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Complete List of Authors:	Fecchio, Rafael; University of São Paulo, Exercise Hemodynamic Laboratory, School of Physical Education and Sport Chehuen, Marcel; University of São Paulo, Exercise Hemodynamic Laboratory, School of Physical Education and Sport Oliveira, Tiago; University of São Paulo, Exercise Hemodynamic Laboratory, School of Physical Education and Sport Cucato, Gabriel; Hospital Israelita Albert Einstein, Israel Institute of Education and Research Albert Einsten Costa, Luiz ; University of São Paulo, School of Physical Education and Sport Leicht, Anthony; James Cook University, Sport and Exercise Science Ritti Dias, Raphael; Hospital Israelita Albert Einstein, IIEP Wolosker, Nelson; Albert Einstein Israelite Hospital, Israel Institute of Education and Research Albert Einsten Forjaz, Cláudia; University of Sao Paulo, Exercise Hemodynamic Laboratory, School of Physical Education and Sport
Key Words:	peripheral arterial disease, exercise, autonomic function, reliability, agreement

REPRODUCIBILITY OF HEART RATE RECOVERY IN PATIENTS WITH INTERMITTENT CLAUDICATION

Short title: heart rate recovery reproducibility in intermittent claudication.

Rafael Fecchio[1], Marcel Chehuen[1], Tiago Peçanha[1], Gabriel Grizzo Cucato[1,2], Luiz Augusto Riani Costa[1], Anthony Scott Leicht[3], Raphael Mendes Ritti-Dias[2], Nelson Wolosker[2], Cláudia Lúcia de Moraes Forjaz[1].

[1] School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil; cforjaz@usp.br

[2] Albert Einstein Israelite Hospital, Brazil; raphaelritti@gmail.com

[3] College of Healthcare Sciences, James Cook University, Townsville, Australia; anthony.leicht@jcu.edu.au

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Address for correspondence:

Cláudia Lúcia de Moraes Forjaz

Av. Prof. Mello Moraes, 65, Butantã, São Paulo/SP - 05508-030 - Brazil

Phone: +55+11 30913136; FAX: +55+11 30913136 Email: cforjaz@usp.br

Background. Post-exercise heart rate recovery (HRR) is a non-invasive tool for cardiac autonomic function assessment. Reproducibility of HRR has been established in healthy subjects; however, no study has evaluated this reproducibility in clinical populations who may present autonomic dysfunction. Patients with peripheral artery disease and intermittent claudication (IC) often present altered cardiac autonomic function and HRR could be an interesting tool for evaluating autonomic responses to interventions in this population. Therefore, the reproducibility of HRR should be determined in this specific population.

Objective. To determine the reproducibility of HRR indices in patients with IC.

Methods. Nineteen men with IC underwent two repeated maximal treadmill tests. Raw HR and relative HRR (difference to exercise peak) indices measured at 30, 60, 120, 180, 240 and 300s of recovery were evaluated. The presence of systematic bias was assessed by comparing test and retest mean values via paired *t*-test. Reliability was assessed by intraclass correlation coefficient (ICC), and agreement by typical error (TE), coefficient of variation (CV) and minimal detectable difference (MDD).

Results. There were no significant differences between the test and retest values of all raw HR and relative HRR indices ($p \geq 0.05$), except for HR120s ($p=0.032$). All indices exhibited excellent reliability ($ICC \geq 0.78$). Raw HR and relative HRR indices showed $TE \leq 6.4$ bpm and $MDDs \leq 17.8$ bpm. In addition, all indices showed CVs $\leq 13.2\%$, except HRR30s ($CV=45.6\%$).

Conclusions. The current results demonstrated that most HRR indices were highly reproducible with no systematic error, excellent reliability and good agreement in patients with IC following maximal graded exercise.

Keywords: peripheral arterial disease, exercise, autonomic function, reliability, agreement.

