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Hemodynamic and autonomic effects of low-dose glyceryl trinitrate used to test endothelium-independent vasodilation of the brachial artery

Lorenzo Ghiadoni[a], Martina Francesconi[a,b,*], Stefano Taddei[a], Rosa Maria Bruno[a]

[a] Department of Clinical and Experimental Medicine, University of Pisa, Italy
[b] Institute of Clinical Physiology, CNR, Pisa, Italy

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ABSTRACT

Smooth muscle function is explored by sublingual glyceryl trinitrate (GTN) administration to compare with endothelium-dependent vasodilation of the brachial artery by flow-mediated-dilation (FMD). This study compared the hemodynamic and autonomic effects of the two most often used GTN dosages.

In 80 essential hypertensive patients (HT) and 60 normotensive subjects (NT), FMD of the brachial artery and endothelium-independent response to sublingual GTN (25 μg and 400 μg) were evaluated by high-resolution ultrasound and automated image analysis. In 10 HT, muscle sympathetic nerve activity (MSNA) was also assessed by microneurography.

HT showed significantly ($p < .01$) lower FMD (5.5 ± 3.3%) compared to NT (6.9 ± 2.2%). The response to GTN 25 μg tended to be lower (HT:7.2 ± 3.3%; NT:7.9 ± 2.9%; $p = .06$), whereas response to GTN 400 μg was similar (HT:14.3 ± 4.8%, NT:14.5 ± 4.7%, p = ns). Blood pressure (BP) reduction induced by GTN 400 μg (systolic-BP:-3.2 ± 7.7 mm Hg, diastolic-BP:-4.7 ± 5.0 mm Hg) was greater ($p < .001$) compared to GTN 25 μg (systolic-BP:-0.7 ± 5.8 mm Hg, diastolic-BP:-0.7 ± 4.4 mm Hg). Changes in heart rate were also greater (+5.6 ± 6.4 bpm versus −0.2 ± 5.4 bpm, $p < .001$). This behaviour was similar in either NT or HT. MSNA was significantly increased by GTN 400 μg (31 ± 7bursts/min to 41 ± 6bursts/min, $p < .001$) but not by 25 μg (33 ± 9bursts/min to 37 ± 11bursts/min, $p = .19$).

In conclusion, the administration of low-dose GTN allows exploring endothelium-independent vasodilation in FMD protocols, inducing only modest hemodynamic and sympathetic responses.

1. Introduction

Endothelial dysfunction is the first step in the development of atherosclerosis, which precedes structural vascular alterations. Endothelial function is assessed non-invasively by the flow-mediated dilation (FMD) technique, which is the increase in brachial artery diameter following a post-ischemic increase in blood flow [1,2]. This response has been demonstrated to be largely dependent on local nitric oxide (NO) release [3,4]. However, from the pathophysiological point of view, the degree of FMD might be influenced not only by the shear stimulus and NO availability in the endothelial cells, but also by the functionality of vascular smooth muscle (VSM) cells, representing the target cells for the paracrine action of NO [5]. Since VSM cell dysfunction, of any cause, may reduce their capacity to respond to endogenous as well as exogenous NO, any FMD protocol should include the use of exogenous, in order to quantify the extent of any coexistent endothelium-independent dysfunction [2]. However, few studies have directly assessed so far the issue of standardization of endothelium-independent vasodilation tests, which are necessary to establish the clinical significance of VSM function in humans.

Sublingual glyceryl trinitrite (GTN) 400 μg, which is the lowest marketed dose and the first to be used for this purpose, is still widely administered in clinical studies [6]. Lower dosages (25 μg) were then introduced [7], since they induce a smaller dilatation, more similar to FMD in healthy controls, and less side effects, including systemic hemodynamic changes [8,9]. However, no studies are available so far directly comparing the two most commonly used dosages on hemodynamic and autonomic effects, in health and disease.

Therefore, the aim of this study is to evaluate the effect of 25 and 400 μg GTN dose on blood pressure, heart rate and muscle sympathetic nervous system activity, as well as brachial artery diameter, in healthy individuals and patients with essential hypertension. Furthermore, the relationship between endothelium-dependent and –independent vasodilation will be also explored.

* Corresponding author at: Department of Clinical and Experimental Medicine, University of Pisa, Via Roma, 67, 56100 Pisa, Italy.
E-mail address: martina.francesconi@med.unipi.it (M. Francesconi).

