Original article

REPRODUCIBILITY OF HEMODYNAMIC, CARDIAC AUTONOMIC MODULATION AND BLOOD FLOW ASSESSMENTS IN PATIENTS WITH INTERMITTENT CLAUDICATION

Running head: Cardiovascular function reproducibility in IC

Authors

Aluísio Andrade-Lima[1], Marcel Chehuen[1], Natan Silva Junior[1], Rafael Fecchio[1], Tiago Peçanha[1], Leandro Brito[1], Roberto Miyasato[1], Anthony S. Leicht[2], Cláudia L. M. Forjaz[1].

Affiliations

[1]Exercise Hemodynamic Laboratory, School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil;

[2]College of Healthcare Sciences, James Cook University, Townsville, Australia.

Corresponding author

Aluísio Andrade-Lima, MS, School of Physical Education and Sport, University of São Paulo, Av. Professor Melo Moraes, 65, 05508-030 São Paulo, Brazil. Tel: + 55 11 9 5847 1567; e-mail: aluisiolima@live.com

STRUCTURED ABSTRACT

Objective: To identify, in patients with peripheral artery disease and intermittent claudication (IC), the reproducibility of heart rate (HR), blood pressure (BP), rate pressure product (RPP), heart rate variability (HRV), and forearm and calf blood flow (BF) and vasodilatory assessments.

Methods: Twenty-nine patients with IC underwent test and retest sessions, 8-12 days apart. During each session, HR, BP, HRV, BF and vasodilatory responses were measured by electrocardiogram, auscultation, spectral analysis of HRV (low frequency, LF_{R-R}; high frequency, HF_{R-R}) and strain gauge plethysmography (baseline BF, post-occlusion BF, post-occlusion area under the curve, AUC). Reproducibility was determined by intraclass coefficient correlation (ICC), typical error, coefficient of variation (CV) and limits of agreement.

Results: The ICC for HR and BP were > 0.8 with CV < 9%. For most HRV measures, ICC were > 0.9 while CV were < 7%, except for LF/HF (ICC = 0.737; CV = 93.8%). The ICC for forearm and calf baseline BF assessments were > 0.9 while CV were < 19%; variable ICC and CV for vasodilatory responses were exhibited for calf (0.653 – 0.770; 35.2 – 37.7%) and forearm (0.169 – 0.265; 46.2 – 55.5%).

Conclusions: In male patients with IC, systemic hemodynamic (HR and BP), cardiac autonomic modulation (LF_{R-R} and HF_{R-R}) and forearm and calf baseline BF assessments exhibited excellent reproducibility, whereas the level of reproducibility for vasodilatory responses were moderate to poor. Assessment reproducibility has highlighted appropriate clinical tools for the regular monitoring of disease/intervention progression in patients with IC.

Key-words: peripheral artery disease; reliability; agreement; cardiovascular system; hemodynamic variables.

1. INTRODUCTION

Peripheral artery disease (PAD) affects 200 million people worldwide with this population suffering from a partial or complete artery occlusion, primarily in the lower limbs [1]. Due to severe ischemia caused by increased oxygen demand, patients with PAD usually present with pain in the legs during walking that ceases with rest [2, 3]; a symptom referred to as intermittent claudication (IC) [4]. Patients who experience IC exhibit a reduced walking capacity and quality of life compared to the general healthy population [5].

Apart from limb-specific impairments, patients with IC also present with several traditional cardiovascular risk factors [6, 7], which contribute towards their high rates of cardiovascular events and mortality [7, 8]. Recent studies have examined other physiological mechanisms that may be linked with the increased cardiovascular disease burden in PAD. Compared to healthy adults, augmented blood pressure (BP) [8, 9], decreased parasympathetic and increased sympathetic modulations to the heart [10] as well as impairments in blood flow (BF) and vasodilation [11] have been reported in patients with IC. Significant associations between hemodynamic, autonomic and vascular impairments with cardiovascular disease and mortality in PAD [12, 13] have increased the clinical interest in evaluating these impairments to assist with early identification of increased cardiovascular risk for patients. However, the reproducibility of the tools for assessing these impairments must be confirmed before they can be consistently utilised in clinical practice.

Reproducibility is an important methodological psychometric property, especially for tracking changes over time, and crucial for identifying clinical meaningful changes for individuals [14]. In healthy [15, 16] and clinical populations, such as those with hypertension [17, 18] and diabetes mellitus [19], electrocardiogram (ECG), auscultation, heart rate variability (HRV) and strain gauge plethysmography have been used reliably to assess heart rate (HR), BP, cardiac autonomic modulation, and BF and vasodilatory response, respectively. However, some of these measures have

been reported to be irreproducible in populations such as cardiac transplant [20] and chronic heart failure [21] patients suggesting that reproducibility may be population-specific. Furthermore, patients with IC usually present with a myriad of cardiometabolic risk factors [6, 7], which further aggravates hemodynamic, autonomic and vascular dysfunction [8-11]. This particular disease presentation may lead to unique vascular and cardiac autonomic responses that may affect the reproducibility of assessments, and subsequent clinical relevance of these measures and future cardiovascular risk prediction. Currently, the reproducibility of these important prognostic assessment tools for IC patients is unknown. Thus, the aim of this study was to evaluate the reproducibility of the assessments of HR, BP, rate pressure product (RPP), HRV and upper limb and leg BF and vasodilatory responses in patients with PAD and IC. These assessment tools provide clinicians with reliable indicators for the monitoring of disease progression and cardiovascular risk in patients with IC.