INTRODUCTION

Immediately after exercise, heart rate (HR) exponentially decays towards its pre-exercise levels with this response mediated by the autonomic nervous system (Perini et al., 1989; Imai et al., 1994; Coote, 2010; Peçanha et al., 2014). This decay represents a normal response to return the body to resting homeostatic function (Luttrell & Halliwill, 2015). The kinetics of the post-exercise heart rate recovery (HRR) reveals two phases with different physiological meanings. The first phase consists of a rapid reduction of post-exercise HR that is primarily determined by parasympathetic reactivation (Imai et al., 1994), while the second phase consists of a slow reduction that is predominantly determined by sympathetic withdrawal (Perini et al., 1989). For this reason, HRR is used as a non-invasive tool to evaluate the integrity of the cardiac autonomic nervous system.

HRR is reduced in several clinical conditions, such as cardiovascular and pulmonary diseases (Morshedi-Meibodi et al., 2002; Racine et al., 2003; Lipinski et al., 2004; Erdogan et al., 2011; Gupta et al., 2013), autoimmune diseases (Dogdu et al., 2010; Akgul et al., 2011), neurodegenerative disorders (Kanegusuku et al., 2016), among others. Despite these well-reported reduced HRR in clinical populations, there is scarce information about the reproducibility of HRR. To the best of our knowledge, studies assessing HRR reproducibility have been limited to healthy populations (Yawn et al., 2003; Bosquet et al., 2008; Al Haddad et al., 2011; Arduini et al., 2011; Tulumen et al., 2011; Dupuy et al., 2012; Boullosa et al., 2014). As lower HRR has been associated with greater overall (Cole et al., 1999; Mora et al., 2003) and cardiovascular (Mora et al., 2003) mortality rates, a greater understating of the reproducibility of HRR for clinical populations

is vital. This knowledge is fundamental for research and in clinical settings, since the level of reproducibility of HRR indices will affect their capacity to predict mortality and to detect real effects of interventions (Atkinson & Nevill, 1998).

Peripheral artery disease (PAD) is a clinical condition characterized by a narrowing of the arteries of the lower limbs that results in ischemia and pain in the leg during walking that releases with rest, known as intermittent claudication (IC) (Hirsch et al., 2006; Kröger et al., 2006; Makdisse et al., 2008). Patients with IC often present altered cardiac autonomic function (Goernig et al., 2008; Lima et al., 2016) characterized by increased cardiac sympathetic and decreased cardiac parasympathetic modulations (Goernig et al., 2008). This impaired function might contribute to the high cardiovascular risk in this population (Imai et al., 1994). In addition, a previous study (Mahé et al., 2011) showed an inverse relationship between exercise-induced lower-limb ischemia and HRR. Therefore, HRR could be an interesting tool for these patients.

Based on previous background, the current study was designed to determine the reproducibility of different indices of HRR in patients with IC.

METHODS

Participants

Patients with IC registered at a tertiary center specialized in vascular disease participated in this study. Due to the differences in the exercise responses between males and females, and the possible influence of menstrual cycle phase on the reproducibility of the physiological responses to exercise, which imposes a special study design for

evaluating women, the current study only included men. All patients met the following criteria: 1) age ≥ 50 years; 2) diagnosed with Fontaine stage II of PAD; 3) ankle brachial index at rest ≤ 0.90 in at least one lower limb; 4) capacity to walk on a treadmill at 2.0 mph for at least two minutes; 5) resting systolic and diastolic blood pressures lower than 160/105 mmHg, respectively; 6) absence of revascularization surgery or angioplasty in the previous year; and 7) not taking β-blockers or non-dihydropyridine calcium channel antagonists. The exclusion criteria were: 1) walking capacity limited by factors other than claudication (e.g. orthopedic limitations, neurological impairment or dyspnea); 2) electrocardiographic abnormalities suggestive of myocardial ischemia or arrhythmia during a graded maximal treadmill test; and 3) presence of diabetes with clinic cardiovascular autonomic neuropathy.

The study's protocol was registered at the Brazilian Clinical Trials (http://www.ensaiosclinicos.gov.br, RBR-7m3d8w), was approved by the Joint Committee on Ethics of Human Research of the School of Physical Education and Sport at the University of São Paulo (process 2008/55), and written informed consent was obtained from the patients.