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2. Materials and methods

The study population included 80 essential hypertensive patients (HT) and 60 normotensive individuals (NT). Individuals with history of cardiovascular events, current smoking and ethanol abuse, diabetes, impaired renal function (eGFR < 60 ml/min), severe hypercholesterolemia (LDL cholesterol > 190 mg/dl) were excluded from the study.

Normotensive controls were recruited among university employees. Inclusion criteria were: age > 18 years, written informed consent; blood pressure (BP) values below 140/90 mm Hg on several occasions. Exclusion criteria were: diseases, medications or supplements known to impact vascular function according to clinical judgment.

Essential hypertensive patients, defined as hypertensive according to ESC/ESH guidelines [10], were recruited from consecutive outpatients with a recent (< 6 months) history of hypertension. They were never treated or had received discontinuous antihypertensive treatment, with at least one month of wash-out. Secondary forms of hypertension were excluded by routine screening.

The protocol was approved by the Ethical Committee of the University of Pisa and all patients gave written consent to the study.

2.1. Experimental design

This study adopted a randomized, crossover design. The experimental session took place in the morning after an overnight fasting, as described elsewhere [11]. Medical history, body weight and height, as well as blood samples were collected. Lipid profile, plasma glucose and serum creatinine were determined according to standard laboratory procedures. Vascular function tests were performed in a quiet air-conditioned room (22–24 °C). Brachial BP was measured three times at 2-min intervals by automatic oscillometric device (OMRON-705IT), with the patients resting in a supine position for at least 10 min by a trained operator. Then FMD was performed. Then patients were randomized to receive sequentially, with 1-h time span between, sublingual 25 or 400 μg GTN dosages. A subgroup of HT underwent an additional experimental session in a different day, in which muscle sympathetic nerve activity (MSNA) was also assessed by microneurography under the same protocol.

2.1.1. Endothelium-dependent and independent vasodilation in the brachial artery

Brachial artery flow meditated dilation (FMD) was performed using high-resolution ultrasound with a 10 MHz linear array transducer (MyLab25, ESAOTE, Florence, Italy), following recent guidelines as previously described [12]. FMD and the response to GTN were calculated as the maximal percentage increase in diameter. Endothelium-independent dilation was obtained by sublingual administration of 25 and 400 μg GTN.

Analysis of changes in brachial artery diameter was performed using a real-time computerized edge detection system, which is independent of investigator bias [13,14] by a single investigator (L.G.) blinded to patient's allocation to treatment. Diameter was assessed for 1-min baseline, until after 3 min after cuff deflation, and until 5 min after GTN administration. Hyperemic stimulus was quantified by baseline and hyperemic shear rate (SR = 8 x mean flow velocity / brachial artery diameter).

2.1.2. Muscle sympathetic nerve activity

Multiunit recording of efferent postganglionic muscle sympathetic nerve activity (MSNA) of the peroneal nerve was obtained using microneurography. Briefly, a tungsten microelectrode with an uninsulated 1–5-μm-diameter tip (Medical Instruments, University of Iowa) was transcutaneously inserted in the peroneal nerve just posterior to the fibular head, as previously described [15]. A reference electrode was inserted subcutaneously 1 to 3 cm from the recording site. The signal was integrated with a 0.1-s time constant, amplified with a gain of 50,000–80,000, band-pass filtered (700–2000 Hz), and acquired at 1000 Hz through a digital acquisition system (ACQ-16; Gould Electronics). MSNA was identified according to previously outlined criteria [15,16]. Obtained neurograms were recorded together with BP and heart rate by means of dedicated computer software (Ponemah; LDS). Recordings were considered acceptable if the signal-to-noise ratio exceeded the value of 3. MSNA responses were measured at rest and during the administration of the two GTN doses. Data were quantified as bursts/min (burst frequency) and bursts/100 heart beats (burst incidence). MSNA was analysed by visual inspection by a single investigator (R.M.B.) blinded to the patient's allocation to treatment.

2.2. Statistical analysis

All statistical analyses were conducted using NCSS 8 software (NCSS; Kaysville, Utah; USA). Descriptive statistics are presented as means and standard deviation (SD). The differences in vascular parameters between hypertensive and normotensive individuals were analysed using Wilcoxon test; ANCOVA was also performed on selected variables in order to adjust for age. Hemodynamic changes between baseline and after GTN administration were analysed using Wilcoxon test. Univariate and multivariate linear regression analysis was performed to explore correlation between endothelium-independent and -dependent vasodilation. The change in outcome parameters of the microneurographic measurements in hypertensive individuals were analysed using repeated measures ANOVA with treatment as between-subject variable and time as within-subject variable. All differences were considered statistically significant at $p < .05$.