2. MATERIAL AND METHODS

2.1. Participants

Patients with PAD and IC symptoms and enrolled in a tertiary center specialized in vascular disease were invited to participate in this study. Patients were included if they met the following criteria: (1) male; (2) aged ≥ 50 years; (3) diagnosed with Fontaine stage II of PAD [22]; (4) ankle brachial index at rest ≤ 0.90 in at least 1 lower limb; (5) resting systolic and diastolic BP lower than 160 and 105 mmHg, respectively; and (6) not receiving β-blockers or non-dihydropyridine calcium channel antagonists. The study's protocol was conducted in accordance with the Declaration of Helsinki, registered with the Brazilian Clinical Trials (http://www.ensaiosclinicos.gov.br, RBR-3pq58k), and approved by the Joint Committee on Ethics of Human Research of the institution

(process 667.382). Written informed consent was obtained from all patients prior to study commencement.

2.2. Procedures

Figure 1 shows the timeframe of the four visits to the laboratory completed by participants during study. During the first visit, they were interviewed, and ankle brachial index, anthropometric and resting BP measures were taken. The interview identified age, the presence of cardiovascular disease and risk factors, comorbid conditions, and current medication treatment. Ankle brachial index was evaluated as previously described [23], and anthropometric measures of body mass and height (Welmy, 110, São Paulo, Brazil) were used to calculate body mass index (BMI). Auscultatory BP was measured in triplicate using a mercury column sphygmomanometer (Unitec, São Paulo, Brazil). Measures were taken after 5 min of rest in the seated position. During the second visit, resting BP was measured again and the mean of the 6 measures (visit 1 and 2) was defined as each participant's resting BP. For the third and fourth visits (test and retest sessions), HR, BP, HRV, BF and vasodilatory response were measured 8-12 days apart and assessed for reproducibility.

INSERT FIGURE 1

2.3. Experimental protocol

The participants were instructed to avoid physical exercise for the previous 48 h, caffeinated and alcoholic beverages for the previous 24 h and smoking on the day of the sessions. They were also instructed to take their medication as prescribed by their physicians. The sessions were conducted throughout the year, with some participants taken part of the study in all the year seasons. Data was collected in a temperature-controlled laboratory (21 – 22°C).

The sessions started at 8:00 a.m and patients initially rested in the supine position for 50 min, including an initial stabilisation period of 10 min. ECG and breathing rate were recorded between 10 and 20 min, while BP and HR were measured in triplicate between 20 and 25 min with the mean value used for analysis. Lower and upper limb BF and vasodilatory response were determined during the last 25 min of supine rest.

2.4. Measurements

ECG (EMG System do Brazil, EMG 030110/00B, Brazil) was continuously monitored while auscultatory BP was measured in the dominant arm using a mercury sphygmomanometer (Unitec, São Paulo, Brazil). Mean BP was calculated as the sum of diastolic BP and one-third of pulse pressure (systolic – diastolic BP), while HR was recorded from the ECG immediately after BP measurement. RPP was calculated as HR x systolic BP.

For cardiac autonomic evaluation, R-R intervals from the ECG and respiratory signal from a thoracic piezoelectric belt (UFI, Pneumotrace2, California, USA) were inputted into a data acquisition system (WinDaq, DI-720, Akron, USA) at a sampling rate of 500 Hz/channel. Stationary segments (250 – 300 beats) of the HR series were assessed for HRV via spectral analysis (Heart Scope, version 1.3.0.1, New York, USA), employing an autoregressive method in accordance with Task Force recommendations [24]. Low-frequency (LF_{RR}) (0.04 – 0.15 Hz) and high-frequency (HF_{RR}) (0.15 – 0.4 Hz) components of HRV were expressed in normalized units (nu) with these representing the relative contribution of each component within the total power spectrum minus the very low frequency component [24]. Total variance (TV_{R-R}) (0 – 0.4 Hz) and LF/HF ratio were also calculated.