Procedures

Initially, the patients underwent a preliminary visit comprising of an interview and physical examination for the collection of patients' characteristics, such as age, height, mass, body mass index, presence of cardiovascular risk factors, comorbidities conditions and current medications. In addition, all patients underwent, at least, two practice graded treadmill tests before the formal collection period. This practice procedure was essential to

ensure optimal performances as assessment of reproducibility has been shown to be influenced by the degree of familiarization with the exercise testing procedures (Gardner et al., 1991).

After familiarization, all patients underwent two repeated maximal cardiopulmonary treadmill tests performed on different occasions with an interval of at least 3 weeks between them. Both test and retest were conducted by the same research team, at the same time of day, and in a laboratory with controlled temperature (20 to 22^{o}C). Patients were instructed to maintain the same daily habits before all repeated tests as follow: having a light meal 2 hours before the tests, not smoking nor ingesting caffeine for at least 12 hours, and avoiding intense physical efforts in the previous 24 hours before tests.

Maximal Treadmill Exercise Test

The graded maximal treadmill exercise tests followed a protocol previously utilized for patients with IC (Gardner et al., 1991). Treadmill speed was maintained at 2.0 mph with grade commencing at 0% and increased by 2% every 2 minutes until patients were unable to walk because of leg pain (i.e. maximal claudication pain). Immediately after this exercise phase, patients lay down and remained resting in supine position during 5 minutes of recovery. HR was continuously assessed, and recorded by electrocardiography (Cardio Perfect MD, Netherlands) at rest, during exercise and in the recovery phase. Blood pressure was also assessed at rest and every two minutes during the exercise. Peak HR was determined as the highest value achieved during the exercise phase.

Heart rate recovery analysis

HRR was expressed by both raw and relative indices. Raw HR indices were defined as HR values measured at 30 (HR30s), 60 (HR60s), 120 (HR120s), 180 (HR180s), 240 (HR240s) and 300s (HR300s) of recovery. Relative HRR indices were defined as the absolute differences between peak HR and the HR values measured at 30 (HRR30s), 60 (HRR60s), 120 (HRR120s), 180 (HRR180s), 240 (HRR240s) and 300s (HRR300s) of recovery.

Statistical Analyses

Values are expressed as mean ± standard deviation (SD). The normality of all data was checked by the Shapiro-Wilk test, and the presence of heteroscedasticity by the coefficient of correlation between the mean values of the test and retest and the absolute difference between them (Atkinson & Nevill, 1998). The reproducibility of raw HR and relative HRR indices was assessed by the following parameters: 1) presence of systematic bias; 2) reliability; and 3) agreement (Atkinson & Nevill, 1998; de Vet et al., 2006).

The presence of systematic bias was assessed by comparing the test and retest values via a paired *t* test, and considering $p \leq 0.05$ as significant.

Reliability was examined via intraclass coefficient correlation (ICC) with reliability considered poor if ICC was < 0.40, fair to good if ICC was between 0.40 and 0.74, and excellent if ICC was ≥ 0.75 (Rosner, 2011).

As all variables exhibited a normal distribution and an absence of heteroscedasticity, agreement was evaluated by typical error (TE) expressed in beats per minute (bpm), with a small TE representing good agreement (Atkinson & Nevill, 1998; Hopkins, 2000; de Vet et al., 2006). In addition, to improve the comparison between the

different HRR indices (Atkinson & Nevill, 1998), agreement was also expressed by coefficient of variation (CV), which was calculated by the quotient between TE and the mean value of the test and retest values (Hopkins, 2000). Minimal difference detectable (MDD), which is the minimal difference that can be attributed to a real difference between repeated tests and not to random variation, was also derived from TE, using the following formula: $1.96 \times \sqrt{2} \times TE$ (de Vet et al., 2006). Finally, Bland and Altman plots with 95% limits of agreement (LOA) were also calculated and demonstrated as previously described (Bland & Altman, 1986).

RESULTS

Eighty-four patients were invited to participate and 56 agreed and underwent the preliminary procedures. In this phase, two subjects were excluded because of the presence of diabetes with clinic cardiovascular autonomic neuropathy. Thus, 54 subjects performed the maximal cardiopulmonary treadmill test and another five were excluded (3 presented electrocardiographic abnormalities and 2 did not present intermittent claudication symptom). Thus, of the remaining 49 patients, 20 were randomly selected to perform the second maximal cardiopulmonary treadmill test. Lastly, one patient was excluded due to technical problems that precluded data analysis. Therefore, 19 patients with IC formed the sample of this study, and their clinical characteristics are shown in Table 1. There were no significant differences in the test results between the included and non-included patients (data not shown).

