# BAROREFLEX SENSITIVITY IN TYPE 2 DIABETES MELLITUS AND END-STAGE LIVER DISEASE

**PhD thesis**


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**Table of Contents**

**List of Abbreviations**

A, end-diastolic lumen cross-sectional area

AVP, vasopressin

BMI, body mass index

BP, blood pressure

BPLM, blood pressure lowering medication

BRS, baroreflex sensitivity

$BRS_{ESLD}$, integrated baroreflex sensitivity in the End-Stage Liver Disease Study

CC, compliance coefficient

CVLM, caudal ventrolateral medulla

D, diameter

$DBP_c$, carotid diastolic blood pressure

DC, distensibility coefficient

$D_{ed}$, external end-diastolic diameter

ΔA, change in lumen area during systole

ΔD, pulsatile distension

$\Delta D_{10\text{-}90}$, change in diameter between 10% and 90% of the systolic rise

$\Delta t_{10\text{-}90}$, duration of diameter change between 10% and 90% of the systolic rise

$D_m$, mean external diameter

DMNX, dorsal motor nucleus of the nervus vagus

DR, distension rate

ECG, electrocardiogram

eGFR, estimated glomerular filtration rate

ESLD, end-stage liver disease

FFT, Fast Fourier Transformation

fr, frequency

GLM, glucose lowering medication

HCV, hepatitis C virus

HMR, high metabolic risk

HR, heart rate

IFG, impaired fasting glucose

IML, intermediolateral

IMT, intima-media thickness

$K_b$, K factor at the brachial artery

$K_c$, K factor at the carotid artery

LF, low frequency

LLM, lipid lowering medication

mBRS, mechanical baroreflex sensitivity

$mBRS_{ESLD}$, mechanical baroreflex sensitivity in the End-Stage Liver Disease Study

$mBRS_{PPS3}$, mechanical baroreflex sensitivity in the Paris Prospective Study III

MetS, metabolic syndrome

NA, nucleus ambiguous

nBRS, neural baroreflex sensitivity

$nBRS_{ESLD}$, neural baroreflex sensitivity in the End-Stage Liver Disease Study

$nBRS_{PPS3}$, neural baroreflex sensitivity in the Paris Prospective Study III

NGM, normal glucose metabolism

NO, nitric oxide

NTS, nucleus tractus solitarius

$PP_b$, pulse pressure in the brachial artery

$PP_c$, pulse pressure in the carotid artery

PPS3, Paris Prospective Study III

PVN, paraventricular nucleus

PWVc, carotid pulse wave velocity

$\rho$, density of blood

RRI, RR interval

RVLM, rostral ventrolateral medulla

$SBP_c$, carotid systolic blood pressure

SON, supraoptic nucleus

t, time

T2D, type 2 diabetes mellitus

WCSA, wall cross sectional area

# 1. Introduction

## 1.1. Physiology of Arterial Baroreflex

Arterial baroreflex plays a major role in short-term blood pressure (BP) regulation. The main function of the arterial baroreflex is the maintenance of a nearly constant BP – following a negative feedback manner – which is essential for the normal function of different organs.

Increasing arterial BP is sensed indirectly by the high-pressure baroreceptors embedded in the wall of carotid sinus and aortic arch. These baroreceptors are mechanosensitive nerve endings responding to deformation not pressure per se (1, 2). The firing frequency of these baroreceptors changes within the range of 50 mmHg and 200 mmHg following a sigmoid pattern as shown by Figure 1 (3). No action potential can be observed on the baroreceptor afferents below approximately 50-60 mmHg and the firing frequency reaches its maximum around 200 mmHg. The system is the most efficient around 100 mmHg which represents the normal operating range of arterial BP.

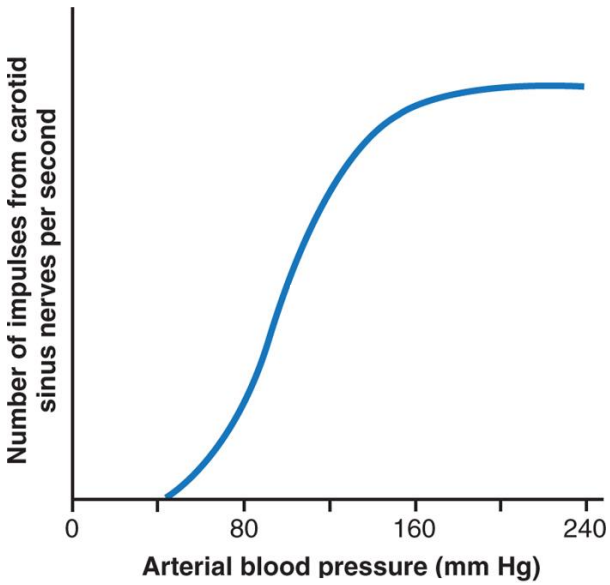


***Figure 1. Carotid Sinus Baroreceptor Activity at Different Arterial Pressure Levels.*** *(Modified after (3).)*

The afferent signal of the baroreceptors is transmitted toward the nucleus tractus solitarius (NTS) via the nervus glossopharyngeus and nervus vagus from the area of carotid sinus and aortic arch, respectively. Figure 2 shows the whole reflex loop with the detailed illustration of the neuronal network that processes the input signal and regulates the efferent mechanisms. Accordingly, elevated BP results in increased parasympathetic activity to the heart leading to lower heart rate (HR), slower atrioventricular conduction and decreased atrial contractility. Furthermore, sympathetic activity decreases to the heart, the vessels in the systemic circulation, the adrenal medulla and the kidney; as a result, HR, myocardial contractility and cardiac excitability decreases, atrioventricular conduction will be slower, resistance in systemic arteries and arterioles decreases, epinephrine and norepinephrine level in the blood decreases and renin secretion decreases. Besides, vasopressin (AVP) secretion also decreases. These regulatory mechanisms decrease cardiac output, total peripheral resistance and blood volume, therefore, BP will decrease and return to the normal value. Decreasing arterial BP elicits responses with opposite direction. Regarding the renin and AVP secretion, the regulatory influence of the low-pressure baroreceptors located primarily in the two venae cavae and the pulmonary large vessels is more pronounced compared with the high-pressure baroreceptors (1). Furthermore, higher centers like the medial prefrontal cortex and insular cortex have modulatory effects on baroreflex function (4).

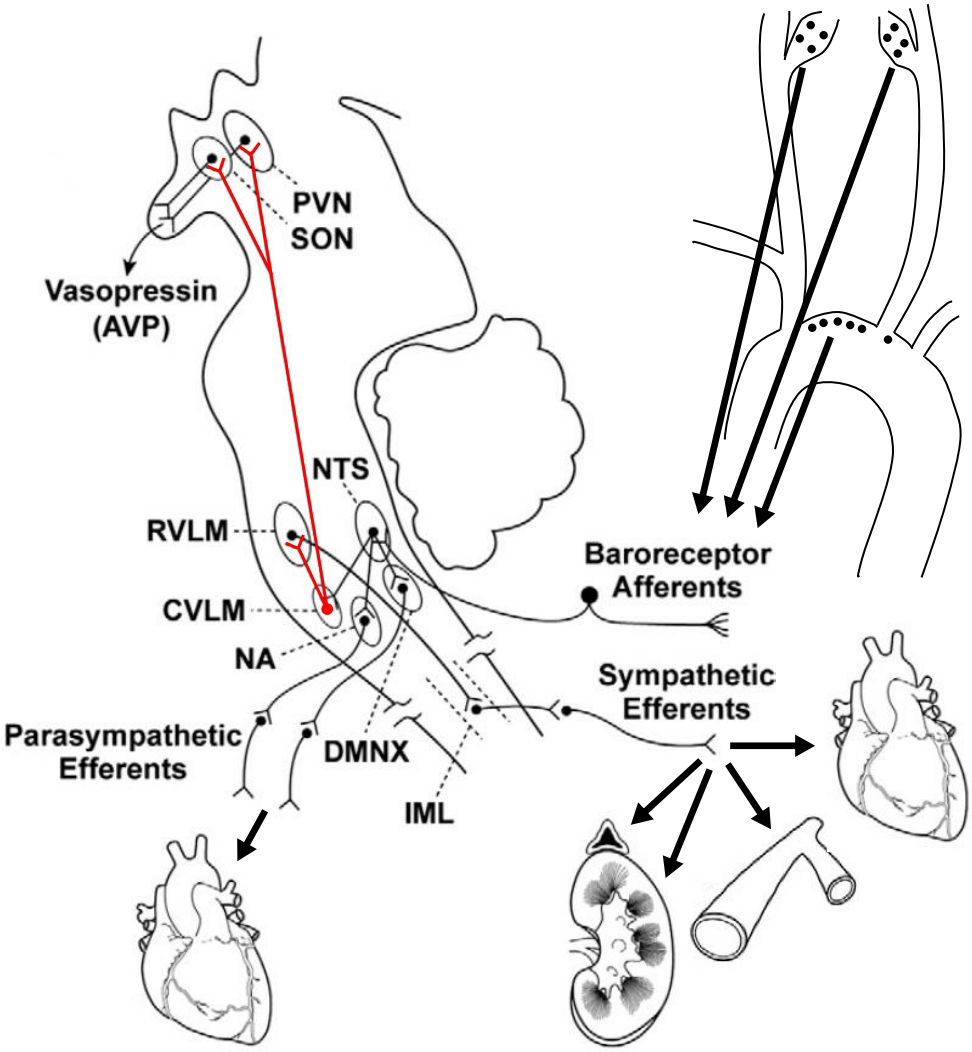


***Figure 2. The arterial baroreflex loop.***

*The information of increasing blood pressure is transmitted to the nucleus tractus solitarius (NTS) by baroreceptor afferents resulting in the activation of the dorsal motor nucleus of the nervus vagus (DMNX), the nucleus ambiguous (NA) and the caudal ventrolateral medulla (CVLM). Activation of DMNX and NA will lead to increased parasympathetic activity to the heart. CVLM will inhibit the constitutive activity of rostral ventrolateral medulla (RVLM) leading to decreased sympathetic activity to the heart, the systemic arteries, the adrenal medulla and the kidney through the decreased activation of the thoracolumbar intermediolateral (IML) neurons. CVLM also inhibits the secretion of vasopressin (AVP) from the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus. Black circles represent the baroreceptors; red colour represents inhibitory connections. (Modified after (1).)*

## 1.2. Measurement of Baroreflex Function – Determination of Baroreflex Sensitivity

There are several methods suitable for the examination of baroreflex function. The majority of these techniques focus on the cardiac chronotropic effects (regulation of HR) from the efferent mechanisms and examine the relationship between the input signal (BP change) and the output signal (change in cardiac cycle length – usually estimated by RR-interval (RRI)).

The first methods developed for the quantification of baroreflex function required different interventions performed on the subjects (e.g.: carotid sinus massage, head-up tilting, lower-body negative pressure application, intravenous injection of vasoactive drugs) or manoeuvre performed by the subject (e.g.: Valsalva manoeuvre). The most widespread and most accepted, "gold standard" method from these techniques is the pharmacological method (intravenous injection of vasoactive drugs) until these days. Smyth et al developed this technique in Oxford, therefore it is also called Oxford method (5). During the application of this procedure blood pressure and ECG are recorded, and a small dose of a pressor agent (originally angiotensin II was used, nowadays phenylephrine is preferred because it does not have direct effects in the central nervous system) is administered intravenously as a bolus. During the analysis of the recordings, the section with increasing BP is selected and then RRI is plotted against systolic BP as shown in Figure 3. The slope of the fitted regression line will give the baroreflex sensitivity (BRS), the quantitative measure of baroreflex function. Essentially, BRS gives the change in RRI in ms evoked by 1 mmHg change in systolic BP. Since in some individuals the RRI-systolic BP relationship is not linear within the range of the systolic BP change because the operating point of the reflex is close to the saturation level, the modified Oxford technique is frequently used (6). During this procedure, administration of phenylephrine is preceded by intravenous bolus injection of sodium nitroprussid (nitric oxide (NO) donor, vasodilator substance). As a result, the initial systolic BP will be lower before the phenylephrine injection and a large linear portion of the RRI-systolic BP relationship could be observed and analysed during the determination of BRS.