Upper and lower limb BF were simultaneously determined in the dominant forearm and the leg with the lowest ankle brachial index. BF was assessed by venous occlusion plethysmography (Hokanson, AI6, Bellevue, USA), as previously described [25]. Briefly, BF to the hand and the foot were interrupted by limb occlusion via cuffs inflated to 200 mmHg around the wrist and the ankle,

149 respectively. Other cuffs were placed on the upper arm and the thigh and were rapidly inflated for 10
150 s at 40 – 60 mmHg, followed by 10 s of deflation of both cuffs. Increases in limb volumes were
151 detected by mercury strain gauges positioned at the forearm and calf using specialised software
152 (NIVP3; Hokanson, Bellevue, WA). Measurements were taken during 4 min (12 x 20 s cycles) and
153 the mean was used to determine BF. Vascular resistance (VR) was determined as the ratio between
154 mean BP and BF in each limb. Vasodilatory response to reactive hyperaemia was also assessed [26].
155 After BF determination, BF to each limb was totally occluded for 5 min by inflating the thigh and
156 arm cuffs to 200 mmHg. The cuff was then released and post-occlusion BF was measured for 4 min
157 as described above. The vasodilatory response was calculated by: 1) the first BF measured
158 immediately after cuff release (post-occlusion BF) and 2) the differences in area under the curve
159 (AUC) between post- and pre-hyperaemia BF measurements.
160
161 **2.5. Statistical Analysis**
162 Data normality was confirmed by Shapiro-Wilk test while the presence of heteroscedasticity
163 was verified by significant correlations between the test-retest mean values and their absolute
164 differences. Variables violating these assumptions were transformed via natural logarithm prior to
165 further analysis.
166 Reproducibility was examined using the following parameters: 1) presence of systematic
167 bias; 2) inter-test reliability; 3) within individual variation; and 4) bias with limits of agreement.
168 Systematic bias was assessed as the difference between test and retest mean values using a paired t-
169 test. Inter-test reliability was evaluated by the intraclass correlation coefficient (ICC) with values <
170 0.40 considered poor, 0.40 – 0.75 considered moderate to good, and > 0.75 considered excellent [27].
171 Within-individual variation was evaluated by the typical error (TE) between test and retest values,
172 expressed both in absolute and relative terms (e.g. coefficient of variation – CV) with CV < 20%
173 considered desirable and > 30% undesirable [28]. Lastly, agreement was evaluated by the mean bias ±

95% limits of agreement (LOA) as previously described by Bland & Altman [29]. For the logarithm-transformed data, the ratio bias */÷ 95% LOA was calculated as previously recommended [30].

3. RESULTS

Twenty-nine male patients with IC volunteered for this study and their characteristics are summarized in Table I. In general, patients were elderly (> 60 years), had an ankle brachial index between 0.45 and 0.90 (i.e. mild to moderate PAD), presented with several cardiovascular risk factors, and were taking a range of medications such as aspirin, statins, and anti-hypertensives.

INSERT TABLE I

Mean values of systemic hemodynamic variables measured at test and retest, and their reproducibility indices are shown in Table II. There were no significant differences between the mean values of any variable ($P > 0.05$), while ICCs ranged from 0.833 (diastolic BP) to 0.908 (HR) and CVs ranged from 5.0 (mean BP) to 8.3% (RPP). For each variable, the TE and mean/ratio bias were small while the LOA varied (Table II).

INSERT TABLE II

Mean values of all HRV variables were not significantly different between test and retest ($P > 0.05$, Table III) with ICCs ranging from 0.929 (ln TVR-R) to 0.986 [HF_{R-R} (nu)], and CVs from 4.9 (ln TV_{R-R}) to 93.8% (LF/HF). For each variable, the TE and mean/ratio bias were small while the LOA varied (Table III).

INSERT TABLE III

The results for BF variables are shown in Table IV. The mean values of most variables did not significantly differ between test and retest with the exception of calf BF that was significantly higher at retest (P = 0.004). Reproducibility of the forearm BF variables varied with ICCs ranging from 0.169 (post-occlusion BF) to 0.982 (BF), and CVs from 8.2 (BF) to 55.5% (post-occlusion BF). For the calf BF variables, ICCs ranged between 0.653 (AUC) to 0.928 (VR), and CVs between 17.8 (VR) and 37.7% (AUC). In addition, the TE and mean bias were small while the LOA varied each forearm and calf BF variable (Table IV).

INSERT TABLE IV

4. DISCUSSION

The main findings of this study were that in patients with PAD and IC, systemic hemodynamic, cardiac autonomic modulation and baseline BF assessments exhibited excellent reproducibility as reflected by ICC, CV and mean bias ± LOA. In contrast, reproducibility of the vasodilatory response was moderate for calf and poor for forearm. These assessment tools provide clinicians with reliable indicators for the monitoring of disease progression and cardiovascular risk in PAD patients with IC.

4.1. Systemic hemodynamic variables

Few studies have evaluated the reproducibility of resting BP, HR and RPP in clinical populations [17, 31, 32]. This is surprising given the utility of these measures in guiding disease management and their positive relationship with cardiovascular morbidity and mortality [12, 33].

Previous studies have reported good to excellent reliability for auscultatory systolic and diastolic BP in patients with borderline hypertension (ICC: 0.61 – 0.83) [18] and untreated adults with elevated BP (ICC: 0.76 – 0.77) [17]. Similarly, the current study identified excellent reproducibility (ICC > 0.80 and CV < 9%) for systolic BP, diastolic BP, HR and RPP in patients with IC. These results confirm the utility of these clinical measures to assist with cardiovascular risk screening, and monitoring of disease progression and treatment in patients with PAD and IC.