In general, patients were elderly (age > 60 years old), with several cardiovascular risk factors and presented with an ankle brachial index between 0.41 and 0.90 (mild to

moderate PAD). Patients were taking different medications, e.g. aspirins, statins, anti-hypertensives and maintained their medication regime throughout this study.

Test duration, total walked distance, raw HR and relative HRR indices obtained in the test and retest, as well as the differences between them are shown in Table 2. There were no significant differences (systematic bias) between test and retest mean values for all variables ($p > 0.05$), except for HR120s ($p = 0.032$).

Reliability and agreement data for HRR are shown in Table 3. The raw HR indices showed ICCs ranging from 0.820 to 0.938, while the relative HRR indices exhibited ICCs between 0.781 and 0.926. Regarding agreement, the raw HR indices exhibited TEs and MDDs ranging from 5.4 to 6.4 bpm and 14.9 to 17.8 bpm, while the relative HRR indices presented TEs and MDDs ranging from 3.0 to 4.0 bpm and 8.2 to 11.1 bpm. When agreement was expressed by CV, the raw HR indices presented CVs ≤ 7.2 %, while relative HRR indices presented CVs ≤ 13.2, with the exception of HRR30s (CV = 45.6 %).

The Bland & Altman's plots for the raw HR and relative HRR indices are shown in Figures 1 and 2, respectively. The visual inspection of Bland-Altman plots reinforces the absence of heteroscedasticity and systematic bias of HRR indices.

DISCUSSION

The main finding of this study was that most raw HR and relative HRR indices were highly reproducible in patients with IC.

As there were no significant differences between test and retest mean values for all indices of HRR, except HR120s, it is possible to state that, in general, there is no systematic error between test and retest. Similarly, previous studies with healthy subjects

also reported absence of systematic bias in raw HR (Bosquet et al., 2008; Tulumen et al., 2011; Boullosa et al., 2014) and relative HRR (Bosquet et al., 2008; Al Haddad et al., 2011; Arduini et al., 2011; Tulumen et al., 2011; Dupuy et al., 2012; Boullosa et al., 2014) indices.

The current study observed an excellent reliability (ICC ≥ 0.78) for all raw HR and relative HRR indices, suggesting that all indices can be used for distinguishing patients with IC from each other (Atkinson & Nevill, 1998; de Vet et al., 2006). Similarly, the study of Dupuy et al. (2012) also demonstrated an excellent reliability (ICC ≥ 0.80) for both raw HR and relative HRR indices in healthy subjects. Moreover, Boullosa et al. (2014) also showed excellent reliability for all these indices (ICC ≥ 0.78), except for HRR60s.

Regarding agreement, both raw HR and relative HRR indices showed good agreement represented by low TEs ≤ 6.4 bpm. The raw HR indices showed slightly higher TEs (5.4 to 6.4 bpm) than the relative HRR indices (3.0 to 4.0 bpm) that resulted in higher MDDs for the raw HR indices. The TE found for the HRR60s (3.0 bpm) was similar to previously reported by Dupuy et al. (2012) (HRR60s = 4.0 bpm), but is lower than the reported by Bosquet et al. (2008) (HRR60s = 10.3 bpm). Differences in the recovery protocols might explain such divergence as previously recovery consisted of 1 minute in the standing position (Bosquet et al., 2008) that likely resulted in varied absolute changes in HR leading to a greater TE. In contrast the current study employed a supine recovery period that likely resulted in more stable and reliable changes in HR and smaller TE. The determination of these agreement parameters has important implications. TE is employed for estimating sample sizes required in clinical studies, while MDD represents the minimal difference necessary between repeated tests to consider a real change when evaluating

individual responses to interventions (de Vet et al., 2006). Thus, the specific TE and MDD determined here for each HRR index should be considered in research and clinical settings involving HRR evaluation in patients with IC.