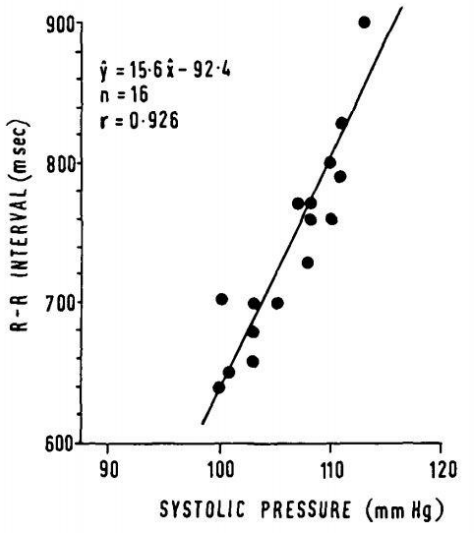


***Figure 3. Determination of baroreflex sensitivity (BRS).***

*RR intervals are plotted against systolic pressure values from the section of the recording where systolic BP increases. The slope of the regression line provides BRS which is 15.6 ms/mmHg in the presented analysis. (Modified after (5).)*

During the last decades, other procedures were developed for the examination of BRS. These techniques focus on the RRI changes evoked by spontaneous fluctuations of BP. Since during the measurement of BRS using these techniques external intervention is not required and the reproducibility of these techniques is good (7, 8), they become very popular and widely accepted and used in the scientific community (9). The detailed description of different spontaneous methods can be found in the methods section.

The ATRAMI study was the first large, longitudinal study that showed the real clinical significance of BRS measurement using the Oxford method. La Rovere et al followed 1284 patients with recent (<28 days) myocardial infarction for 21±8 months. They showed that low BRS (<3 ms/mmHg) had a prognostic value for cardiac mortality which was independent from the known predictors like decreased left ventricular ejection fraction or the presence of frequent ventricular premature complexes (10). La Rovere et al also examined the BRS of 247 patients with heart failure using the Oxford method. After a median follow-up of 29 months they demonstrated that low BRS (<3 ms/mmHg) was associated with an increased risk of cardiac death independently from other clinical variables (11).

The prognostic value of BRS measurement using non-invasive methods was also proved in different patient populations. Robinson et al determined BRS by a spontaneous method in patients with confirmed acute ischemic stroke. They performed the measurements after the stroke (within 72 hours) and followed the patients for 1508 days (median). BRS at the baseline was decreased in patients compared with controls. The subgroup of patients with BRS values ≤ median value of the patient group had significantly higher mortality rate compared with the other part of the patient group. This long-term prognostic value was independent from admission stroke severity (12). Johansson et al examined the prognostic value of BRS measurement in hypertensive patients with renal failure. They followed their subjects for 41±15 months and according to their results low BRS measured by a spontaneous method was an independent predictor of sudden death (13). The aforementioned studies underline the clinical importance of BRS measurements in different diseased states.

### 1.3. Components of Baroreflex Sensitivity

The abovementioned BRS is also called global or integrated BRS because it is calculated using the 2 ends of the reflex loop (BP change and the evoked change in RRI). Integrated BRS is composed of two components: mechanical and neural (6). The mechanical component (mBRS) represents the mechanical transduction of BP change into diameter change of baroreceptor vessels. Therefore, mBRS is importantly dependent on the elastic behaviour of barosensory vessels and frequently estimated by the measurement of different elastic parameters of baroreceptor vessels (14-16). The neural component (nBRS) represents the function of the other parts of the reflex loop ((I) responsiveness of baroreceptors; (II) conduction in afferent nerves; (III) signal processing in the central neural network; (IV) conduction in efferent nerves; (V) reaction of pacemaker cells in the sinoatrial node).

Hunt et al described a method for the quantification of integrated BRS and its components shown in Figure 4 (6). They used the modified Oxford method. However, beside the measurement of BP and RRI they also measured carotid diameter (D) using ultrasound. The gain (slope of the fitted regression line) of the RRI-systolic BP

relationship gave integrated BRS, while the mBRS was determined as the gain of the systolic D-systolic BP relationship and nBRS was calculated as the gain of RRI-systolic D relationship. The product of the 2 components provides integrated BRS.

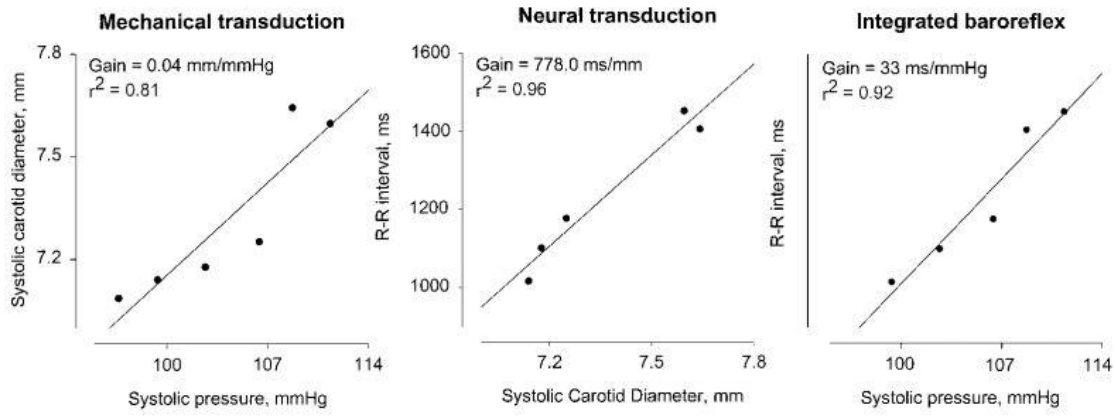


***Figure 4. Determination of integrated baroreflex sensitivity and its components*** *(6). The regression slope of the systolic diameter-systolic pressure relationship gives the mechanical component. The gain of the RR interval-systolic diameter relationship provides the neural component. Integrated baroreflex sensitivity is calculated using the regression slope of the RR interval-systolic pressure relationship.*

Other techniques suitable for the determination of the components of BRS will be described later in details during the methods section.

Decreased integrated BRS could be the consequence of altered mBRS and/or nBRS. Our laboratory showed that impaired elastic function of the common carotid artery (which could represent mBRS) partly explained the damaged baroreflex function in patients with Tetralogy of Fallot (16). Szili-Török et al suggested that altered central and peripheral neural mechanisms are responsible for decreased integrated BRS in patients with Parkinson's and Alzheimer's disease (17). Furthermore, stiffening of baroreceptor vessels and impairment of neural control mechanisms are both responsible for the deterioration of baroreflex function in older individuals (18, 19).

### 1.4. Baroreflex Sensitivity in Patients with Type 2 Diabetes Mellitus

Integrated BRS determined by different spontaneous methods was decreased in patients with type 2 diabetes mellitus (T2D) (20, 21). Decreased BRS could show imbalance in the cardiovascular autonomic nervous system in an early stage of diabetes when the abnormalities are undetectable by tests routinely used in clinical practice (22). A longitudinal study performed by Okada et al showed that major adverse cardiovascular events were independently predicted by depressed BRS (determined by the Oxford method) in patients with T2D (23). The background of impaired BRS is not well understood. While the results about different mBRS parameters are contradictory (24-26), direct examination of nBRS have not yet been performed.

Different prediabetic states like impaired fasting glucose (IFG) or metabolic syndrome (MetS) could have different influence on the two components of BRS. Based on earlier findings, no baroreflex impairment was shown in subjects with IFG (27). However, IFG was associated with carotid stiffening in elder individuals (28). No information is available about the neural component of BRS in IFG subjects. The results of the studies focusing on global baroreflex sensitivity or carotid elasticity – potential estimator of the mechanical component – in MetS were controversial (29-31). One substudy of the Paris Prospective Study III showed deteriorated nBRS in MetS (14).

### 1.5. Baroreflex Sensitivity in Patients with End-Stage Liver Disease

Cardiovascular autonomic neuropathy measured with standard clinical tests is a frequently observed complication in chronic liver disease independently from aetiology (32). The surgical risk is higher during liver transplantation in patients with definite autonomic dysfunction (33). Furthermore, Hendrickse et al showed that vagal neuropathy was an independent predictor of mortality in chronic liver disease (34). BRS was decreased in cirrhotic patients and inversely correlated with the Child-score showing that severe hepatic damage is associated with worse baroreflex function (35, 36). Furthermore, more severely damaged baroreflex regulation was associated with higher mortality in cirrhotic patients (37). In line with these findings, our laboratory

showed impaired integral BRS in patients with chronic hepatitis C virus (HCV) infection (38). In end-stage liver disease, the production of vasodilator substances increases; among these vasodilators, NO plays an essential role (39, 40). Smith et al demonstrated that experimental augmentation of NO-level in the NTS reduced BRS in anesthetized cats (41). Based on these results, increased NO-level could be a potential link between advanced liver disease and impaired baroreflex function. However, whether damaged neural signal processing and/or altered mechanical transduction is responsible for the deteriorated baroreflex regulation is not clarified.

## 2. Objectives

We aimed to determine mBRS and nBRS in subjects with normal glucose metabolism (NGM), subjects at high metabolic risk (HMR) and patients with T2D within the confines of a cross-sectional population study. We hypothesized that a stepwise deterioration would be observable from NGM towards T2D in both nBRS and mBRS.

Our other goal was the measurement of integrated BRS and its components in patients with end-stage liver disease (ESLD) in a cross-sectional case-control study. Based on previous results, we hypothesized that impaired neural signal processing (decreased nBRS) would be responsible for the deteriorated baroreflex function in ESLD.

## 3. Methods

### 3.1. Paris Prospective Study III

#### 3.1.1. Study Participants and Overview

The Paris Prospective Study III (PPS3) is a large prospective study that focuses on the relationship between carotid stiffness, novel HR parameters and sudden death among other cardiovascular diseases (42). 10,157 participants aged 50-75 years were enrolled in the study between June 2008 and May 2012. The recruitment was performed by the Centre d'Investigations Préventives et Cliniques in Paris (France). During the first visit, a fasting blood sample was taken for the determination of standard blood biomarkers. Then, the volunteers completed self-administered questionnaires about their lifestyle (smoking, diet, alcohol consumption, physical activity - evaluated by the Baecke questionnaire (43)) and medical history. Afterwards, they underwent a standard clinical examination which contained a high-precision carotid echotracking to determine the components of BRS. Written informed consent was provided by the subjects and the Ethics Committee of the Cochin Hospital (Paris) approved the study protocol. The number of the study in the international trial registry is NCT00741728.

#### 3.1.2. Study groups

Carbohydrate metabolism status was determined according to the current WHO criteria (44). NGM was defined as fasting glucose level <110 mg/dl and no antidiabetic treatment. When the fasting glucose level ≥110 mg/dl and <126 mg/dl and no hypoglycaemic medication was used, the participants were diagnosed with IFG. T2D was defined as fasting glucose level ≥126 mg/dl and/or use of antidiabetic treatment. The non-T2D population was further subdivided based on the MetS status. The diagnosis of MetS was based on the harmonized MetS definition described by Alberti et al (45) and we used it in line with the recommendation of the WHO expert consultation (46). Since MetS is a premorbid state according to the mentioned WHO expert consensus, patients with T2D or cardiovascular disease history were excluded from this category. MetS was defined by the presence of 3 or more of the following criteria: (I) waist circumference ≥94 cm in men and ≥80 cm in women; (II) triglycerides ≥150

mg/dl or nicotinic acid or fibrate or high-dose ω-3 fatty acid treatment; (III) high density lipoprotein cholesterol level <40 mg/dl in men and <50 mg/dl in women or nicotinic acid or fibrate treatment; (IV) systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or usage of antihypertensive treatment; (V) fasting glucose level ≥110 mg/dl and <126 mg/dl and no hypoglycaemic medication use. We changed the cut point from 100 mg/dl to 110 mg/dl for fasting glycaemia to remain coherent with the WHO guideline about the diagnosis of glucose metabolism disorders. We created a HMR group with subjects having IFG and/or MetS. Since we excluded 2321 subjects due to missing data and 210 subjects due to prior cardiovascular diseases (CVD) – we wanted to eliminate the potential effects of previous CVD on the components of BRS – our final study population consisted of the following groups as it is shown in Figure 5: 5857 subjects with NGM, 1450 subjects with HMR and 319 patients with T2D.