4.2. Cardiac autonomic modulation

HRV has been used as a non-invasive method to quantify parasympathetic and sympathetic modulations to the heart [24]. HRV alterations, mainly characterized by decreased TV_{R-R} and HF_{R-R}, and increased LF_{R-R} and LF/HF, have been reported in patients with PAD and IC compared to healthy adults [10] with this reduce HRV associated with greater mortality [34]. However, to the best of our knowledge, the current study has been the first to verify the reproducibility of resting HRV in patients with IC. It is well known that reproducibility of HRV is population-specific with lower reproducibility generally obtained in clinical populations compared to healthy adults [20, 35-37]. However, the present results showed excellent reproducibility (ICC > 0.90 and CV < 9%) for almost all indices, except for LF/HF. Previous studies of patients with a history of myocardial infarction (ICC: 0.77 – 0.81) [38] and type 2 diabetes mellitus (ICC: 0.58 – 0.71) [19] reported poor reliability for the HRV indices. In addition, the current study demonstrated good within-individual variation for TV_{R-R}, LF_{R-R} and HF_{R-R} (CV < 9.0%). Previous studies have reported greater variation for these variables, even in healthy adults (CV: 20.1 – 23.0%) [39]. Differences for the within-individual variation of HRV may reflect differences in resting HRV levels with healthy individuals exhibiting greater HRV compared to those with chronic diseases [20, 35-37]. Regardless, the current study has demonstrated excellent reproducibility (i.e. excellent ICC and desirable CV) for some common HRV measures used for patients with IC. On the other hand, the LF/HF exhibited a high CV (93.8%) that

might be related to the low test and retest mean values with changes producing greater variation in the measure compared to other HRV variables. Subsequently, LF/HF should be cautiously used for the monitoring of PAD patients with IC due to its poorer reproducibility.

It is important to highlight that a range of factors may influence reproducibility (reliability and agreement) of HRV variables. Differences in internal (e.g. physiological state, mood, alertness, mental activity, etc) and external (e.g. time of day, temperature, etc) factors have been reported to influence cardiac autonomic modulation [24, 40], and may have an impacted on the reproducibility of test-retest measurements. A rigorous set of procedures had been implemented in the current study (e.g. control of physical activity, time of day, laboratory temperature and medication) to minimise any potential influencing factors, which may have resulted in better reproducibility indicators than that seen previously [19, 38, 39]. Subsequently, a standardised procedure, such as that adopted in the current study, may be beneficial and vital to ensure reproducible and accurate monitoring of patients with IC. Future studies may confirm the advantages of these standardised procedures for other clinical populations.

4.3. Peripheral hemodynamic variables

Patients with IC have varying degrees of arterial occlusion that consequently result in several physical limitations due to reduced BF in the limbs [1-3]. Assessment of BF via strain gauge plethysmography has been considered the gold standard method for measure distal limb BF [41] with a previous study reporting similar reliability (ICC > 0.90) to that of the current study for baseline forearm and calf BFs in healthy adults [42]. Additionally, within-individual variations for baseline forearm (8.2%) and calf BF (18.0%) were similar to those previously reported in healthy individuals [42, 43]. Others [15, 44] though have reported higher CV for these variables with differences between studies likely a result of different populations and experimental session conditions, such as positioning of the cuff, strain gauge, assessed limb and physiological circadian rhythm. In addition,

274 forearm and VR also presented excellent reproducibility (ICC > 0.90 and CV < 20%) as compared
275 with a previous study [15].

276 Considering vasodilatory response, a poor to moderate reproducibility was observed for post-
277 occlusion BF and AUC for the forearm and calf. In contrast, Altenkirch et al [45] and Thijssen et al [15]
278 reported better reproducibility via CV for forearm post-occlusion BF (10.5% and 8.6%, respectively)
279 and calf post-occlusion BF (7.1% and 6.4%, respectively) in healthy individuals. Differences in
280 reproducibility between studies are most likely due to the presence of vascular disease for PAD
281 patients with post-occlusion vasodilatory responses influenced by several regulating factors (e.g. NO,
282 prostaglandins and others) [46]. Repeated ischemia and reperfusion during ambulation may alter these
283 regulating factors as well as enhance oxidative stress and inflammation [47, 48] that contributes to the
284 poorer reproducibility of vasodilatory response in PAD patients with IC.