When agreement was indicated by CVs (percentage of their respective means), higher CVs were obtained for relative HRR indices (8.6 to 45.6%) than for raw HR indices (4.6 to 7.2%). A similar pattern has been previously reported in healthy subjects (Bosquet et al., 2008; Tulumen et al., 2011; Boullosa et al., 2014) and may reflect a calculation limitation of the measures with the raw HR indices likely to be similar in terms of absolute HR values for the calculation of CV (i.e. numerator and denominator values were similar to each other and hence, smaller CV). In comparison, the HRR indices were calculated from changes in absolute HR with these smaller mean values impacting the calculation of CV to a greater extent. It is important to note that all CVs, except for HRR30s, were acceptable, and can be interpreted as good agreement. The higher CV for HRR30s might be related to the predominance of parasympathetic reactivation in this early phase of recovery (Imai et al., 1994) and variable decline in HR. Similarly, Boullosa et al. (2014) also reported a high CV for HRR30 (22.6%) in healthy individuals with this value approximately half that of the current study. The greater CV for HRR30s in the current study may indicate variable parasympathetic reactivation for patients with IC as a result of their disease and/or progression. Therefore, the use of latter HRR indices may be more reliable and appropriate for this clinical population.

Collectively, the absence of systematic bias, the excellent reliability and the good agreement for most of the raw HR and relative HRR indices demonstrated that HRR was highly reproducible in patients with IC. As high reproducibility of these indices has also

been previously reported in heathy subjects (Buchheit et al., 2008; Arduini et al., 2011; Tulumen et al., 2011; Boullosa et al., 2014), the current results indicate that patients with IC does not present impaired HRR reproducibility.

It was interesting to observe that the HRR indices that are mainly used for clinical evaluation of HRR and cardiovascular risk, HRR60s, HRR120s and HRR300s (Peçanha et al., 2014) showed excellent reliability (ICCs $\geq$ 0.879) and good agreements (TEs $\leq$ 4.0 bpm) in the current study. However, as stated before, some concern is raised regarding the employment of HRR30s in clinical settings and research with patients with IC since it presented high reliability (ICC =0.781) but poor agreement (TE = 3.7, MDD = 10.4 and CV = 45.6). Thus, the present results suggest that HRR60s, HRR120s and HRR300s might be better indices for evaluating HRR in patients with IC.

While the current study identified novel findings for HRR reproducibility in a clinical population, the results should be cautiously. The current study examined patients with IC that were well familiarized with the maximal graded exercise test, which might have contributed to more reproducible results (Gardner et al., 1991). Secondly, the current study only included men, which limits the extrapolation of its results to women who should be studied in the future. Thirdly, the patients were not in use of medications that directly affected HR responses to exercise. Lastly, it is still not known if the present results are applicable to other exercise protocols. Patients with other characteristics, using other medications and submitted to other exercise protocols should be investigated by future studies. However, it is import to highlight that by our knowledge, the current study is the first to document the reproducibility of HRR in patients with IC.

Conclusion

The vast majority of raw HR and relative HRR indices following maximal graded exercise were highly reproducible in male patients with IC. Therefore, these indices may be used in research and clinical settings with this population for assessing differences between individuals and effects of interventions on the integrity of autonomic cardiac function and on cardiovascular risk.

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CONFLICT OF INTEREST

The authors have no conflicts of interest

REFERENCES

Akgul E, Kutuk U, Ertek S, Cesur M, Cehreli S, Tore HF, Erdogan G. Cardiac autonomic function and global left ventricular performance in autoimmune eauthyroid chronic thyroiditis: Is treatment necessary at the euthyroid stage? *Echocardiography* (2011);**28**:15–21.

Al Haddad H, Laursen PB, Chollet D, Ahmaidi S, Buchheit M. Reliability of resting and postexercise heart rate measures. *Int J Sports Med* (2011);**32**:598–605.

Arduini A, Gomez-Cabrera MC, Romagnoli M. Reliability of different models to assess heart rate recovery after submaximal bicycle exercise. *J Sci Med Sport* (2011);**14**:352–7.

Atkinson G, Nevill A. Statistical Methods for Assssing Measurement Error (Reliability) in Variables Relevant to Sports Medicine. *Sports Med* (1998);**26**:217–38.