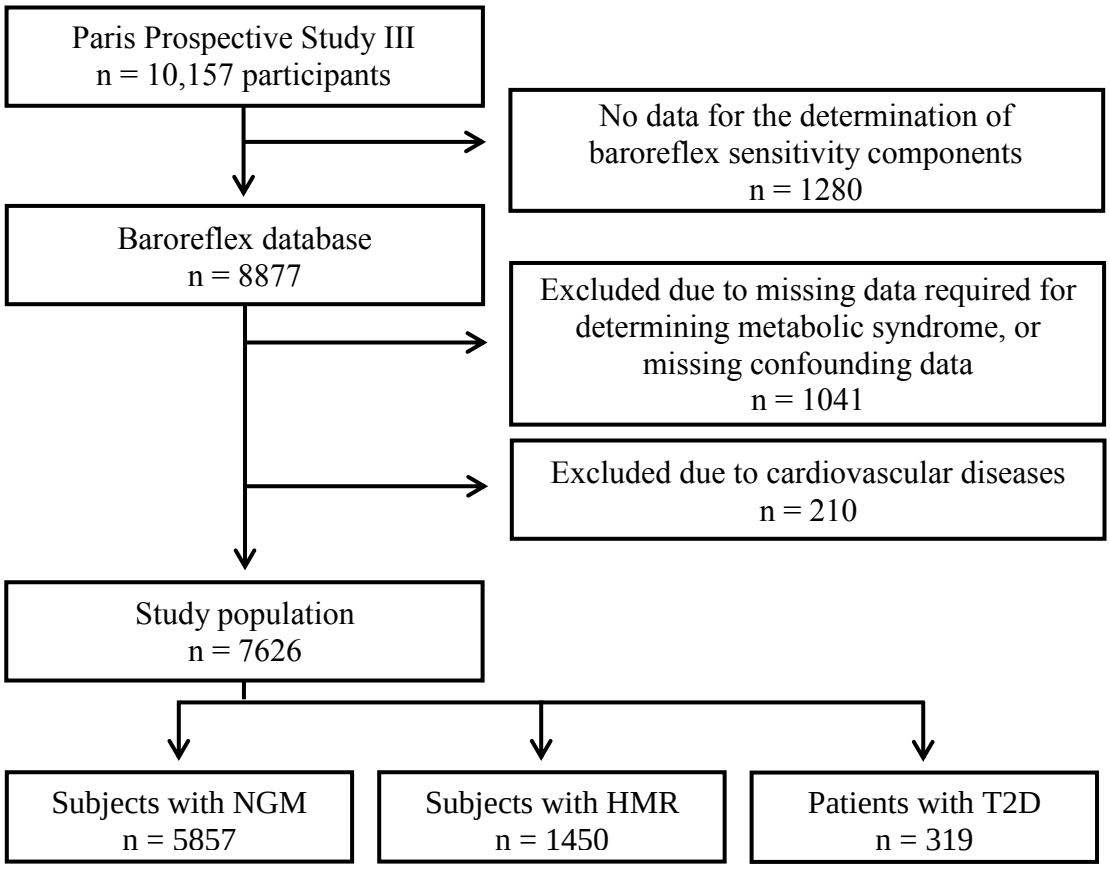


***Figure 5. Flowchart showing the selection and categorization of our participants.*** *NGM, normal glucose metabolism; HMR, high metabolic risk; T2D, type 2 diabetes mellitus.*

### 3.1.3. Measurement of Carotid Parameters

These examinations were performed in a temperature controlled (22 ± 1 ºC) and quiet room. The subjects had to fast for at least 4 hours before the measurements. After a 10-minute-long resting period in supine position, brachial systolic and diastolic BP were measured with an oscillometric method (Omron 705C). Pulse pressure in the brachial artery ($PP_b$) was calculated as follows: $PP_b$ = systolic BP – diastolic BP. Mean BP was determined as diastolic BP + $PP_b/3$. Common carotid artery external end-diastolic diameter ($D_{ed}$) and intima-media thickness (IMT) were measured in B-mode (60 Hz, 128 radiofrequency lines), pulsatile distension (ΔD) was measured in fast B-mode (600Hz, 14 radiofrequency lines) 2 cm proximal to the carotid bulb with a high-resolution echotracking device (ART.LAB®, Esaote, Maastricht, Netherlands) using a conventional ultrasound scanner (7.5 MHz linear array). A 6-second-long recording was made both in B-mode and in fast B-mode and a 5-minute-long recording was performed in fast B-mode afterwards. We determined carotid pulse pressure ($PP_c$) based on the method reported by Van Bortel et al by the calibration of the recorded carotid distension wave (47). This calibration process is built on the observation that the difference between mean BP and diastolic BP is constant in the large arteries. Therefore, $PP_c$ is calculated as follows: $PP_c = PP_b \times K_c/K_b$, where $K_c$ and $K_b$ are K factors at the carotid and brachial arteries, respectively. $K_c$ is defined as $(D_m - D_{ed})/\Delta D$ (where $D_m$ is the mean external diameter calculated by dividing the area under the distension wave by time) and $K_b$ is calculated as (mean BP – diastolic BP)/$PP_b$.

### 3.1.4. Determination of Mechanical Baroreflex Sensitivity

We used the Bramwell-Hill equation to calculate carotid pulse wave velocity (PWVc) representing mBRS as follows: $mBRS_{PPS3} = PWVc = \sqrt{1/(\rho \times DC)}$, where ρ is the density of blood and DC is the distensibility coefficient of the carotid artery (48). DC provides 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. Local carotid pulse wave velocity is shown in meters per second (m/s) by our $mBRS_{PPS3}$ parameter which is

a widely used and accepted marker of local arterial stiffness (14, 26, 48). The higher the pulse wave velocity, the stiffer the artery, the lesser the stretch for similar changes in BP. We also determined other elastic parameters of the carotid artery representing other metrics of the mechanical component of BRS according to an international guideline (48). Beyond the determination of the mentioned DC, we calculated compliance coefficient (CC) as $\Delta A/PP_c$. It gives information about the buffering capacity of the vessel as a whole. Young's elastic modulus was also determined as $[3\times(1+A/WCSA)]/DC$, where WCSA stands for wall cross-sectional area. This parameter provides information about the vessel wall material.

### 3.1.5. Determination of Neural Baroreflex Sensitivity

The neural component of BRS ($nBRS_{PPS3}$) was determined by the method developed by Kornet et al (49). Common carotid diameter curve was recorded in fast B-mode for 5 minutes. RR intervals were determined as the time difference between the feet of consecutive distension waves as shown in Figure 6. Carotid distension rate (DR) was determined as the ratio of the diameter change between 10% and 90% of the systolic rise and the associated rise time (Figure 6).

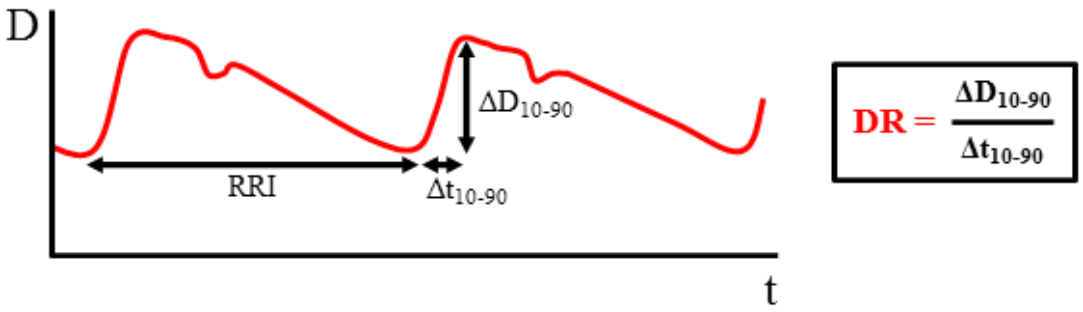


***Figure 6. Determination of RR interval (RRI) and distension rate (DR).***
*D, diameter; $\Delta D_{10\text{-}90}$, change in diameter between 10% and 90% of the systolic rise; $\Delta t_{10\text{-}90}$, duration of diameter change between 10% and 90% of the systolic rise; t, time.*

Figure 7 shows the determination process of $nBRS_{PPS3}$. After the 5-minute-long recording we created the time series of DR and RRI and selected a section of 256 heart beats for analysis. Fast Fourier Transformation was used to obtain the power spectrum

of DR and RRI. The $nBRS_{PPS3}$ was defined as the low frequency (LF) gain: the mean cross-spectral transfer gain between DR and RRI signals in the low frequency band (0.04-0.15 Hz).

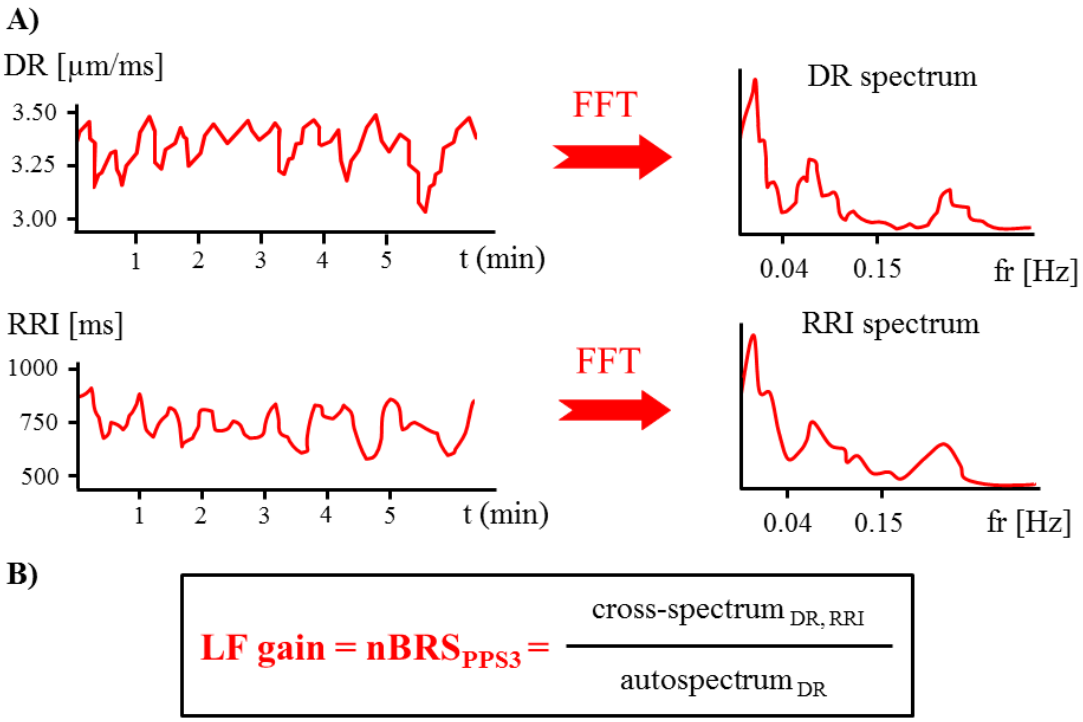


***Figure 7. Determination of neural baroreflex sensitivity ($nBRS_{PPS3}$) by the Kornet method.***

*A) Power spectrum of distension rate (DR) and RR interval (RRI) was created from the time series of DR and RRI by Fast Fourier Transformation (FFT). t, time; fr, frequency. B) The ratio of the cross-spectrum between DR and RRI and the autospectrum of DR in the low frequency (LF) band provided $nBRS_{PPS3}$.*

### 3.1.6. Statistical Analysis

SAS software 9.4 (Statistical Analysis System, Cary, NC, USA) was used to perform the statistical analyses. While variables with normal distribution are expressed as mean±SD, variables with skewed distribution underwent logarithmic transformation and are presented as median (interquartile range). A special ln-transformation was used in the case of neural baroreflex sensitivity: $nBRS_{PPS3}$, normalized units (NU) = $\ln(10^2 \times LF\ gain)$. Armitage chi-square test or linear regression was used for unadjusted test for

trend across the groups for categorical and continuous variables, respectively. We used multivariable linear regression with Tukey's post hoc test to quantify the associations between our subject groups and the arterial parameters. The association of HMR/T2D with $nBRS_{PPS3}$ and $mBRS_{PPS3}$ was adjusted for potential confounders (age, sex, body mass index (BMI), smoking, alcohol consumption and physical activity score) firstly. Potential confounding factors could have distinct influence on the dependent and independent variables (50). As a second step, we made further adjustments for suspected mediators identified from the literature (mean BP, statin use, estimated glomerular filtration rate (eGFR), and additionally, $mBRS_{PPS3}$ in the case of $nBRS_{PPS3}$, HR in the case of $mBRS_{PPS3}$ – we did not adjust for HR when investigating $nBRS_{PPS3}$ to avoid potential collinearity). Potential mediators could explain the association between the dependent and the independent variables (50). To discriminate between the influence of IFG and other metabolic disturbances, we split the HMR group into the following subgroups: (I) IFG alone, no MetS; (II) MetS without IFG; (III) MetS with IFG. Then, we adjusted our analysis for these subgroups instead of the HMR group. To verify the robustness of our findings we performed several sensitivity analyses. First, we excluded subjects treated by insulin (suspected to have type 1 diabetes mellitus). Second, to assess the confounding influence of antihypertensive treatment, we adjusted the analysis for antihypertensive medication (yes/no) and then for antihypertensive drug classes. Third, we repeated the analyses using other metrics of the mechanical component of BRS (CC, DC and Young's elastic modulus). Last, since models adjusted for age, sex and mean BP are frequent in other studies, we built similar models to ease the comparisons (24, 26). The continuous variables were used in standardized forms as z-scores in our multivariable models. The threshold for statistical significance was $p<0.05$.