285

286 *4.4. Practical applications*

287 The current findings have important applications for both research and clinical settings. In
288 general, excellent reproducibility (ICC > 0.75 and CV < 20%) was identified for systemic
289 hemodynamics, cardiac autonomic modulation and baseline BF and VR in patients with IC that
290 confirms their valuable and practical use to monitor disease and/or intervention progression in
291 clinical settings. However, vasodilatory responses (i.e. post-occlusion BF and AUC) for calf (ICC >
292 0.40 and CV > 30%) and forearm (ICC < 0.40 and CV > 30%) were moderate-poor that questions
293 the practical relevance of these measures in patients with IC. Finally, the current study determined
294 the specific TE for each variable that should be considered when examining smallest worthwhile
295 changes in PAD patient's progression and for calculating minimum sample sizes for research studies
296 with IC patients. Moreover, the specific LOA of each variable could be employed as a cut-
297 off/minimal threshold to identify mean changes when evaluating interventions within this specific
298 population [49].

299 *4.5. Limitations*

300 This study has some limitations, which should be acknowledged. Firstly, the current study
301 was conducted with men only, which restricts extrapolation of the results to women. Secondly,
302 although patients with PAD usually present other cardiovascular diseases, none of the participants of
303 the study presented heart failure. As reproducibility parameters has been shown to be poor in heart
304 failure [21], results might be different in patients with this comorbidity. Third, different medications
305 might affect cardiovascular reproducibility. The current study included medication regimes typically
306 used in patients with PAD excepted for β-blockers and non-dihydropyridine calcium channel
307 antagonists because these drugs directly affect the assessment of heart rate variability. Therefore, the
308 results were not applicable to patients receiving these specific drugs. Finally, as seasonal variations
309 may impact on cardiovascular parameters and test and retest had been conducted at the same season
310 of the year, the reproducibility indices obtained in the current study are valid for within season
311 measures but may be different if test and retest were conducted at different seasons. The limitations
312 of the present study suggest that future study should investigate the reproducibility indices in patients
313 with PAD who are women, have heart failure, and are receiving β-blockers or non-dihydropyridine
314 calcium channel antagonists. In addition, future studies should address reproducibility between
315 different seasons of the year.

316

317 **5. CONCLUSIONS**

318

319 Systemic hemodynamic (HR and auscultatory BP), cardiac autonomic modulation (TV_{R-R},
320 LF_{R-R} and HF_{R-R}) and baseline BF assessments exhibited excellent reproducibility at rest for male
321 patients with IC with these tools highly appropriate for clinical monitoring of patients. However, the
322 level of reproducibility for vasodilatory responses (post-occlusion BF and AUC) of the calf and

forearm was moderate to poor that highlighted the limitations of these tools for regular monitoring of disease/intervention in PAD patients.

Sources of funding: This study was supported by FAPESP (2015/13800-0) and CNPq (141477/2015- 6).

The authors declare no conflicts of interest to disclose

6. REFERENCES

1. Fowkes FG, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. Lancet. 2013;382:1329-40.

2. Hirsch AT, Haskal ZJ, Hertzer NR, et al. ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation. 2006;113:e463-654.

3. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). Eur J Vasc Endovasc Surg. 2007;33 Suppl 1:S1-75.

348 4. McDermott MM, Mehta S, Greenland P. Exertional leg symptoms other than intermittent
349 claudication are common in peripheral arterial disease. Arch Intern Med. 1999;159:387-92.
350 5. Spronk S, White JV, Bosch JL, et al. Impact of claudication and its treatment on quality of
351 life. Semin Vasc Surg. 2007;20:3-9.
352 6. Cimminiello C, Arpaia G, Polo Friz H, et al. A prospective multicentre study on the treatment
353 of cardiovascular risk factors and claudication symptoms in patients with peripheral artery disease
354 (the IDOMENEO study). Vasa. 2015;44:371-9.
355 7. Criqui MH, Aboyans V. Epidemiology of peripheral artery disease. Circ Res. 2015;116:1509-
356 26.
357 8. Bhatt DL, Steg PG, Ohman EM, et al. International prevalence, recognition, and treatment of
358 cardiovascular risk factors in outpatients with atherothrombosis. JAMA. 2006;295:180-9.
359 9. Carbayo JA, Divison JA, Escribano J, et al. Using ankle-brachial index to detect peripheral
360 arterial disease: prevalence and associated risk factors in a random population sample. Nutr Metab
361 Cardiovasc Dis. 2007;17:41-9.
362 10. Goernig M, Schroeder R, Roth T, et al. Peripheral arterial disease alters heart rate variability
363 in cardiovascular patients. Pacing Clin Electrophysiol. 2008;31:858-62.
364 11. Ogren M, Hedblad B, Engstrom G, et al. Leg blood flow and long-term cardiovascular
365 prognosis in men with typical and atypical intermittent claudication. Eur J Vasc Endovasc Surg.
366 2003;26:272-9.
367 12. Jelnes R, Gaardsting O, Hougaard Jensen K, et al. Fate in intermittent claudication: outcome
368 and risk factors. Br Med J (Clin Res Ed). 1986;293:1137-40.
369 13. Huang AL, Silver AE, Shvenke E, et al. Predictive value of reactive hyperemia for
370 cardiovascular events in patients with peripheral arterial disease undergoing vascular surgery.
371 Arterioscler Thromb Vasc Biol. 2007;27:2113-9.