3. Results

Clinical characteristics of the study population were reported in

Table 1
Clinical characteristics of the normotensive subjects and essential hypertensive patients (BP: blood pressure; HDL: high density lipoprotein; LDL: low density lipoprotein).

	Normotensive subjects (n = 60)	Essential hypertensive patients (n = 80)	p value
Male sex (n, %)	28 (46.7)	45 (55.6%)	0.30
Age (years)	44.8 ± 11.6	51.2 ± 10.4	0.001
Systolic BP (mm Hg)	121.9 ± 10.2	151.2 ± 16.1	0.001
Diastolic BP (mm Hg)	77.6 ± 6.3	94.0 ± 8.9	0.001
Heart rate (beats/min)	67.9 ± 11.5	70.3 ± 11.6	0.34
Body mass index (g/m^2)	25.2 ± 3.1	26.2 ± 3.4	0.14
Serum creatinine (mg/dl)	0.82 ± 0.18	0.88 ± 0.23	0.12
Plasma glucose (mg/dl)	91.9 ± 9.9	95.9 ± 11.3	0.11
Total cholesterol (mg/dl)	199.5 ± 38.4	220.1 ± 38.3	0.008
HDL cholesterol (mg/dl)	56.3 ± 16.8	56.9 ± 19.7	0.92
LDL cholesterol (mg/dl)	115.6 ± 44.7	139.7 ± 40.8	0.001
Triglycerides (mg/dl)	110.0 ± 69.9	112.4 ± 50.2	0.30

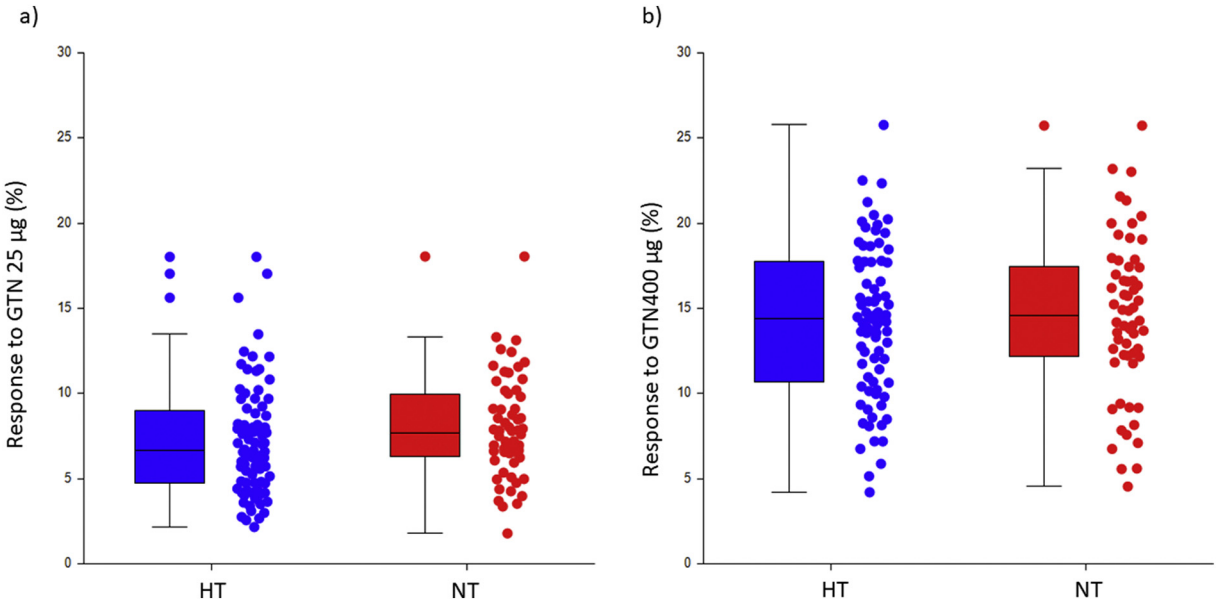

Fig. 1. Dot plots and box plots showing the percentage change in brachial artery diameter to glyceryl trinitrate (GTN) 25 µg (a) and 400 µg (b) in hypertensive (HT, blue) and normotensive (NT, red) individuals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1. Normotensive individuals were younger, with lower BP values and total and LDL cholesterol values.