### 3.2. End-Stage Liver Disease Study

#### 3.2.1. Study Participants and Overview

24 patients with ESLD awaiting liver transplantation were recruited into our study from the Department of Transplantation and Surgery, Semmelweis University, Budapest,

Hungary. 19 patients suffered from chronic active hepatitis-related liver disease (3 hepatitis B, 12 hepatitis C, 1 autoimmun and 3 cryptogenic), 3 patients had alcoholic liver disease and 2 had primary biliary cirrhosis. All patients had portal hypertension confirmed by computer tomography, duplex ultrasonography and/or endoscopic examination. Patients with permanent pacing, atrial fibrillation or more than 2 ectopic beats/minute during data acquisition, recent gastrointestinal bleeding, hepatic encephalopathy above grade I or clinical instability within the preceding two months were excluded. A control group of 23 healthy volunteers with similar age and sex distribution was also examined. All subjects gave their written informed consent and the Ethical Committee of the Semmelweis University approved the study protocol (ethical permission number: 571/09).

The measurements were performed at the Clinical Cardiovascular Laboratory, Department of Physiology, Semmelweis University, Budapest, Hungary. Similarly to the PPS3, the measurements were performed in a temperature controlled (22 ± 1 ºC), quiet room. The subjects were asked to abstain from smoking and fast for at least 2 hours prior to the study. Besides, they had to refrain from drinking caffeine containing beverages and alcohol and performing strenuous physical exercise for at least 24 hours before the measurements. During their visit, a detailed medical history was documented by the examiner and anthropometric parameters (height, weight) were recorded. Then, the subjects rested in a supine position after instrumentation until the stabilization of HR and BP. Brachial systolic and diastolic BP were measured with an oscillometric device (Colin CBM-7000, Colin Corporation, Komaki City, Japan). Mean BP was calculated similarly to the PPS3 study. Afterwards, carotid echotracking and applanation tonometry was performed to determine the mechanical component of BRS that was followed by a simultaneous 10-minute-long recording of ECG and BP to determine integrated BRS.

### 3.2.2. Determination of Integrated Baroreflex Sensitivity

The spontaneous sequence method was used for the assessment of integrated BRS ($BRS_{ESLD}$) (9, 51). ECG was recorded in Einthoven lead II for 10 minutes.

Simultaneous, beat-to-beat BP recording was performed non-invasively using a special plethysmographic cuff (Finapres 2300, Ohmeda, Helsinki, Finland) placed around the right middle finger. The analysis was made by the WinCPRS software that generated the systolic BP and RRI time series. The determination of integrated BRS is shown in Figure 8. The built-in algorithm detected the sequences in which systolic BP and RRI concurrently increased over 3 or more consecutive beats. 1 mmHg was the minimal accepted change for a spontaneous rise in SBP and 5 ms for the lengthening in RRI within the sequences. The slope of the RRI-systolic BP relationship provided the BRS value of the sequence. The sequences were considered valid when the correlation coefficient was >0.85. The $BRS_{ESLD}$ was calculated as the mean of the slopes of the valid sequences. During the measurement, respiration rate was controlled with a metronome at 15/minute frequency.

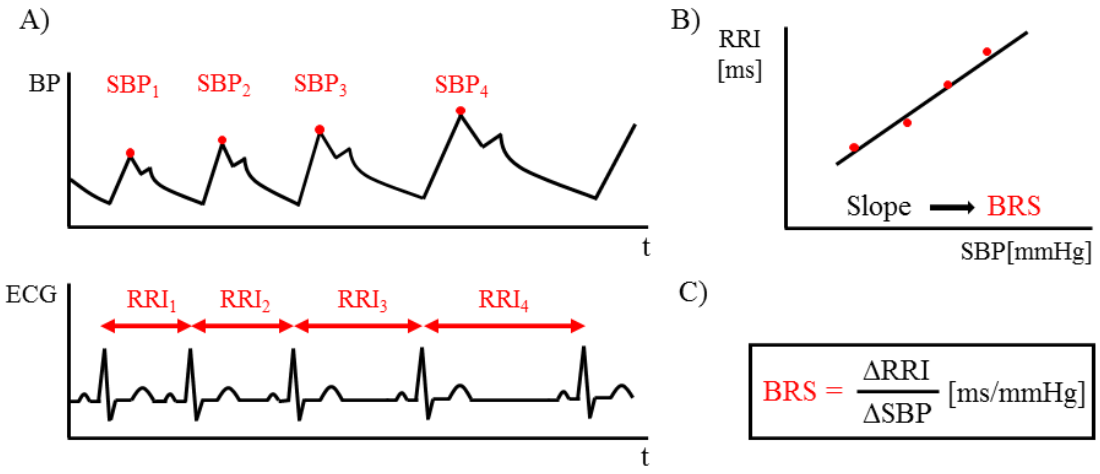


***Figure 8. The determination of integrated baroreflex sensitivity (BRS) by the spontaneous sequence method.***

*A) The sequence is identified where increasing systolic blood pressure (SBP) values are followed by lengthening RR intervals (RRI). BP, blood pressure; ECG, electrocardiogram; t, time. B) BRS is defined as the slope of the RRI-SBP relationship. C) The mathematical formula that provides the BRS value of the given sequence.*

### 3.2.3. Determination of Mechanical Baroreflex Sensitivity

Similarly to the PPS3 project, IMT, $D_{ed}$ and $\Delta D$ were measured in B-mode and fast B-mode with the same echotracking system (ART.LAB®, Esaote, Maastricht, Netherlands) using a conventional ultrasound scanner (10 MHz linear scanner, L10-5, 40 mm, Picus Pro, Esaote, Maastricht, Netherlands).

Carotid BP values were determined using applanation tonometry (Figure 9). Due to the flattening of the artery by the tonometer (SPT-301, Millar Instruments, Houston, TX, USA) the circumferential pressures were equalized and high-fidelity pressure waveforms were recorded. Since mean and diastolic BP can be considered constant in the large arteries (52) the carotid pressure curves were calibrated with brachial mean and diastolic BP values using the fact that the area under the pressure curve divided by the associated time interval provides mean pressure (48). The SphygmoCor device (AtCor, Sydney, Australia) was used for the analysis of the tonometric data.

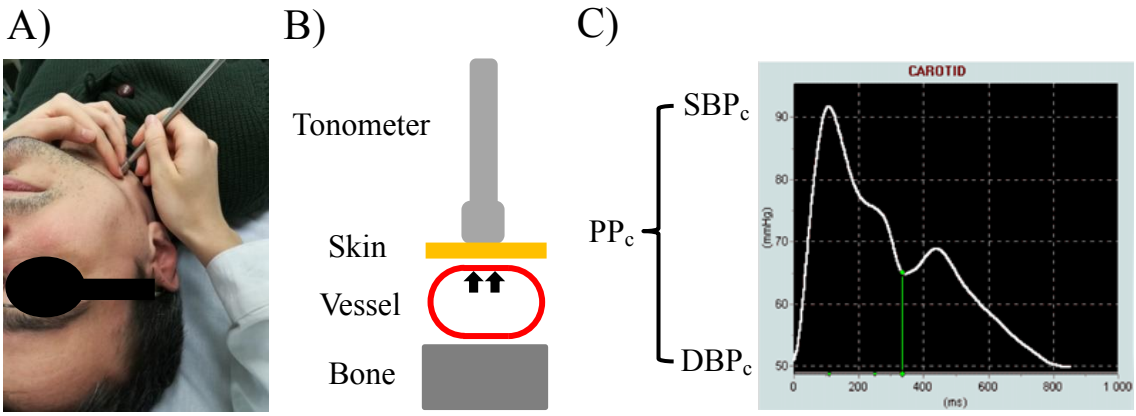


***Figure 9. Determination of carotid blood pressure by applanation tonometry.***

*A) The tonometer is placed onto the skin above the common carotid artery. B) The pressure wave is recorded properly due to the flattening of the artery. C) The calibration of the recorded signal provides carotid systolic pressure ($SBP_c$), diastolic pressure ($DBP_c$) and pulse pressure ($PP_c$). (Recording from the SphygmoCor program.)*

We used carotid distensibility coefficient for the estimation of the mechanical component of BRS ($mBRS_{ESLD}$). We calculated $mBRS_{ESLD}$ according to the guideline written by Van Bortel et al as follows: $mBRS_{ESLD} = (2\times\Delta D\times D_{ed}+\Delta D^2)/(PP_c\times D_{ed}^2)$ (53).

We also determined CC, DC, PWVc and Young's elastic modulus similarly to the PPS3 project according to the recommendation of Laurent et al (48) to be able to compare the main findings of the 2 study in reference to different metrics of the mechanical component of BRS.

### 3.2.4. Determination of Neural Baroreflex Sensitivity

Since integrated BRS is the simple product of the mechanical and the neaural components (6), we estimated neural BRS ($nBRS_{ESLD}$) using the ratio of $BRS_{ESLD}$ and $mBRS_{ESLD}$.

### 3.2.5. Statistical Analysis

IBM SPSS v. 22 (IBM Corporation, Somers, NY, USA) was used to perform the statistical analyses. Data are presented as mean±SD for normally distributed variables and median (interqurtile range) for variables with skewed distribution. We tested the normal distribution of data with the Shapiro-Wilk test. Variables with skewed distribution were logarithmically transformed as follows: normalized unit = lg(100×variable). Between group comparisons were made by chi-square test and independent samples t-test for categorical and continuous variables, respectively. $P<0.05$ was the threshold for statistical significance.

As it was mentioned earlier, our laboratory demonstrated that BRS was 7.1±3.4 and 11.5±6.5 ms/mmHg for patients with chronic HCV infection and healthy controls, respectively (38). Based on these results, the calculated Cohen's d effect size was 0.848. Using a two sided independent samples t-test with significance level set at 0.05 and power of 0.80, the sample size calculation indicated that the recruitment of at least 23 patients and 23 control subjects was required in the present study.

## 4. Results

### 4.1. Paris Prospective Study III

The baseline characteristics of our groups are presented in Table 1. The mean age was 60 years in the whole population and 40% of the participants were women. Subjects with HMR and patients with T2D had significantly higher BMI, BP and HR, were more likely to be men, take BP and lipid lowering medication compared with the subjects with NGM. Besides, $nBRS_{PPS3}$ decreased and $mBRS_{PPS3}$ increased significantly across the groups as shown in Table 2. Similar results were seen when other carotid elastic parameters representing other metrics of the mechanical component of BRS were examined (Table 3).

Compared with NGM subjects, $nBRS_{PPS3}$ was significantly lower in T2D patients and the association was borderline significant in HMR subjects after adjustment for confounding factors (Table 4). Both HMR and T2D subjects had significantly higher $mBRS_{PPS3}$ compared with NGM subjects after similar adjustment (Table 4). After further adjustment for mediating factors, $nBRS_{PPS3}$ was significantly lower in HMR and in T2D subjects compared with NGM subjects as shown in Table 5. However, no significant association was found between HMR or T2D status and $mBRS_{PPS3}$. Age, sex, BMI, smoking, physical activity score, mean BP, eGFR and $mBRS_{PPS3}$ were significant associates of $nBRS_{PPS3}$. Factors significantly associated with $mBRS_{PPS3}$ were age, sex, BMI, alcohol consumption, mean BP, HR and eGFR.