14. Al Haddad H, Laursen PB, Chollet D, et al. Reliability of resting and postexercise heart rate measures. Int J Sports Med. 2011;32:598-605.

15. Thijssen DH, Bleeker MW, Smits P, et al. Reproducibility of blood flow and post-occlusive reactive hyperaemia as measured by venous occlusion plethysmography. Clin Sci (Lond). 2005;108:151-7.

16. Farah BQ, Lima AH, Cavalcante BR, et al. Intra-individuals and inter- and intra-observer reliability of short-term heart rate variability in adolescents. Clin Physiol Funct Imaging. 2016;36:33-9.

17. Stergiou GS, Baibas NM, Gantzarou AP, et al. Reproducibility of home, ambulatory, and clinic blood pressure: implications for the design of trials for the assessment of antihypertensive drug efficacy. Am J Hypertens. 2002;15:101-4.

18. Reeves RA, Leenen FH, Joyner CD. Reproducibility of nurse-measured, exercise and ambulatory blood pressure and echocardiographic left ventricular mass in borderline hypertension. J Hypertens. 1992;10:1249-56.

19. Sacre JW, Jellis CL, Marwick TH, et al. Reliability of heart rate variability in patients with type 2 diabetes mellitus. Diabet Med. 2012;29:e33-40.

20. Lord SW, Senior RR, Das M, et al. Low-frequency heart rate variability: reproducibility in cardiac transplant recipients and normal subjects. Clin Sci (Lond). 2001;100:43-6.

21. Sandercock GR, Bromley PD, Brodie DA. The reliability of short-term measurements of heart rate variability. Int J Cardiol. 2005;103:238-47.

22. Fontaine R, Kim M, Kieny R. [Surgical treatment of peripheral circulation disorders]. Helv Chir Acta. 1954;21:499-533.

23. Gardner AW, Montgomery PS. Comparison of three blood pressure methods used for determining ankle/brachial index in patients with intermittent claudication. Angiology. 1998;49:723-8.

24. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996;93:1043-65.

25. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. Br J Clin Pharmacol. 2001;52:631-46.

26. Thijssen DH, Black MA, Pyke KE, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol. 2011;300:H2-12.

27. Szklo R, Nieto F. Epidemiology: Beyond the Basis. Gaithersburg, MD: Aspen Publications; 2000.

28. Rosner B. Fundamentals of Biostatistics. 7. Boston: Brooks/Cole, Cengage Learning; 2011.

29. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1:307-10.

30. Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med. 1998;26:217-38.

31. Dunne JF. Variation of blood-pressure in untreated hypertensive outpatients. Lancet. 1969;1:391-2.

32. Bottini PB, Carr AA, Prisant LM, et al. Variability and similarity of manual office and automated blood pressures. J Clin Pharmacol. 1992;32:614-9.

33. Bordejevic DA, Caruntu F, Mornos C, et al. Prognostic impact of blood pressure and heart rate at admission on in-hospital mortality after primary percutaneous intervention for acute myocardial infarction with ST-segment elevation in western Romania. Ther Clin Risk Manag. 2017;13:1061-8.

34. Badarau S, Siriopol D, Drugus D, et al. Electrocardiogram abnormalities and heart rate variability in predicting mortality and cardiovascular events among hemodialyzed patients. Int Urol Nephrol. 2015;47:1703-8.

422 35. Delgado G, Estanol B, Rodriguez-Violante M, et al. Cardiovascular variability in Mexican
423 patients with Parkinson's disease. Arq Neuropsiquiatr. 2014;72:762-7.
424 36. Kunz VC, Souza RB, Takahashi AC, et al. The relationship between cardiac autonomic
425 function and clinical and angiographic characteristics in patients with coronary artery disease. Rev
426 Bras Fisioter. 2011;15:503-10.
427 37. Giordano M, Manzella D, Paolisso G, et al. Differences in heart rate variability parameters
428 during the post-dialytic period in type II diabetic and non-diabetic ESRD patients. Nephrol Dial
429 Transplant. 2001;16:566-73.
430 38. Maestri R, Raczak G, Danilowicz- Szymanowicz L, et al. Reliability of heart rate variability
431 measurements in patients with a history of myocardial infarction. Clin Sci (Lond). 2009;118:195-
432 201.
433 39. Kobayashi H. Inter- and intra-individual variations of heart rate variability in Japanese males.
434 J Physiol Anthropol. 2007;26:173-7.
435 40. Malliani A, Pagani M, Lombardi F, et al. Cardiovascular neural regulation explored in the
436 frequency domain. Circulation. 1991;84:482-92.
437 41. Joyner MJ, Dietz NM, Shepherd JT. From Belfast to Mayo and beyond: the use and future of
438 plethysmography to study blood flow in human limbs. J Appl Physiol (1985). 2001;91:2431-41.
439 42. Engelke KA, Halliwill JR, Proctor DN, et al. Contribution of nitric oxide and prostaglandins
440 to reactive hyperemia in human forearm. J Appl Physiol (1985). 1996;81:1807-14.
441 43. Roberts DH, Tsao Y, Breckenridge AM. The reproducibility of limb blood flow
442 measurements in human volunteers at rest and after exercise by using mercury-in-Silastic strain
443 gauge plethysmography under standardized conditions. Clin Sci (Lond). 1986;70:635-8.
444 44. Petrie JR, Ueda S, Morris AD, et al. How reproducible is bilateral forearm plethysmography?
445 Br J Clin Pharmacol. 1998;45:131-9.