Baseline brachial artery diameter was not significantly different in hypertensive patients as compared to normotensive controls. FMD was significantly lower reduced in hypertensive patients compared to healthy controls (5.5 ± 3.3% vs 6.9 ± 2.2%, $p < .01$). This difference was attenuated after adjustment for age ($p < .05$). No difference was found in hyperemic SR between normotensive subjects (1213 ± 440 s^{-1}) and essential hypertensive patients (1126 ± 451 s^{-1}; $p = .15$).

Brachial artery % change in response to 25 µg GTN tended to be lower in hypertensive patients ($p = .06$), whereas response to GTN 400 was similar to controls (Fig. 1). The difference in brachial artery dilatation to GTN 25 µg was attenuated after adjustment for age ($p = .16$). Timing of maximal response to GTN was similar in hypertensive and normotensive individuals (GTN 25 µg: 239 ± 53 vs 233 ± 46 s, $p = .73$; GTN 400 µg 230 ± 46 vs 229 ± 42 s, $p = .67$).

In normotensive individuals, FMD was not correlated either to response to GTN 400 ($r = -0.13$, $p = .30$) or to GTN 25 µg. ($r = -0.05$, $p = .68$). In hypertensive patients FMD was significantly correlated to response to GTN 400 µg ($r = 0.35$, $p = .01$) but not to GTN 25 µg ($r = 0.17$, $p = .14$). However, in the multivariate analysis the significance was lost (beta 0.11, 95% C.I.: 0.05–0.27; $p = .18$) after adjustment for baseline diameter, age and sex.

In the overall population, GTN 400 µg induced a significantly greater percent brachial artery dilation than GTN 25 µg (14.39 ± 4.70 vs 7.53 ± 3.16, $p < .0001$). Brachial artery diameter and percent dilation to the GTN doses was not influenced by their sequence of administration 1 h apart (data not shown).

In the overall population, GTN 400 µg induced a significantly greater reduction in systolic and diastolic BP and a greater increase in HR (Table 2). Similar effects were observed in the hypertensive group, whereas reduction in systolic BP was not different between 25 e 400 µg GTN in normotensive individuals. Noteworthy, changes in BP and HR induced by 25 µg GTN were negligible either in normotensive (systolic BP: 119.5 ± 10.7 to 118.7 ± 10.1 mm Hg, $p = .28$; diastolic BP: 77.5 ± 6.9 to 76.3 ± 7.8 mm Hg, $p = .05$; HR: 65.1 ± 9.5 to 65.7 ± 9.5 bpm, $p = .16$) or in hypertensive individuals (systolic BP: 144.8 ± 15.6 to 144.2 ± 15.7 mm Hg, $p = .37$; diastolic BP 91.2 ± 9.6 to 90.8 ± 9.3 mm Hg, $p = .46$; HR 67.6 ± 10.0 to 67.5 ± 10.1, $p = .77$).

Table 2
Absolute changes in hemodynamic variables in normotensive subjects and essential hypertensive patients after glyceril trinitrate (GTN) 25 and 400 µg (BP: blood pressure; HDL: high density lipoprotein; LDL: low density lipoprotein).

	GTN 25 µg	GTN 400 µg	*p* value
Total population			
Delta SBP (mm Hg)	−0.71 ± 5.82	−3.17 ± 7.66	0.001
Delta DBP (mm Hg)	−0.72 ± 4.44	−4.75 ± 5.04	0.001
Delta HR (bpm)	0.15 ± 4.60	5.49 ± 6.02	0.001
Normotensives			
Delta SBP (mm Hg)	−0.85 ± 5.37	−2.03 ± 6.90	0.35
Delta DBP (mm Hg)	−1.22 ± 4.61	−5.63 ± 4,39	0.001
Delta HR (bpm)	0.59 ± 3.29	5.27 ± 5.52	0.001
Hypertensives			
Delta SBP (mm Hg)	−0.62 ± 6.16	−4.00 ± 8.11	0.001
Delta SBP (mm Hg)	−0.36 ± 4.31	−4.11 ± 5.41	0.001
Delta HR (bpm)	−0.17 ± 5.36	5.65 ± 6.39	0.001