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

Bosquet L, Camelin FX, Berthoin S. Reliability of postexercise heart rate recovery. *Int J Sports Med* (2008);**29**:238–43.

Boullosa DA, Barros ES, Del Rosso S, Nakamura FY, Leicht AS. Reliability of heart rate measures during walking before and after running maximal efforts. *Int J Sports Med* (2014);**35**:999–1005.

Buchheit M, Millet GP, Parisy A, Pourchez S, Laursen PB, Ahmaidi S. Supramaximal training and postexercise parasympathetic reactivation in adolescents. *Med Sci Sport Exer* (2008);**40**:362–71.

Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-Rate Recovery

Immediately after Exercise as a Predictor of Mortality. *New Engl J Med* (1999);**341**:1351–7.

Coote JH. Recovery of heart rate following intense dynamic exercise. *Exp Physiol* (2010);**95**:431–40.

De Vet HCW, Terwee CB, Knol DL, Bouter LM. When to use agreement versus reliability measures. *J Clin Epidemiol* (2006);**59**:1033–9.

Dogdu O, Yarlioglues M, Kaya MG, Ardic I, Oguzhan N, Akpek M, Sahin O, Akyol L, Kelesoglu S, Koc F, Ozdogru I, Oguzhan A. Deterioration of heart rate recovery index in patients with systemic lupus erythematosus. *J Rheumatol* (2010);**37**:2511–5.

Dupuy O, Mekary S, Berryman N, Bherer L, Audiffren M, Bosquet L. Reliability of heart rate measures used to assess post-exercise parasympathetic reactivation. *Clin Physiol Funct Imaging* (2012);**32**:296–304.

Erdogan D, Gonul E, Icli A, Yucel H, Arslan A, Akcay S, Ozaydin M. Effects of normal blood pressure, prehypertension, and hypertension on autonomic nervous system function. *Int J Cardiol* (2011);**151**:50–3.

Gardner AW, Skinner JS, Cantwell BW, Smith LK. Progressive vs single-stage treadmill tests for evaluation of claudication. *Med Sci Sport Exer* (1991);**23**:402–8.

Goernig M, Schroeder R, Roth T, Truebner S, Palutke I, Figulla HR, Leder U, Voss A. Peripheral arterial disease alters heart rate variability in cardiovascular patients. *PACE* (2008);**31**:858–62.

Gupta M, Bansal V, Chhabra SK. Abnormal heart rate recovery and chronotropic incompetence on exercise in chronic obstructive pulmonary disease. *Chron Respir Dis* (2013);**10**:117–26.

Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WRC, Olin JW, Puschett JB, Rosenfield KA, Sacks D, Stanley JC, Taylor LM, White CJ, White J, White RA, Antman EM, Smith SC, Adams CD, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Halperin JL, Hiratzka LF, Hunt SA, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA 2005 Practice Guidelines for the Management of Patients With Peripheral Arterial Disease (Lower Extremity, Renal, Mesenteric, and Abdominal Aortic). *Circulation* (2006);**113**:e463–5.

Hopkins WG. Measures of reliability in sports medicine and science. *Sports Med* (2000);**30**:1–15.

Imai K, Sato H, Hori M, Kusuoka H, Ozaki H, Yokoyama H, Takeda H, Inoue M, Kamada T. Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J Am Coll Cardiol* (1994);**24**:1529–35.

Kanegusuku H, Silva-Batista C, Peçanha T, Nieuwboer A, Jr.Silva ND, Costa LA, de Mello MT, Piemonte ME, Ugrinowitsch C, Forjaz CL. Blunted Maximal and Submaximal Responses to Cardiopulmonary Exercise Tests in Patients With Parkinson Disease. *Arch Phys Med Rehabil* (2016);**97**:720–5.

Kröger K, Stang A, Kondratieva J, Moebus S, Beck E, Schmermund A, Möhlenkamp S, Dragano N, Siegrist J, Jöckel KH, Erbel R. Prevalence of peripheral arterial disease - Results of the Heinz Nixdorf Recall Study. *Eur J Epidemiol* (2006);**21**:279–85.