The subgroup analysis showed that decreased $nBRS_{PPS3}$ in HMR subjects was observed in subjects with MetS without IFG, in subjects with MetS with IFG, but not in subjects with IFG without MetS as compared with the NGM group (Table 6). Significantly altered $mBRS_{PPS3}$ was seen only in those HMR subjects who had both MetS and IFG.

Table 7 shows that the exclusion of patients under insulin treatment resulted in essentially unaltered results. Table 8 shows the number of subjects receiving different BP lowering drug classes in different groups. Based on these descriptive results, we created the following subgroups: (I) beta blocking agents, (II) calcium channel blockers,

(III) agents acting on the renin-angiotensin system, (IV) diuretics and other antihypertensive agents. Neither the adjustment for antihypertensive treatment (yes/no) nor the adjustment for antihypertensive drug classes changed our main results (Table 9 and 10). Our results were similar when other carotid elastic parameters were used in the models instead of $mBRS_{PPS3}$ (Table 11). Figure 10 shows that after adjustment for age, sex and mean BP, $nBRS_{PPS3}$ decreased, $mBRS_{PPS3}$ increased across the main study groups.

***Table 1. Participant Characteristics.***

*Data are mean±SD or median (interquartile range). [*]indicates significant difference compared with subjects with NGM; [†]indicates significant difference compared with subjects with HMR. NGM, normal glucose metabolism; HMR, high metabolic risk; T2D, type 2 diabetes mellitus; BP, blood pressure; BPLM, blood pressure lowering medication; LLM, lipid lowering medication; GLM, glucose lowering medication; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate.*

| | **NGM (n = 5857)** | **HMR (n = 1450)** | **T2D (n = 319)** | **p trend** |
|---|---|---|---|---|
| Age (years) | 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 |
| Physical activity score | 6.9±1.5 | 6.8±1.6[*] | 6.6±1.6[*] | <0.0001 |
| Systolic BP (mmHg) | 129±16 | 136±15[*] | 137±16[*] | <0.0001 |
| Diastolic BP (mmHg) | 75±9 | 79±10[*] | 78±10[*] | <0.0001 |
| Mean BP (mmHg) | 93±11 | 98±10[*] | 98±10[*] | <0.0001 |
| Resting heart rate (bpm) | 68±10 | 71±12[*] | 73±13[*†] | <0.0001 |
| BPLM, n (%) | 710 (12) | 343 (24)[*] | 136 (43)[*†] | <0.0001 |
| LLM, n (%) | 560 (10) | 306 (21)[*] | 103 (32)[*†] | <0.0001 |
| GLM, 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 |

***Table 2. The Components of Baroreflex Sensitivity in Subjects with Normal Glucose Metabolism (NGM), Subjects with High Metabolic Risk (HMR) and Patients with Type 2 Diabetes Mellitus (T2D).***

*Data are mean±SD. *indicates significant difference compared with subjects with NGM. $nBRS_{PPS3}$, neural baroreflex sensitivity; $mBRS_{PPS3}$, mechanical baroreflex sensitivity – estimated by the determination of carotid pulse wave velocity.*

| | **NGM (n = 5857)** | **HMR (n = 1450)** | **T2D (n = 319)** | **p trend** |
|---|---|---|---|---|
| $nBRS_{PPS3}$ (NU) | 2.96±0.63 | 2.89±0.63* | 2.80±0.67* | <0.0001 |
| $mBRS_{PPS3}$ (m/s) | 7.0±1.3 | 7.4±1.4* | 7.6±1.4* | <0.0001 |

***Table 3. Carotid Elastic Parameters Other than the Main Mechanical Baroreflex Sensitivity Parameter ($mBRS_{PPS3}$) in Subjects with Normal Glucose Metabolism (NGM), Subjects with High Metabolic Risk (HMR) and Patients with Type 2 Diabetes Mellitus (T2D).***

*Data are mean±SD. *indicates significant difference compared with NGM.*

| | **NGM (n = 5857)** | **HMR (n = 1450)** | **T2D (n = 319)** | **p trend** |
|---|---|---|---|---|
| Compliance coefficient ($m^2\ kPa^{-1}\ 10^{-6}$) | 0.60±0.24 | 0.57±0.22* | 0.56±0.21* | <0.0001 |
| Distensibility coefficient ($10^{-3}$/kPa) | 22.6±8.2 | 20.0±7.3* | 19.0±6.7* | <0.0001 |
| Young's elastic modulus (kPa) | 480±211 | 539±242* | 554±215* | <0.0001 |

***Table 4. Multivariable Associations between High Metabolic Risk (HMR; n = 1450) or Type 2 Diabetes Mellitus (T2D; n = 319) and Neural Baroreflex Sensitivity ($nBRS_{PPS3}$) or Mechanical Baroreflex Sensitivity ($mBRS_{PPS3}$) as Compared with Normal Glucose Metabolism (NGM; n = 5857) after Adjustment for Confounding Factors.***

*Data are unstandardized regression coefficients and 95% confidence intervals. The continuous variables were included in the models in standardized forms as z-scores. Confounding factors: age, sex, body mass index, smoking, alcohol consumption and physical activity score. ref, reference group.*

| | **$nBRS_{PPS3}$** | **$mBRS_{PPS3}$** |
|---|---|---|
| NGM | ref | ref |
| HMR | -0.06 (-0.12, 0.00), p=0.059 | 0.17 (0.11, 0.23), p<0.0001 |
| T2D | -0.16 (-0.28, -0.05), p=0.006 | 0.20 (0.09, 0.31), p=0.0003 |
| Age | -0.11 (-0.13, -0.08), p<0.0001 | 0.24 (0.22, 0.27), p<0.0001 |
| Sex | 0.02 (-0.03, 0.07), p=0.40 | -0.04 (-0.09, 0.00), p=0.067 |
| Body mass index | -0.05 (-0.07, -0.03), p=0.0001 | 0.17 (0.15, 0.19), p<0.0001 |
| Smoking | -0.12 (-0.19, -0.06), p=0.0002 | -0.03 (-0.09, 0.03), p=0.33 |
| Alcohol consumption | 0.04 (-0.03, 0.10), p=0.32 | -0.08 (-0.14, -0.01), p=0.024 |
| Physical activity score | 0.02 (0.00, 0.05), p=0.067 | -0.01 (-0.04, 0.01), p=0.30 |

***Table 5. Multivariable Associations between High Metabolic Risk (HMR; n = 1450) or Type 2 Diabetes Mellitus (T2D; n = 319) and Neural Baroreflex Sensitivity ($nBRS_{PPS3}$) or Mechanical Baroreflex Sensitivity ($mBRS_{PPS3}$) as Compared with Normal Glucose Metabolism (NGM; n = 5857) after Adjustment for Confounding and Mediating Factors.***

*Data are unstandardized regression coefficients and 95% confidence intervals. The continuous variables were included in the models in standardized forms using z-scores. Confounding factors: age, sex, body mass index, smoking, alcohol consumption and physical activity score. Mediating factors: mean blood pressure, statin use, estimated glomerular filtration rate (eGFR), and additionally, $mBRS_{PPS3}$ in the case of $nBRS_{PPS3}$, heart rate in the case of $mBRS_{PPS3}$. ref, reference group.*

| | **$nBRS_{PPS3}$** | **$mBRS_{PPS3}$** |
|---|---|---|
| NGM | ref | ref |
| HMR | -0.07 (-0.12, -0.01), p=0.029 | 0.04 (-0.01, 0.10), p=0.12 |
| T2D | -0.18 (-0.29, -0.07), p=0.002 | 0.08 (-0.02, 0.18), p=0.12 |
| Age | -0.16 (-0.19, -0.14), p<0.0001 | 0.21 (0.19, 0.24), p<0.0001 |
| Sex | 0.09 (0.04, 0.14), p=0.0003 | -0.06 (-0.11, -0.02), p=0.007 |
| Body mass index | -0.07 (-0.10, -0.05), p<0.0001 | 0.11 (0.08, 0.13), p<0.0001 |
| Smoking | -0.11 (-0.17, -0.04), p=0.001 | -0.01 (-0.06, 0.05), p=0.88 |
| Alcohol consumption | 0.05 (-0.02, 0.12), p=0.16 | -0.08 (-0.14, -0.02), p=0.015 |
| Physical activity score | 0.03 (0.01, 0.05), p=0.015 | -0.01 (-0.03, 0.02), p=0.69 |
| Mean blood pressure | -0.14 (-0.16, -0.12), p<0.0001 | 0.30 (0.28, 0.33), p<0.0001 |
| Heart rate | - | 0.11 (0.09, 0.14), p<0.0001 |
| Statin use | -0.04 (-0.12, 0.03), p=0.26 | -0.03 (-0.10, 0.04), p=0.44 |
| eGFR | -0.05 (-0.07, -0.03), p<0.0001 | -0.07 (-0.09, -0.04), p<0.0001 |
| $mBRS_{PPS3}$ | 0.25 (0.23, 0.27), p<0.0001 | - |

***Table 6. Multivariable Associations between High Metabolic Risk Subgroups (n = 1450) or Type 2 Diabetes Mellitus (T2D; n = 319) and Neural Baroreflex Sensitivity ($nBRS_{PPS3}$) or Mechanical Baroreflex Sensitivity ($mBRS_{PPS3}$) as Compared with Normal Glucose Metabolism (NGM; n = 5857) after Adjustment for Confounding and Mediating Factors.***

*Data are unstandardized regression coefficients and 95% confidence intervals. The continuous variables were included in the models in standardized forms using z-scores. Confounding factors: age, sex, body mass index, smoking, alcohol consumption and physical activity score. Mediating factors: mean blood pressure, statin use, estimated glomerular filtration rate (eGFR), and additionally, $mBRS_{PPS3}$ in the case of $nBRS_{PPS3}$, heart rate in the case of $mBRS_{PPS3}$. ref, reference group; IFG, impaired fasting glucose; MetS, metabolic syndrome.*

| | **$nBRS_{PPS3}$** | **$mBRS_{PPS3}$** |
|---|---|---|
| NGM | ref | ref |
| IFG, no MetS (n = 420) | 0.05 (-0.05, 0.14), p=0.33 | -0.06 (-0.15, 0.03), p=0.17 |
| MetS, no IFG (n = 624) | -0.10 (-0.18, -0.02), p=0.019 | 0.06 (-0.02, 0.14), p=0.11 |
| MetS with IFG (n = 406) | -0.15 (-0.25, -0.05), p=0.004 | 0.14 (0.05, 0.24), p=0.002 |
| T2D | -0.18 (-0.30, -0.07), p=0.001 | 0.09 (-0.02, 0.19), p=0.095 |
| Age | -0.16 (-0.19, -0.14), p<0.0001 | 0.21 (0.19, 0.24), p<0.0001 |
| Sex | 0.08 (0.04, 0.13), p=0.0007 | -0.06 (-0.10, -0.01), p=0.012 |
| Body mass index | -0.06 (-0.09, -0.04), p<0.0001 | 0.10 (0.08, 0.12), p<0.0001 |
| Smoking | -0.11 (-0.17, -0.04), p=0.001 | -0.01 (-0.06, 0.05), p=0.87 |
| Alcohol consumption | 0.05 (-0.02, 0.12), p=0.16 | -0.08 (-0.14, -0.02), p=0.016 |
| Physical activity score | 0.03 (0.01, 0.05), p=0.020 | 0.00 (-0.03, 0.02), p=0.75 |
| Mean blood pressure | -0.14 (-0.16, -0.11), p<0.0001 | 0.30 (0.28, 0.32), p<0.0001 |
| Heart rate | - | 0.11 (0.09, 0.14), p<0.0001 |
| Statin use | -0.04 (-0.12, 0.03), p=0.27 | -0.03 (-0.10, 0.04), p=0.43 |
| eGFR | -0.05 (-0.07, -0.03), p<0.0001 | -0.07 (-0.09, -0.04), p<0.0001 |
| $mBRS_{PPS3}$ | 0.25 (0.23, 0.28), p<0.0001 | - |

