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1 **Original article**

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

5 **Running head:** Cardiovascular function reproducibility in IC

6

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26 **STRUCTURED ABSTRACT**

27

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

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

37 **Results:** The ICC for HR and BP were > 0.8 with $CV < 9\%$. For most HRV measures, ICC were $>$
38 0.9 while CV were $< 7\%$, except for LF/HF (ICC = 0.737; CV = 93.8%). The ICC for forearm and
39 calf baseline BF assessments were > 0.9 while CV were $< 19\%$; variable ICC and CV for
40 vasodilatory responses were exhibited for calf (0.653 – 0.770; 35.2 – 37.7%) and forearm (0.169 –
41 0.265; 46.2 – 55.5%).

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

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

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50

51 1. INTRODUCTION

52

53 Peripheral artery disease (PAD) affects 200 million people worldwide with this population
54 suffering from a partial or complete artery occlusion, primarily in the lower limbs¹. Due to severe
55 ischemia caused by increased oxygen demand, patients with PAD usually present with pain in the
56 legs during walking that ceases with rest^{2,3}; a symptom referred to as intermittent claudication (IC)
57⁴. Patients who experience IC exhibit a reduced walking capacity and quality of life compared to the
58 general healthy population⁵.

59 Apart from limb-specific impairments, patients with IC also present with several traditional
60 cardiovascular risk factors^{6,7}, which contribute towards their high rates of cardiovascular events and
61 mortality^{7,8}. Recent studies have examined other physiological mechanisms that may be linked with
62 the increased cardiovascular disease burden in PAD. Compared to healthy adults, augmented blood
63 pressure (BP)^{8,9}, decreased parasympathetic and increased sympathetic modulations to the heart¹⁰
64 as well as impairments in blood flow (BF) and vasodilation¹¹ have been reported in patients with IC.
65 Significant associations between hemodynamic, autonomic and vascular impairments with
66 cardiovascular disease and mortality in PAD^{12,13} have increased the clinical interest in evaluating
67 these impairments to assist with early identification of increased cardiovascular risk for patients.
68 However, the reproducibility of the tools for assessing these impairments must be confirmed before
69 they can be consistently utilised in clinical practice.

70 Reproducibility is an important methodological psychometric property, especially for
71 tracking changes over time, and crucial for identifying clinical meaningful changes for individuals¹⁴.
72 In healthy^{15,16} and clinical populations, such as those with hypertension^{17,18} and diabetes mellitus
73¹⁹, electrocardiogram (ECG), auscultation, heart rate variability (HRV) and strain gauge
74 plethysmography have been used reliably to assess heart rate (HR), BP, cardiac autonomic
75 modulation, and BF and vasodilatory response, respectively. However, some of these measures have

76 been reported to be irreproducible in populations such as cardiac transplant²⁰ and chronic heart
77 failure²¹ patients suggesting that reproducibility may be population-specific. Furthermore, patients
78 with IC usually present with a myriad of cardiometabolic risk factors^{6,7}, which further aggravates
79 hemodynamic, autonomic and vascular dysfunction⁸⁻¹¹. This particular disease presentation may
80 lead to unique vascular and cardiac autonomic responses that may affect the reproducibility of
81 assessments, and subsequent clinical relevance of these measures and future cardiovascular risk
82 prediction. Currently, the reproducibility of these important prognostic assessment tools for IC
83 patients is unknown. Thus, the aim of this study was to evaluate the reproducibility of the
84 assessments of HR, BP, rate pressure product (RPP), HRV and upper limb and leg BF and
85 vasodilatory responses in patients with PAD and IC. These assessment tools provide clinicians with
86 reliable indicators for the monitoring of disease progression and cardiovascular risk in patients with
87 IC.

88

89 **2. MATERIAL AND METHODS**

90

91 **2.1. Participants**

92 Patients with PAD and IC symptoms and enrolled in a tertiary center specialized in vascular
93 disease were invited to participate in this study. Patients were included if they met the following
94 criteria: (1) male; (2) aged ≥ 50 years; (3) diagnosed with Fontaine stage II of PAD²²; (4) ankle
95 brachial index at rest ≤ 0.90 in at least 1 lower limb; (5) resting systolic and diastolic BP lower than
96 160 and 105 mmHg, respectively; and (6) not receiving β -blockers or non-dihydropyridine calcium
97 channel antagonists. The study's protocol was conducted in accordance with the Declaration of
98 Helsinki, registered with the Brazilian Clinical Trials (<http://www.ensaiosclnicos.gov.br>, RBR-
99 3pq58k), and approved by the Joint Committee on Ethics of Human Research of the institution

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

102

103 **2.2. Procedures**

104 Figure 1 shows the timeframe of the four visits to the laboratory completed by participants
105 during study. During the first visit, they were interviewed, and ankle brachial index, anthropometric
106 and resting BP measures were taken. The interview identified age, the presence of cardiovascular
107 disease and risk factors, comorbid conditions, and current medication treatment. Ankle brachial
108 index was evaluated as previously described²³, and anthropometric measures of body mass and
109 height (Welmy, 110, São Paulo, Brazil) were used to calculate body mass index (BMI). Auscultatory
110 BP was measured in triplicate using a mercury column sphygmomanometer (Unitec, São Paulo,
111 Brazil). Measures were taken after 5 min of rest in the seated position. During the second visit,
112 resting BP was measured again and the mean of the 6 measures (visit 1 and 2) was defined as each
113 participant's resting BP. For the third and fourth visits (test and retest sessions), HR, BP, HRV, BF
114 and vasodilatory response were measured 8-12 days apart and assessed for reproducibility.