A subgroup of hypertensive individuals underwent a second experimental session for microneurographic assessment. In 2 individuals adequate nerve recordings could not be obtained, while in 3 individuals nerve signal was not stable along the whole experiment. Thus, beat-to-beat BP and HR were available for 15 individuals, while MSNA was analysed in 10 individuals. In this population, s, non-invasive beat-to-beat systolic BP was significantly reduced under GTN 400 µg but not after GTN 25 µg (Fig. 2a), with a significant time*treatment interaction. A similar behaviour was observed for diastolic BP (GTN 25 diastolic BP 91.6 ± 9.6 to 90.4 ± 9.3 mm Hg; GTN 400 diastolic BP 91.8 ± 9.3 to 81.5 ± 13.5 mm Hg, time*treatment interaction $p = .004$). HR and MSNA were significantly increased only after GTN 400 µg (Fig. 2b and c). Consequently, MSNA did not show a different behaviour when calculated as burst incidence (GTN 25 MSNA 53.2 ± 11.6 to 60.5 ± 18.7 bursts/100HR; GTN 400 MSNA 50.4 ± 11.8 to 61.6 ± 12.1 bursts/100HR, time*treatment interaction $p = .005$).

4. Discussion

This study compared the two most commonly used dosages of GTN for the assessment of endothelium-independent vasodilation in FMD

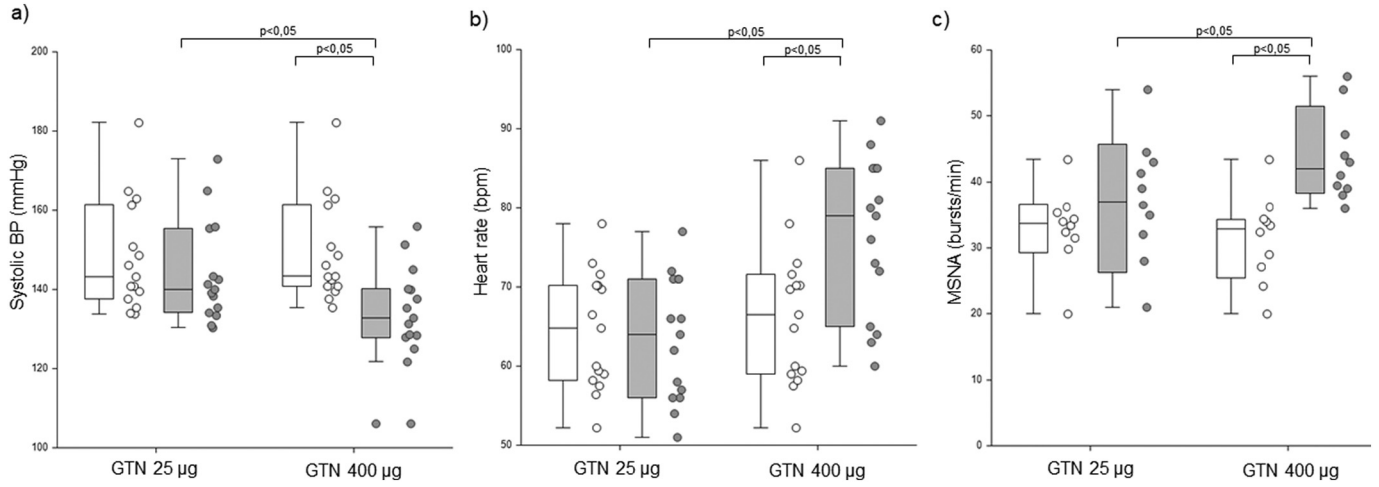

Fig. 2. Dot plots and box plots showing the behaviour of systolic blood pressure (BP, a), heart rate (b) and muscle sympathetic nerve activity (MSNA, c), before (in white) and after (in black) sublingual administration of glyceryl trinitrate GTN 25 µg and GTN 400 µg in a subgroup of hypertensive patients. *P* values are for Bonferroni post-hoc.

protocols. The main result of the study is that administration of GTN at the dose of 25 µg induced a significant increase in brachial artery diameter, which is comparable to that one obtained by reactive hyperemia, with only modest hemodynamic and sympathetic response. Conversely, GTN at the dose of 400 µg is able to exert a marked vasodilation, which is more than twofold compared to FMD, inducing a not negligible hemodynamic impact, accompanied by reflex sympathetic activation.