***Table 7. Multivariable Associations between High Metabolic Risk (HMR; n = 1450) or Type 2 Diabetes Mellitus (T2D; n = 311) and Neural Baroreflex Sensitivity ($nBRS_{PPS3}$) or Mechanical Baroreflex Sensitivity ($mBRS_{PPS3}$) as Compared with Normal Glucose Metabolism (NGM; n = 5857) after the Exclusion of Patients Treated by Insulin (n = 8) and after Adjustment for Confounding and Mediating Factors.***

*Data are unstandardized regression coefficients and 95% confidence intervals. The continuous variables were included in the models in standardized forms using z-scores. [*]Confounding factors: age, sex, body mass index, smoking, alcohol consumption, physical activity score. [†]Mediating factors: mean blood pressure, statin use, estimated glomerular filtration rate, and additionally, $mBRS_{PPS3}$ in the case of $nBRS_{PPS3}$, heart rate in the case of $mBRS_{PPS3}$. ref, reference group.*

| | **$nBRS_{PPS3}$** | **$mBRS_{PPS3}$** |
|---|---|---|
| *Adjusted for confounding factors[*] and mediating factors[†]* | | |
| NGM | ref | ref |
| HMR | -0.07 (-0.12, -0.01 ), p=0.029 | 0.04 (-0.01, 0.10), p=0.117 |
| T2D | -0.18 (-0.28, -0.06), p=0.003 | 0.08 (-0.02, 0.19), p=0.123 |

***Table 8. Distribution of Antihypertensive Drug Classes in Subjects with Normal Glucose Metabolism (NGM), Subjects with High Metabolic Risk (HMR) and Subjects with Type 2 Diabetes Mellitus (T2D).***

*RAS, renin-angiotensin system.*

| | **NGM** | **HMR** | **T2D** |
|---|---|---|---|
| Beta blocking agents, n (%) | 179 (3.1) | 104 (7.2) | 37 (11.6) |
| Calcium channel blockers, n (%) | 122 (2.1) | 69 (4.8) | 25 (7.8) |
| Agents acting on the RAS, n (%) | 403 (6.9) | 210 (14.5) | 92 (28.8) |
| Diuretics, n (%) | 64 (1.1) | 27 (1.9) | 12 (3.8) |
| Other antihypertensive agents, n (%) | 17 (0.3) | 4 (0.3) | 3 (0.9) |

***Table 9. Multivariable Associations between High Metabolic Risk (HMR; n = 1450) or Type 2 Diabetes Mellitus (T2D; n = 319) and Neural Baroreflex Sensitivity ($nBRS_{PPS3}$) or Mechanical Baroreflex Sensitivity ($mBRS_{PPS3}$) as Compared with Normal Glucose Metabolism (NGM; n = 5857) after Adjustment for Confounding Factors, Mediating Factors and Antihypertensive Treatment (yes/no).***

*Data are unstandardized regression coefficients and 95% confidence intervals. The continuous variables were included in the models in standardized forms using z-scores. Confounding factors: age, sex, body mass index, smoking, alcohol consumption and physical activity score. Mediating factors: mean blood pressure, statin use, estimated glomerular filtration rate (eGFR), and additionally, $mBRS_{PPS3}$ in the case of $nBRS_{PPS3}$, heart rate in the case of $mBRS_{PPS3}$. ref, reference group.*

| | **$nBRS_{PPS3}$** | **$mBRS_{PPS3}$** |
|---|---|---|
| NGM | ref | ref |
| HMR | -0.06 (-0.12, 0.00), p=0.049 | 0.04 (-0.02, 0.09), p=0.17 |
| T2D | -0.15 (-0.27, -0.04), p=0.008 | 0.06 (-0.04, 0.17), p=0.25 |
| Age | -0.16 (-0.18, -0.13), p<0.0001 | 0.21 (0.18, 0.23), p<0.0001 |
| Sex | 0.09 (0.04, 0.13), p=0.0006 | -0.06 (-0.10, -0.01), p=0.011 |
| Body mass index | -0.07 (-0.09, -0.04), p<0.0001 | 0.10 (0.08, 0.12), p<0.0001 |
| Smoking | -0.11 (-0.17, -0.05), p=0.0007 | 0.00 (-0.06, 0.06), p=0.92 |
| Alcohol consumption | 0.05 (-0.02, 0.12), p=0.16 | -0.08 (-0.14, -0.01), p=0.016 |
| Physical activity score | 0.03 (0.01, 0.05), p=0.020 | 0.00 (-0.03, 0.02), p=0.76 |
| Mean blood pressure | -0.14 (-0.16, -0.11), p<0.0001 | 0.30 (0.28, 0.32), p<0.0001 |
| Heart rate | - | 0.12 (0.09, 0.14), p<0.0001 |
| Statin use | -0.02 (-0.10, 0.05), p=0.58 | -0.05 (-0.12, 0.03), p=0.21 |
| eGFR | -0.05 (-0.07, -0.03), p<0.0001 | -0.06 (-0.08, -0.04), p<0.0001 |
| $mBRS_{PPS3}$ | 0.25 (0.23, 0.28), p<0.0001 | - |
| Antihypertensive treatment | -0.11 (-0.18, -0.05), p=0.0005 | 0.09 (0.04, 0.15), p=0.002 |

***Table 10. Multivariable Associations between High Metabolic Risk (HMR; n = 1450) or Type 2 Diabetes Mellitus (T2D; n = 319) and Neural Baroreflex Sensitivity ($nBRS_{PPS3}$) or Mechanical Baroreflex Sensitivity ($mBRS_{PPS3}$) as Compared with Normal Glucose Metabolism (NGM; n = 5857) after Adjustment for Confounding Factors, Mediating Factors and Antihypertensive Drug Classes.***

*Data are unstandardized regression coefficients and 95% confidence intervals. The continuous variables were included in the models in standardized forms using z-scores. Confounding factors: age, sex, body mass index, smoking, alcohol consumption and physical activity score. Mediating factors: mean blood pressure, statin use, estimated glomerular filtration rate (eGFR), and additionally, $mBRS_{PPS3}$ in the case of $nBRS_{PPS3}$, heart rate in the case of $mBRS_{PPS3}$. ref, reference group; RAS, renin-angiotensin system.*

| | **$nBRS_{PPS3}$** | **$mBRS_{PPS3}$** |
|---|---|---|
| NGM | ref | ref |
| HMR | -0.06 (-0.12, 0.00), p=0.0497 | 0.04 (-0.02, 0.09), p=0.17 |
| T2D | -0.15 (-0.27, -0.04), p=0.007 | 0.07 (-0.04, 0.17), p=0.21 |
| Age | -0.16 (-0.18, -0.13), p<0.0001 | 0.21 (0.19, 0.23), p<0.0001 |
| Sex | 0.09 (0.04, 0.14), p=0.0004 | -0.06 (-0.10, -0.02), p=0.009 |
| Body mass index | -0.07 (-0.09, -0.04), p<0.0001 | 0.10 (0.08, 0.13), p<0.0001 |
| Smoking | -0.11 (-0.17, -0.05), p=0.0008 | 0.00 (-0.06, 0.05), p=0.91 |
| Alcohol consumption | 0.05 (-0.02, 0.12), p=0.16 | -0.08 (-0.14, -0.01), p=0.016 |
| Physical activity score | 0.03 (0.01, 0.05), p=0.019 | 0.00 (-0.03, 0.02), p=0.76 |
| Mean blood pressure | -0.14 (-0.16, -0.11), p<0.0001 | 0.30 (0.28, 0.32), p<0.0001 |
| Heart rate | - | 0.12 (0.10, 0.14), p<0.0001 |
| Statin use | -0.02 (-0.10, 0.06), p=0.58 | -0.04 (-0.11, 0.03), p=0.26 |
| eGFR | -0.05 (-0.07, -0.03), p<0.0001 | -0.06 (-0.09, -0.04), p<0.0001 |
| $mBRS_{PPS3}$ | 0.25 (0.23, 0.28), p<0.0001 | - |
| Beta blocking agents | -0.08 (-0.19, 0.03), p=0.14 | 0.09 (-0.02, 0.19), p=0.095 |
| Calcium channel blockers | -0.01 (-0.15, 0.12), p=0.86 | 0.03 (-0.09, 0.15), p=0.63 |
| Agents acting on the RAS | -0.10 (-0.18, -0.02), p=0.011 | 0.04 (-0.03, 0.12), p=0.24 |
| Diuretics and other antihypertensive agents | -0.08 (-0.26, 0.09), p=0.35 | 0.02 (-0.15, 0.18), p=0.86 |

***Table 11. Multivariable Associations between High Metabolic Risk (HMR; n = 1450) or Type 2 Diabetes Mellitus (T2D; n = 319) and Carotid Elastic Parameters Other than the Main Mechanical Baroreflex Sensitivity Parameter ($mBRS_{PPS3}$) as Compared with Normal Glucose Metabolism (NGM; n = 5857) after Adjustment for Confounding and Mediating Factors.***

*Data are unstandardized regression coefficients and 95% confidence intervals. The continuous variables were included in the models in standardized forms using z-scores. Confounding factors: age, sex, body mass index, smoking, alcohol consumption and physical activity score. Mediating factors: mean blood pressure, heart rate, statin use and estimated glomerular filtration rate (eGFR), ref, reference group.*

| | **Young's elastic modulus** | **Distensibility coefficient** | **Compliance coefficient** |
|---|---|---|---|
| NGM | ref | ref | ref |
| HMR | 0.03 (-0.03, 0.08), p=0.37 | -0.05 (-0.10, 0.01), p=0.095 | -0.04 (-0.10, 0.02), p= 0.19 |
| T2D | 0.04 (-0.07, 0.15), p=0.50 | -0.10 (-0.20, 0.01), p=0.074 | -0.07 (-0.18, 0.04), p= 0.23 |
| Age | 0.12 (0.09, 0.14), p<0.0001 | -0.20 (-0.22, -0.17), p<0.0001 | -0.10 (-0.12, -0.07), p<0.0001 |
| Sex | 0.08 (0.03, 0.13), p=0.001 | 0.03 (-0.02, 0.07), p=0.25 | 0.44 (0.39, 0.48), p<0.0001 |
| Body mass index | 0.07 (0.05, 0.09), p<0.0001 | -0.11 (-0.13, -0.09), p<0.0001 | -0.02, (-0.04, 0.00), p=0.099 |
| Smoking | -0.01 (-0.07, 0.05), p=0.71 | 0.04 (-0.02, 0.10), p=0.20 | 0.07 (0.01, 0.13), p=0.032 |
| Alcohol consumption | -0.06 (-0.13, -0.00), p=0.052 | 0.06 (-0.01, 0.12), p=0.069 | 0.03 (-0.04, 0.09), p=0.40 |
| Physical activity score | -0.00 (-0.03, 0.02), p=0.75 | 0.00 (-0.02, 0.03), p=0.72 | 0.01 (-0.02, 0.03), p=0.70 |
| Mean blood pressure | 0.28 (0.26, 0.30), p<0.0001 | -0.29 (-0.31, -0.26), p<0.0001 | -0.19 (-0.21, -0.17), p<0.0001 |
| Heart rate | 0.11 (0.09, 0.13), p<0.0001 | -0.12 (-0.14, -0.10), p<0.0001 | -0.10 (-0.12, -0.08), p<0.0001 |
| Statin use | -0.02 (-0.10, 0.05), p=0.51 | 0.01 (-0.06, 0.08), p=0.84 | -0.03 (-0.11, 0.04), p=0.36 |
| eGFR | -0.05 (-0.07, -0.02), p=0.0001 | 0.06 (0.04, 0.08), p<0.0001 | 0.08 (0.06, 0.10), p<0.0001 |