446 45. Altenkirch HU, Fransson L, Koch G. Assessment of arterial and venous circulation in upper
447 and lower extremities by venous occlusion strain gauge plethysmography. Normal values and
448 reproducibility. Vasa. 1989;18:140-5.
449 46. Vitale V, Monami M, Mannucci E. Prostanoids in patients with peripheral arterial disease: A
450 meta-analysis of placebo-controlled randomized clinical trials. J Diabetes Complications.
451 2016;30:161-6.
452 47. Signorelli SS, Mazzarino MC, Di Pino L, et al. High circulating levels of cytokines (IL-6 and
453 TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral
454 arterial disease at rest and after a treadmill test. Vasc Med. 2003;8:15-9.
455 48. Silvestro A, Scopacasa F, Oliva G, et al. Vitamin C prevents endothelial dysfunction induced
456 by acute exercise in patients with intermittent claudication. Atherosclerosis. 2002;165:277-83.
457 49. de Vet HCW, Terwee CB, Mokkink LB, et al. Measurement in medicine: A practical guide:
458 Cambridge Universty Press; 2010.
459

460 **LEGENDS TO ILLUSTRATION**

461

462 **Figure 1. Timeframe of the 4 visits. BMI – Body Mass Index; ABI – Ankle Brachial Index; BP**

463 **– Blood Pressure; HR – Heart Rate; HRV – Heart Rate Variability; BF – Blood Flow.**

464

Table

Table I. Clinical characteristics of patients

n	29
Age, y	67 ± 11
Height, m	1.67 ± 0.07
Weight, kg	71.0 ± 10.1
Body mass index, kg/m^2	25.3 ± 3.3
Ankle brachial index	0.62 ± 0.13
Cardiovascular measures	
Systolic blood pressure (mmHg)	136 ± 15
Diastolic blood pressure (mmHg)	82 ± 9
Heart rate (bpm)	72 ± 11
Cardiovascular risk factors and comorbidities	
Obesity, %	6.9
Hypertension, %	69.0
Diabetes mellitus, %	27.6
Dyslipidaemia, %	93.1
Current smoker, %	34.5
Heart disease, %	17.2
Medications	
Aspirin, %	93.1
Statin, %	93.1
Angiotensin-converting enzyme inhibitor, %	44.8
Diuretic, %	24.1
Dihydropyridine calcium channel antagonist, %	20.7

Oral hypoglycaemic, %	27.6

Continuous data are expressed as mean ± SD. Categorical data are expressed as percentage of occurrence (%). Obesity was defined as body mass index ≥ 30 kg/m. Hypertension, diabetes mellitus, dyslipidaemia and heart disease were defined by previous physician diagnosis.

Table

Table II. Mean ± SD values and reproducibility parameters of systemic hemodynamic variables

	Test	**Retest**	**P value**	**ICC**	**TE**	**CV %**	**Bias ± 95%LOA**
Systolic BP (mmHg)	134 ± 13	132 ± 16	0.409	0.863	7.2	5.4	1.6 ± 20.0
Diastolic BP (mmHg)	78 ± 8	77 ± 9	0.362	0.833	4.8	6.2	1.1 ± 13.2
Mean BP (mmHg)	96 ± 9	95 ± 10	0.306	0.845	4.8	5.0	1.3 ± 13.2
HR (bpm)	66.6 ± 9.1	64.6 ± 8.8	0.062	0.908	3.7	5.6	2.0 ± 10.2
RPP (mmHg.bpm)	8879 ± 1347	8494 ± 1520	0.060	0.856	720	8.3	385 ± 1994

BP – blood pressure; HR – heart rate; RPP – rate pressure product; ICC – intraclass correlation coefficient; TE – typical error; CV – coefficient of variation; LOA – limits of agreement

Table

Table III. Mean ± SD values and reproducibility parameters of autonomic variables

	Test	**Retest**	**P value**	**ICC**	**TE**	**CV %**	**Bias** ± or */÷ **95% LOA**
ln TV_{R-R} (ms^2)	6.8 ± 1.0	6.9 ± 0.9	0.459	0.929	0.3	4.9	0.9 */÷ 2.6
LF_{R-R} (nu)	47.8 ± 19.9	47.5 ± 18.8	0.831	0.979	4.0	8.3	0.3 ± 11.0
HF_{R-R} (nu)	44.7 ± 20.7	45.5 ± 19.4	0.535	0.986	3.3	7.3	-0.7 ± 9.1
ln LF/HF	0.18 ± 0.87	0.17 ± 0.88	0.737	0.982	0.16	93.8	1.0 */÷ 1.6

ln – natural logarithm; TV – total variance; LF – low frequency component; HF – high frequency component; nu – normalized units; ICC – intraclass correlation coefficient; TE – typical error; CV – coefficient of variation; LOA – limits of agreement