As already introduced, response to GTN is an accepted proxy for VSM function [5]. However, its clinical relevance is still under debate. A meta-analysis demonstrated a significantly impaired response to GTN in type 2 diabetic patients in the micro- but not in the macrocirculation, though the brachial artery was studied in only a minority of the studies [17]. Furthermore, recently it was reported that VSM function is impaired also in patients with fibromuscular dysplasia [18]. In the present study vasodilation to GTN 25 µg only tended to be reduced in hypertensive individuals, at variance of the results obtained in a larger cohort [19], thus the impact of classical risk factors on VSM function still need to be explored extensively. In fact, clinical determinants of VSM function are not fully understood. In a large Japanese cohort, brachial artery dilation to sublingual GTN was correlated with most of the classical cardiovascular risk factors and was identified as an independent determinant of FMD [20]. Furthermore, in our study we did not find any significant correlation between endothelium-dependent and –independent vasodilation, confirming that factors associated with FMD and response to GTN might be different [21]. The association between brachial artery diameter response to GTN and lack of nocturnal BP fall [22] and surrogate biomarkers such as coronary artery calcium [23] or microalbuminuria [24] has been demonstrated in small cohorts. However, the prognostic value of brachial artery dilation to GTN was investigated only in few prospective studies, with inconclusive results [25,26].

Many research centers still use sublingual GTN 400 µg, which is the lowest marketed dose and the first to be used in that setting [6]. Lower dosages (25 µg) were then introduced [7] and recommended in 2005 in a ESH statement [8]. Indeed, in a group of 17 healthy men with a mean age of 40 years, GTN dosages from 5 to 400 µg were tested [9]. Whereas GTN 400 µg induced an approximately 10% decrease in BP and about 10% increase in HR, a dose between 8 and 35 µg induced a 4% to 10% change in brachial artery diameter without affecting systemic hemodynamic [9]. Our study confirmed that the extent of dilation with GTN 25 µg was similar to that one induced by FMD and that a significant impact on BP and heart rate occurred only with the 400 µg dose. A novelty of the present study is that in a subgroup of hypertensive patients we were able to show that 25 µg GTN did not cause any significant increase in sympathetic traffic to muscle vasculature, directly assessed by the microneurography technique, while a relevant reflex activation of sympathetic traffic directed both to the muscle vasculature and to the heart was found administering GTN 400 µg. It has been suggested that using higher GTN doses might convey the advantage of assessing the maximal brachial artery vasodilation, which may add information about structural arterial wall alterations [9]. However, it is conceivable that maximal vasodilation cannot be obtained in these conditions, given the counter-regulatory sympathetic activation induced by the systemic hemodynamic effects of higher doses of GTN. Furthermore, even higher GTN doses might be insufficient to induce maximal vasodilation, to our knowledge, the most effective protocol to induce maximal vasodilation require a combination of stimuli, usually nitrate administration plus local heating [27], possibly in order to overcome the counterregulatory sympathethically-mediated vasoconstriction.An additive finding of this study regards the assessment of timing of the maximum dilation to different GTN dosages by the use of continuous automatic diameter measurement [13]. This methodological issue is of relevance, since previous studies showed a non-negligible variability among individuals, both with high [28,29] and with low GTN doses [7]. The direct comparison in the same individuals allows us to conclude that timing of peak vasodilation induced by 25 and 400 µg is similar and after about 4 min. Thus, brachial artery diameter monitoring should be extended at least to 5 min after GTN administration.

This study has some strengths, and in particular it is the first comparing the effect of two different GTN dosages on directly recorded sympathethic nerve traffic, on top of classical hemodynamic variables. We should acknowledge some limitations in this study too. Hypertensive patients and healthy controls were not matched for age and other risk factors as hypercholesterolemia, possibly limiting the value of the comparison between groups. However, comparing responses within groups, it is important to note that a greater hemodynamic effect of higher GTN doses was demonstrated in both groups, indicating that the advantage carried by using lower GTN doses is present regardless of patient CV risk profile. Furthermore, the protocol did not include administration of GTN 75 µg, a dosage for which a large amount of clinical evidence is available, including predictive power for CV events [20,30].

5. Conclusions

In conclusion, the present study suggests, given the neutral effect on systemic hemodynamic, a low-dose GTN is preferable when testing

endothelial-independent vasodilation of the brachial artery.

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Declarations of Competing Interest

None.

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