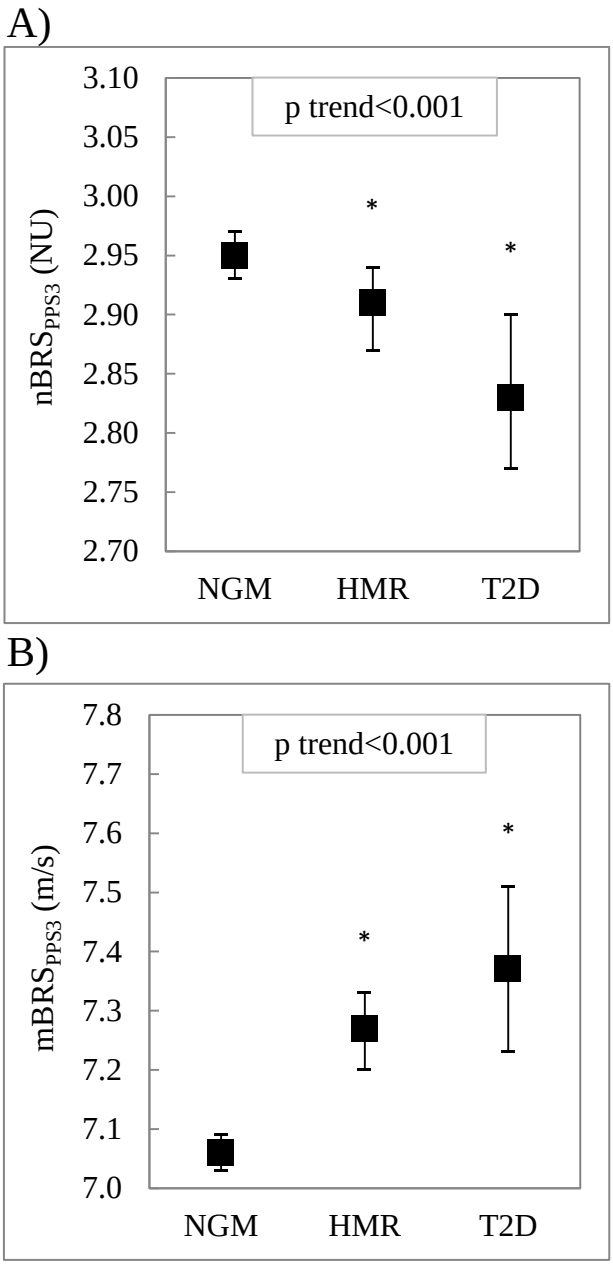


***Figure 10. Distribution of neural baroreflex sensitivity (nBRS$_{PPS3}$; A) and mechanical baroreflex sensitivity (mBRS$_{PPS3}$; B) in subjects with normal glucose metabolism (NGM), subjects with high metabolic risk (HMR) and patients with type 2 diabetes mellitus (T2D).***

*Mean values and 95% confidence intervals are adjusted for age, sex and mean blood pressure. *indicates statistically significant difference compared with subjects with NGM.*

### 4.2. End-Stage Liver Disease Study

Table 12 shows the basic characteristics of our subject groups. There was no difference in sex distribution, age, BMI and smoking habits between the groups. The patients had lower brachial systolic and diastolic BP compared with controls and no difference was observed in brachial pulse pressure. The difference was at the limit of significance in HR between the two groups.

***Table 12. Participant Characteristics.***

*Data are mean±SD. Independent samples t-test unless otherwise stated. *p value of chi-square test. ESLD, end-stage liver disease; BP, blood pressure; MELD score, Model for End-Stage Liver Disease score.*

| | **ESLD (n = 24)** | **Controls (n = 23)** | ***p* value** |
|---|---|---|---|
| Male, n (%) | 13 (54.2) | 12 (52.2) | 0.891* |
| Age (years) | 55±7 | 55±7 | 0.987 |
| Body mass index (kg/m$^2$) | 27±4 | 27±3 | 0.968 |
| Smoking n (%) | 7 (29) | 8 (35) | 0.680* |
| Brachial systolic BP (mmHg) | 118±10 | 126±9 | <0.01 |
| Brachial diastolic BP (mmHg) | 67±9 | 75±6 | <0.01 |
| Brachial pulse pressure (mmHg) | 51±5 | 51±8 | 0.675 |
| Heart rate (bpm) | 65±7 | 61±7 | 0.050 |
| MELD score | 14±3 | - | - |

17 patients were treated by non-selective betablockers (propranolol), 22 patients received diuretics (spironolactone, amiloride, hydrochlorothiazide, furosemide, ethacrynic acid), 1 patients was taking angiotensin-converting enzyme inhibitor (trandolapril), 1 patient received angiotensin II receptor antagonist (losartan), 9 patients were taking non-absorbable disaccharides (lactulose), 6 patients were treated by antibiotics (rifaximin), 3 patients received antiviral therapy (lamivudine), 2 patients used anticonvulsants (gabapentin, pregabalin), 4 patients were taking ursodeoxycholic acid and 12 patients used proton pump inhibitor (pantoprazole, rabeprazole).

Carotid artery pressures and morphological variables are presented in Table 13. Similarly to brachial blood pressures, carotid artery systolic and diastolic BP were found to be lower in patients compared with controls and there was no difference in carotid pulse pressure between the groups. No differences were found in carotid artery $D_{ed}$, $\Delta D$ and IMT between the groups.

***Table 13. Carotid Artery Blood Pressures and Morphological Data in Patients with End-Stage Liver Disease (ESLD) Compared with the Control Group.***

*Data are mean±SD. Independent samples t-test. BP, blood pressure.*

| | **ESLD** | **Controls** | ***p* value** |
|---|---|---|---|
| Carotid systolic BP (mmHg) | 107±9 | 115±11 | <0.05 |
| Carotid diastolic BP (mmHg) | 67±9 | 75±6 | <0.01 |
| Carotid pulse pressure (mmHg) | 40±5 | 40±8 | 0.902 |
| External end-diastolic diameter (mm) | 6.87±0.62 | 6.81±0.72 | 0.734 |
| Pulsatile distension (mm) | 0.33±0.13 | 0.36±0.11 | 0.373 |
| Intima-media thickness (mm) | 0.58±0.09 | 0.60±0.10 | 0.546 |

$BRS_{ESLD}$ and its components are shown in Table 14. $BRS_{ESLD}$ was lower in patients compared with controls. While $mBRS_{ESLD}$ did not show significant difference between the groups, $nBRS_{ESLD}$ was lower in the patient group.

***Table 14. Integrated Baroreflex Sensitivity ($BRS_{ESLD}$) and its Mechanical and Neural Component ($mBRS_{ESLD}$ and $nBRS_{ESLD}$, Respectively) in Patients with End-Stage Liver Disease (ESLD) and Control Individuals.***

*Data are mean±SD or median (interquartile range). Independent samples t-test.*

| | **ESLD** | **Controls** | ***p* value** |
|---|---|---|---|
| $BRS_{ESLD}$ (ms/mmHg) | 7.00 (5.80, 9.25) | 11.1 (8.50, 14.80) | <0.01 |
| $mBRS_{ESLD}$ ($10^{-3}$/mmHg) | 2.34 (1.81, 2.68) | 2.61 (1.97, 3.84) | 0.215 |
| $nBRS_{ESLD}$ (ms/$10^{-3}$) | 3.54±1.20 | 4.48±1.43 | <0.05 |

Carotid elastic parameters calculated similarly to the PPS3 project are presented in Table 15. There was no difference in CC, DC, PWVc and Young's elastic modulus between patients with ESLD and the control group.

***Table 15. Carotid Elastic Parameters Calculated According to the Guideline Used in the Paris Prospective Study III in Patients with End-Stage Liver Disease (ESLD) and Control Individuals.***

*Data are mean±SD or median (interquartile range). Independent samples t-test.*

| | **ESLD** | **Controls** | ***p* value** |
|---|---|---|---|
| Compliance coefficient ($m^2$ $kPa^{-1}$ $10^{-6}$) | 0.53 (0.40, 0.70) | 0.58 (0.46, 0.86) | 0.367 |
| Distensibility coefficient ($10^{-3}$/kPa) | 21.8 (16.0, 23.7) | 24.9 (18.1, 32.5) | 0.250 |
| Carotid pulse wave velocity (m/s) | 7.0±1.4 | 6.5±1.2 | 0.232 |
| Young's elastic modulis (kPa) | 455 (334, 649) | 417 (247, 577) | 0.199 |

## 5. Discussion

### 5.1. Paris Prospective Study III

Within the confines of this large population study, we have shown that $nBRS_{PPS3}$ is significantly and gradually decreased in subjects with HMR and in patients with T2D compared with subjects with NGM independently from confounding and mediating factors as shown in Table 5. We also found impaired $mBRS_{PPS3}$ in the T2D group which was explained by mediating factors like increased BP, increased HR and eGFR. Furthermore, the subgroup analysis reported in Table 6 revealed that deteriorated $nBRS_{PPS3}$ in the HMR group was mainly explained by the presence of MetS rather than IFG per se and independently altered $mBRS_{PPS3}$ was observed in the HMR group only in the subjects with both MetS and IFG.

Alteration in the neural component of BRS was not frequently examined in diabetic patients. Ruiz et al focused on neuropathy measured at the periphery and carotid distensibility in relation to integrated BRS in patients with T2D. They found that neuropathy was a more important determinant of integrated BRS than the elasticity of the carotid artery (21). However, we have shown impaired neural signal processing in T2D patients in a much larger sample size (at a population level) and we used a method that was specifically developed for the measurement of the neural component of BRS. Based on the basic characteristics of our participants we can conclude that the majority of our diabetic patients had a milder presentation of the disease and we could see that deteriorated neural BRS can be observed in these earlier stages of disease progression. Lipponen et al used methods similar to ours and showed impaired neural BRS in patients with type 1 diabetes mellitus in a small study (54). Since the effect of enhanced glucose control in neuropathy management is modest in T2D (55-57) compared with the substantial effect in type 1 diabetes mellitus (58), our results emphasize the importance of treatment development that is based on pathogenic concepts and underline the significance of lifestyle-modification. Regular physical exercise should be an important pillar in the lifestyle-modification process because beside the other positive effects it ameliorates integrated BRS in T2D patients (59) and according to the results of Deley et al it improves neural BRS even at advanced ages (60). In line with these findings, we have also seen the positive association between the physical activity score and

$nBRS_{PPS3}$. Similarly to earlier observations, we have also shown the negative association between smoking and baroreflex function (61) and a similar relationship for increased BP (62). Consequently, a multifactorial approach should be used to counteract diabetes-associated neural damage. The Steno-2 study showed that intensive multifactorial therapeutic strategy with strict treatment goals in reference to control of weight, BP and glucose level, cessation of smoking, encouragement for performing more physical activity and other interventions profoundly decreased the progression of autonomic neuropathy in T2D patients (63) and the positive effect of this 7.8-year-long intensified treatment on neural functions was still detectable after 21.2 years (64). In line with these findings, Gibbons et al showed the lack of cardiovascular autonomic neuropathy progression within a 3-year-long period in T2D patients with well-controlled risk factors and diabetes (65). Furthermore, the compliance of the diabetic patients could be improved by the early detection of neural damage using the Kornet method (66). According to a pilot study reported by Ptaszynski et al, it was possible to distinguish post-myocardial infarction patients at high and low risk for arrhythmias based on neural BRS data more precisely than with conventional BRS measures (67). The 20-year-long follow-up period of the PPS3 could reveal further information about the role of nBRS measurements in risk stratification in T2D patients.

The number of studies focusing on neural BRS in prediabetic states is limited. Zanoli et al showed decreased neural BRS in patients with MetS in a substudy of the PPS3 (n = 2835) (14). In this present study, decreased $nBRS_{PPS3}$ was observed in patients with HMR (i.e. with IFG and/or MetS) compared with the subjects with NGM after adjustment for confounding and mediating factors. In accordance with the work of Zanoli et al, our subgroup analysis showed that decreased $nBRS_{PPS3}$ in the HMR group was mainly mediated by the accumulation of metabolic disturbances that define MetS and less by IFG per se. Besides, we showed the lack of independent association between IFG and baroreflex impairment similarly to the findings of Wu et al (27).