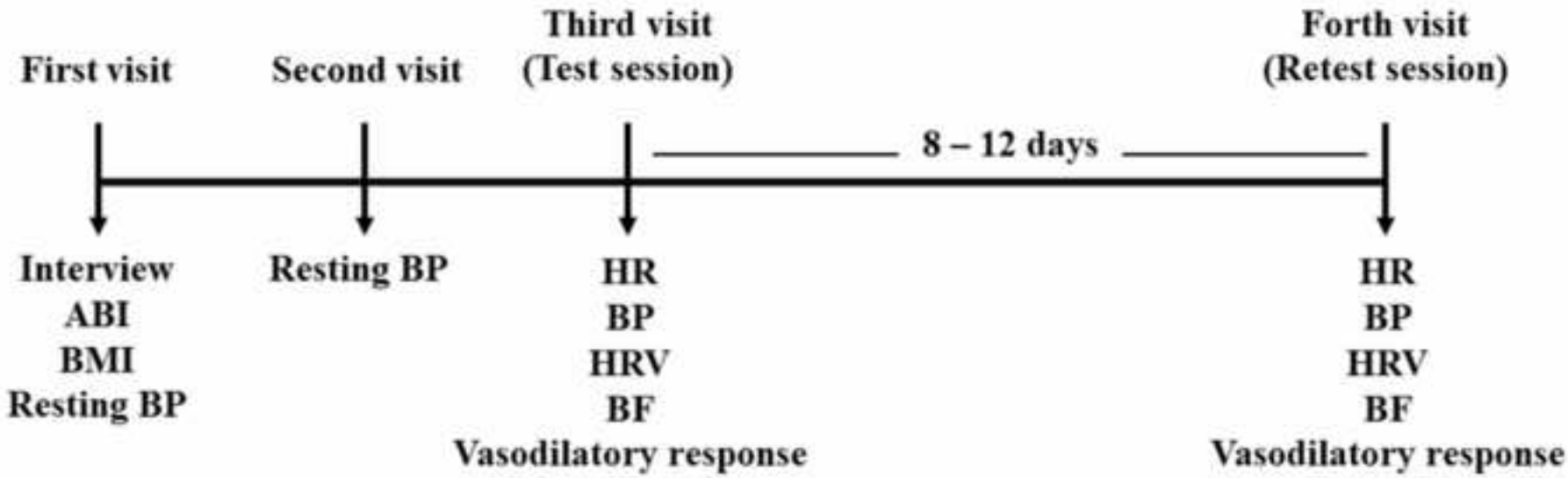
Table

Table IV. Mean ± SD values and reproducibility forearm and calf blood flows and vasodilatory response variables

	Test	Retest	P value	ICC	TE	CV %	Bias ± 95% LOA
Forearm							
BF (ml.100 ml^{-1}.min^{-1})	1.42 ± 0.63	1.41 ± 0.59	0.872	0.982	0.12	8.2	0.00 ± 0.32
VR (U)	75 ± 35	74 ± 32	0.627	0.978	7.0	9.3	1 ± 19
Post-occlusion BF (ml.100 ml^{-1}.min^{-1})	11.4 ± 7.0	11.2 ± 6.1	0.938	0.169	6.3	55.5	0.15 ± 17.37
Post-occlusion AUC BF (U)	1294 ± 676	1085 ± 507	0.232	0.265	550	46.2	209 ± 1524
Calf							
BF (ml.100 ml^{-1}.min^{-1})	1.69 ± 0.72	2.06 ± 0.95	0.004	0.913	0.34	18.0	-0.37 ± 0.94
VR (U)	60 ± 25	57 ± 31	0.362	0.928	10.0	17.8	3 ± 29
Post-occlusion BF (ml.100 ml^{-1}.min^{-1})	5.73 ± 3.18	5.05 ± 3.04	0.297	0.770	1.9	35.2	0.68 ± 5.27
Post-occlusion AUC BF (U)	999 ± 467	1081 ± 616	0.553	0.653	392	37.7	-82 ± 1088

BF – blood flow; VR – vascular resistance; AUC – area under the curve; ICC – intraclass correlation coefficient; TE – typical error; CV – coefficient of variation; LOA – limits of agreement.

Figure


First visit
Second visit
Third visit
(Test session)
Forth visit
(Retest session)
8 – 12 days
Interview
ABI
BMI
Resting BP
Resting BP
HR
BP
HRV
BF
Vasodilatory response
HR
BP
HRV
BF
Vasodilatory response