115

116 ***INSERT FIGURE 1***

117

118 **2.3. Experimental protocol**

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

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

129

130 **2.4. Measurements**

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

136 For cardiac autonomic evaluation, R-R intervals from the ECG and respiratory signal from a
137 thoracic piezoelectric belt (UFI, Pneumotrace2, California, USA) were inputted into a data
138 acquisition system (WinDaq, DI-720, Akron, USA) at a sampling rate of 500 Hz/channel. Stationary
139 segments (250 – 300 beats) of the HR series were assessed for HRV via spectral analysis (Heart
140 Scope, version 1.3.0.1, New York, USA), employing an autoregressive method in accordance with
141 Task Force recommendations²⁴. Low-frequency (LF_{RR}) (0.04 – 0.15 Hz) and high-frequency (HF_{RR})
142 (0.15 – 0.4 Hz) components of HRV were expressed in normalized units (nu) with these representing
143 the relative contribution of each component within the total power spectrum minus the very low
144 frequency component²⁴. Total variance (TV_{R-R}) (0 – 0.4 Hz) and LF/HF ratio were also calculated.

145 Upper and lower limb BF were simultaneously determined in the dominant forearm and the
146 leg with the lowest ankle brachial index. BF was assessed by venous occlusion plethysmography
147 (Hokanson, AI6, Bellevue, USA), as previously described²⁵. Briefly, BF to the hand and the foot
148 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 \pm

174 95% limits of agreement (LOA) as previously described by Bland & Altman ²⁹. For the logarithm-
175 transformed data, the ratio bias \ast/\div 95% LOA was calculated as previously recommended ³⁰.

176

177 3. RESULTS

178

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

183

184 ***INSERT TABLE I***

185

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

191

192 ***INSERT TABLE II***

193

194 Mean values of all HRV variables were not significantly different between test and retest ($P >$
195 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
196 (ln TV_{R-R}) to 93.8% (LF/HF). For each variable, the TE and mean/ratio bias were small while the
197 LOA varied (Table III).

198

199 ***INSERT TABLE III***

200

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

208

209 ***INSERT TABLE IV***

210

211 **4. DISCUSSION**

212

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

219

220 *4.1. Systemic hemodynamic variables*

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

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

230

231 4.2. Cardiac autonomic modulation

232 HRV has been used as a non-invasive method to quantify parasympathetic and sympathetic
233 modulations to the heart²⁴. HRV alterations, mainly characterized by decreased TV_{R-R} and HF_{R-R} ,
234 and increased LF_{R-R} and LF/HF, have been reported in patients with PAD and IC compared to
235 healthy adults¹⁰ with this reduce HRV associated with greater mortality³⁴. However, to the best of
236 our knowledge, the current study has been the first to verify the reproducibility of resting HRV in
237 patients with IC. It is well known that reproducibility of HRV is population-specific with lower
238 reproducibility generally obtained in clinical populations compared to healthy adults^{20, 35-37}.
239 However, the present results showed excellent reproducibility (ICC > 0.90 and CV < 9%) for almost
240 all indices, except for LF/HF. Previous studies of patients with a history of myocardial infarction
241 (ICC: 0.77 – 0.81)³⁸ and type 2 diabetes mellitus (ICC: 0.58 – 0.71)¹⁹ reported poor reliability for
242 the HRV indices. In addition, the current study demonstrated good within-individual variation for
243 TV_{R-R} , LF_{R-R} and HF_{R-R} (CV < 9.0%). Previous studies have reported greater variation for these
244 variables, even in healthy adults (CV: 20.1 – 23.0%)³⁹. Differences for the within-individual
245 variation of HRV may reflect differences in resting HRV levels with healthy individuals exhibiting
246 greater HRV compared to those with chronic diseases^{20, 35-37}. Regardless, the current study has
247 demonstrated excellent reproducibility (i.e. excellent ICC and desirable CV) for some common HRV
248 measures used for patients with IC. On the other hand, the LF/HF exhibited a high CV (93.8%) that

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

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

263

264 *4.3. Peripheral hemodynamic variables*

265 Patients with IC have varying degrees of arterial occlusion that consequently result in several
266 physical limitations due to reduced BF in the limbs¹⁻³. Assessment of BF via strain gauge
267 plethysmography has been considered the gold standard method for measure distal limb BF⁴¹ with a
268 previous study reporting similar reliability (ICC > 0.90) to that of the current study for baseline
269 forearm and calf BFs in healthy adults⁴². Additionally, within-individual variations for baseline
270 forearm (8.2%) and calf BF (18.0%) were similar to those previously reported in healthy individuals
271^{42, 43}. Others^{15, 44} though have reported higher CV for these variables with differences between
272 studies likely a result of different populations and experimental session conditions, such as
273 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 study¹⁵.

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 al⁴⁵ and Thijssen et al¹⁵
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 population⁴⁹.

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

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

325

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328

329 **The authors declare no conflicts of interest to disclose**

330

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

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

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

	Test	Retest	P value	ICC	TE	CV %	Bias \pm or $*/\div$ 95% LOA
$\ln TV_{R-R}$ (ms ²)	6.8 \pm 1.0	6.9 \pm 0.9	0.459	0.929	0.3	4.9	0.9 $*/\div$ 2.6
LF _{R-R} (nu)	47.8 \pm 19.9	47.5 \pm 18.8	0.831	0.979	4.0	8.3	0.3 \pm 11.0
HF _{R-R} (nu)	44.7 \pm 20.7	45.5 \pm 19.4	0.535	0.986	3.3	7.3	-0.7 \pm 9.1
$\ln LF/HF$	0.18 \pm 0.87	0.17 \pm 0.88	0.737	0.982	0.16	93.8	1.0 $*/\div$ 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 IV. Mean \pm SD values and reproducibility forearm and calf blood flows and vasodilatory response variables

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