Previous results about the relationship between carotid elastic properties representing the mechanical component of BRS and T2D are controversial. On the one hand, the Hoorn study and the Maastricht study found independent relationship between carotid

stiffening and T2D (24, 26). In the Hoorn study 55.7%, in the Maastricht study 29% of the T2D patients had earlier documented cardiovascular problems that could show an advanced stage of diabetes with fully developed carotid macroangiopathy. On the other hand, the Asklepios study did not show significant difference in carotid pulse wave velocity – equivalent with our $mBRS_{PPS3}$ parameter – between T2D patients and controls (25). The participants were middle-aged and without a history of cardiovascular disease in the Asklepios study. One possible explanation for our results showing no independent association between $mBRS_{PPS3}$ and T2D that the majority of our diabetic patients were in the early stage of the disease. There were only 8 patients treated by insulin in our population. Additionally, similarly to the Asklepios study, we excluded patients with previous cardiovascular diseases. Another possible explanation that due to the voluntary participation in the PPS3, the most health-oriented people underwent our measurements. In accordance with our assumptions, 4.3% of the PPS3 volunteers had T2D which is much lower than the age-specific prevalence of T2D in France (68-70). Essentially, early stage and good clinical control could be the main factors behind the results that carotid stiffening is not an intrinsic feature in our T2D group; it is mediated by factors like increased BP, increased HR and eGFR. These factors should be in the focus of treatment development. Decreased $nBRS_{PPS3}$ could explain higher HR in our diabetic group. The stiffening action of elevated HR is not well understood (71), however, therapeutic improvement of neural functions could result in ameliorated mechanical BRS through the shift of the sympatho-vagal balance toward vagal activity and the consequential lowering of baseline HR.

Previous studies focusing on carotid elasticity in prediabetic states also showed contradictory results (14, 24, 25, 28, 30, 31, 72-75). Since several diagnostic criteria systems were used in these studies, clear conclusions cannot be drawn about carotid stiffness in patients with HMR. According to our results, decreased carotid elasticity is already present before the fully developed T2D and that is likely due to mediating factors. In line with the results of the Rotterdam study, our subgroup analysis revealed that there was no independent association between carotid stiffening and IFG in subjects younger than 75 years (28). However, subjects with both Mets and IFG had significantly higher $mBRS_{PPS3}$ compared with subjects with NGM. This finding is in

line with the results of Guize et al and the findings of the MARE consortium. Guize et al showed that those three-component combinations of MetS components that include elevated glucose level were associated with higher risk of all-cause mortality in the majority of the cases (76). The goal of the MARE consortium was the identification of MetS component clusters that were significant determinants of extremely stiff arteries (carotid-femoral pulse wave velocity is above $95^{th}$ % of the population). The majority of these clusters included the component of elevated glucose level (77).

We could not observe significant influence of statin therapy or treatment with different classes of antihypertensive medications on our main results. Besides, we did not adjust our analysis for antidiabetic treatment to prevent substantial overfitting.

Traditionally, peripheral BP is measured during the determination of BRS. However, BP measured at the periphery not necessarily represents the pressure at the level of the baroreceptors due to the phenomenon of systolic BP amplification (78). Therefore, we measured local carotid BP, $D_{ed}$, $\Delta D$ and IMT to calculate $mBRS_{PPS3}$. Besides, examination of the spectral relationship between carotid DR and RRI signals provided information about the function of the purely neural parts of the baroreflex loop. By local carotid measurements, we eliminated the possible influence of wave propagation and wave reflection and could get reliable information about the components of BRS. However, it was a great technical challenge to obtain steady, high quality distension data for the determination of $nBRS_{PPS3}$. Therefore, a specialized training was provided for the technicians before the study and every single recording underwent quality control (42).

The presented study has limitations. We would not have been able to recruit a representative sample using the Oxford method with drug injection, therefore we used non-invasive methods. The diagnosis of T2D was only based on a fasting blood glucose level; oral glucose tolerance test and Hemoglobin $A_{1c}$ level measurement were not performed. We could not distinguish between type 1 and type 2 diabetes mellitus. Since the exclusion of patients treated with insulin (suspected to have type 1 diabetes mellitus; n = 8) did not change our main results, this should not represent a significant confound

in this study. The conclusions regarding causality are limited due to the cross-sectional nature of our study. Results about the association between IFG, MetS and the components of BRS are based on subgroup analysis and thus, they should be interpreted cautiously. Finally, our study population was predominantly white.

### 5.2 End-Stage Liver Disease Study

In line with earlier findings, we showed decreased integrated BRS in patients with ESLD (35, 36, 38, 79). Additionally, we examined the 2 components of integrated BRS. To our knowledge we are the first to show that deteriorated baroreflex function in ESLD is the consequence of impaired neural signal processing as shown in Table 14. Besides, the mechanical transduction of BP changes into baroreceptor vessel wall stretch appeared to be preserved in our patient group.

The decreased neural BRS in patients with ESLD could be explained by different factors. In patients with alcohol-related ESLD, earlier results suggest that the direct toxic effect of alcohol or its metabolites leads to the damage of the neural structures in the cardiovascular autonomic nervous system (80, 81). Chronic cholestatic liver diseases could be accompanied by vitamin E deficiency (82) that could lead to deterioration of neural functions (83). However, since impaired cardiovascular autonomic regulation is independent from aetiology according to earlier results (32) and the degree of deterioration is significantly associated with the severity of hepatic dysfunction (35), a common pathophysiological mechanism could be in the background. In ESLD patients, the hyperdynamic circulation is characterized by portal hypertension, increased cardiac output, increased production of vasodilator substances such as NO and inflammatory cytokins (39). These common features and processes could include the link between ESLD and decreased $nBRS_{ESLD}$, therefore, they should be in the focus of future research. As it was mentioned in the Introduction, increased NO-level could play a role in the attenuation of baroreflex regulation by acting in the NTS.

After the publication of our work, Novo et al reported decreased carotid elasticity in patients with compensated HCV-related cirrhosis (84). Regarding the mechanical BRS,

one possible explanation for the results of Novo et al and our laboratory that the final stage of the liver disease is accompanied by severe haemodynamic disturbances: the increased production of vasodilator substances such as the aforementioned NO (39, 40, 85) could have indirect effect on the baroreceptor vessel walls (86). The fact that the elastic walls of baroreceptor vessels have to work at a lower BP range – due to the massive vasodilation in the systemic circulation – could explain the preserved pulsatile distension in ESLD. However, at a compensated stage, the operating range of BP could be higher and a mild deterioration of carotid elasticity could be observed. Larger sample size and adjustment for mean BP could verify this theory. However, preserved carotid IMT in the patient group suggests no advanced damage of the carotid artery vessel wall in our patient group.

We calculated the carotid elastic parameters according to two different guidelines. These guidelines use the same equations for the calculation of the compliance coefficient, distensibility coefficient and carotid pulse wave velocity, however, Van Bortel et al recommended the usage of carotid external end diastolic diameter ($D_{ed}$) which is the distance between the anterior adventitia-media border and the posterior media-adventitia border (53), while the consensus document written by Laurent et al recommended the calculation using carotid internal end diastolic diameter which represent the diameter of the lumen and calculated as $D_{ed}$-(2×IMT) (48). As expected, our results remained similar with the two types of calculations showing the robustness of our findings.

Overall, our results suggest that the mechanical BRS is comparable with healthy controls and the major cause of impaired baroreflex regulation is the deterioration of the neural component in ESLD.

This study has limitations. We wanted to perform the experiments with a risk as low as possible, therefore, we used non-invasive methods. However, the spontaneous sequence technique was proved to be reliable in the measurement of baroreflex function when the invasive method is not advised (87). Although the measurement of integrated BRS provides information about the function of the whole baroreflex loop and does not

require good cooperation from the subjects, the BP measured at the periphery may not inform us about carotid BP perfectly due to the systolic BP amplification (78). Our sample size was suitable for basic statistical analysis. A longitudinal study with larger sample size could make the examination of complex interactions and causality possible in reference to the relationship between hepatic damage and decreased neural BRS. Finally, since it was a non-invasive study, we could not present data about the biochemical profile of our subjects.

## 6. Conclusions

The PPS3 shows a systematic comparison of neural BRS and mechanical BRS between subjects with NGM, subjects with HMR and patients with T2D at a population level. Our final statements are the following:

- Neural BRS is decreased in T2D patients independently from confounding factors (age, sex, BMI, smoking, alcohol consumption and physical activity score) and mediating factors (mean blood pressure, statin use, eGFR, and additionally, $mBRS_{PPS3}$ in the case of $nBRS_{PPS3}$, HR in the case of $mBRS_{PPS3}$);
- The altered mechanical BRS in T2D is explained by mediating factors (BP, HR and eGFR);
- The state of IFG per se is not independently associated with impaired neural BRS or altered mechanical BRS;
- Subjects with MetS have lower neural BRS independently from confounders and mediators;
- Subjects with MetS with the component of IFG have altered mechanical BRS independently from confounding and mediating factors.

The ESLD Study provides information about the integrated BRS and its components in patients with ESLD in a case-control setting. Our final statements are the following:

- Integrated BRS is decreased in patients with ESLD;
- The mechanical BRS of patients with ESLD is comparable with healthy control subjects;
- The neural BRS is decreased in patients with ESLD.

## 7. Summary

Arterial baroreflex plays a major part in short-term regulation of blood pressure (BP). Baroreflex function is estimated frequently by the measurement of baroreflex sensitivity (BRS). Integrated BRS shows the RR interval change in ms elicited by a unit change in systolic BP. Integrated BRS has two components: (I) mechanical BRS gives information about the mechanical transduction of change in BP into baroreceptor vessel wall stretch and dependent on the elastic behaviour of the carotid sinus and aortic arch; (II) neural BRS represents the sensitivity of the neural structures of the reflex loop. Since depressed baroreflex function is an independent predictor of major cardiovascular events in patients with type 2 diabetes mellitus (T2D), and severely damaged baroreflex regulation is associated with higher mortality in patients with advanced liver disease, deeper understanding of the mechanisms causing damage in baroreflex function in these pathological conditions is of crucial clinical interest. Therefore, we aimed to determine whether the alteration of mechanical BRS and/or neural BRS is responsible for the damaged baroreflex regulation in T2D and end-stage liver disease (ESLD). Within the confines of the Paris Prospective Study III, we determined mechanical and neural BRS at a population level using carotid echotracking in 5857 subjects with normal glucose metabolism, 1450 subjects with high metabolic risk (HMR; i.e. with impaired fasting glucose [IFG] and/or metabolic syndrome [MetS]) and 319 patients with T2D. In the ESLD Study, we measured integrated BRS, mechanical and neural BRS by simultaneous recording of BP and ECG and using carotid echotracking and tonometry in 24 patients with ESLD and 23 healthy controls. T2D and HMR were associated with decreased neural BRS independently from confounding and mediating factors. Decreased neural BRS in the HMR group was in great part due to the presence of MetS rather than IFG per se. Besides, altered mechanical BRS in T2D patients was explained by increased BP, increased heart rate and estimated glomerular filtration rate. Furthermore, independent alteration in mechanical BRS was observed only in those HMR subjects who had both MetS and IFG. The ESLD Study showed decreased integrated BRS in patients with ESLD in line with earlier findings. As novel results, we showed decreased neural BRS and preserved mechanical BRS in our patient group.

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## 9. Bibliography of the Candidate's Publications

### 9.1. Publications Related to the Thesis

Cseh D*, Climie RE*, Offredo L, Guibout C, Thomas F, Zanoli L, Danchin N, Sharman JE, Laurent S, Jouven X, Boutouyrie P, Empana JP. (2020) Type 2 Diabetes Mellitus Is Independently Associated With Decreased Neural Baroreflex Sensitivity: The Paris Prospective Study III. Arterioscler Thromb Vasc Biol, 40: 1420-1428.
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Sárközi A, Cseh D, Gerlei Z, Kollai M. (2018) Reduced neural baroreflex sensitivity is related to enhanced endothelial function in patients with end-stage liver disease. Scan J Gastroenterol, 53: 193-199.

### 9.2. Other Publications

Kardos D, Simon M, Vácz G, Hinsenkamp A, Holczer T, Cseh D, Sárközi A, Szenthe K, Bánáti F, Szathmary S, Nehrer S, Kuten O, Masteling M, Lacza Z, Hornyák I. (2019) The composition of hyperacute serum and platelet-rich plasma is markedly different despite the similar production method. Int J Mol Sci, 20: 721.

Simon M, Major B, Vácz G, Kuten O, Hornyák I, Hinsenkamp A, Kardos D, Bagó M, Cseh D, Sárközi A, Horváthy D, Nehrer S, Lacza Z. (2018) The effects of hyperacute serum on the elements of the human subchondral bone marrow niche. Stem Cells Int, 2018: 4854619.

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