Lima AHRA, Soares AHG, Cucato GG, Leicht AS, Franco FGM, Wolosker N, Ritti-Dias RM. Walking Capacity Is Positively Related with Heart Rate Variability in Symptomatic Peripheral Artery Disease. *Eur J Vasc Endovasc* (2016);**52**:82–9.

Lipinski MJ, Vetrovec GW, Froelicher VF. Importance of the first two minutes of heart rate

recovery after exercise treadmill testing in predicting mortality and the presence of coronary artery disease in men. *Am J Cardiol* (2004);**93**:445–9.

Luttrell MJ, Halliwill JR. Recovery from exercise: vulnerable state, window of opportunity, or crystal ball? *Front Physiol* (2015);**6**:204.

Mahé G, Zeenny M, Ouedraogo N, Vielle B, Leftheriotis G, Abraham P. Heart rate recovery after constant-load exercise tests is decreased in proportion to the importance (severity and diffusion) of exercise-induced lower-limb ischaemia. *Clin Physiol Funct Imaging* (2011);**31**:48–53.

Makdisse M, Pereira ADC, Brasil DDP, Borges JL, Machado-Coelho GLL, Krieger JE, Nascimento Neto RM, Chagas ACP, Hearts of Brazil Study and Peripheral Arterial Disease Committee of the Brazilian Society of Cardiology/Funcor, Pereira C, Machado- GLL. Prevalence and risk factors associated with peripheral arterial disease in the Hearts of Brazil Project. *Arq Bras Cardiol* (2008);**91**:370–82.

Mora S, Redberg RF, Cui Y, Whiteman MK, Flaws JA, Sharrett AR, Blumenthal RS. Ability of Exercise Testing to Predict Cardiovascular and All-Cause Death in Asymptomatic Women: A 20-Year Follow-up of the Lipid Research Clinics Prevalence Study. *JAMA* (2003);**290**:1600–7.

Morshedi-Meibodi A, Larson M, Levy D, O'Donnell C, Vasan R. Heart rate recovery after treadmill exercise testing and risk of cardiovascular disease events (The Framingham Heart Study). *Am J Cardiol* (2002);**90**:848–52.

Peçanha T, Silva-Júnior ND, Forjaz CL de M. Heart rate recovery: Autonomic determinants, methods of assessment and association with mortality and cardiovascular diseases. *Clin Physiol Funct Imaging* (2014);**34**:327–39.

Perini R, Orizio C, Comande A, Castellano M, Beschi M, Veicsteinas A. Plasma norepinephrine and heart rate dynamics during recovery from submaximal exercise in man. *Eur J Appl Physiol Occup Physiol* (1989);**58**:879–83.

Racine N, Blanchet M, Ducharme A, Marquis J, Boucher J-M, Juneau M, White M. Decreased heart rate recovery after exercise in patients with congestive heart failure: effect of beta-blocker therapy. *J Card Fail* (2003);**9**:296–302.

Rosner B. Fundamentals of Biostatistics (seventh edition) (2011); **12**: 568-71. Brooks / Cole, Cengage Learning, Boston.

Tulumen E, Khalilayeva I, Aytemir K, Ergun Baris Kaya FESC, Sinan Deveci O, Aksoy H, Kocabas U, Okutucu S, Tokgozoglu L, Kabakci G, Ozkutlu H, Oto A. The reproducibility of heart rate recovery after treadmill exercise test. *Ann Noninvasive Electrocardiol* (2011);**16**:365–72.

Yawn BP, Ammar KA, Thomas R, Wollan PC. Test-retest reproducibility of heart rate recovery after treadmill exercise. *Ann Fam Med* (2003);**1**:236–41.

Table 1. Clinical characteristics of patients with intermittent claudication (n=19).

Characteristic	Value
Age (ys)	64 ± 8
Height (m)	1.68 ± 0.06
Weight (kg)	74.4 ± 11.6
Body mass index (kg/m^2)	26.4 ± 3.6
Ankle brachial index	0.58 ± 0.15
Cardiovascular Risk Factors and Comorbidities Conditions	
Obesity, n (%)	4 (21.1)
Hypertension, n (%)	17 (89.5)
Dyslipidemia, n (%)	18 (94.7)
Diabetes mellitus, n (%)	6 (31.6)
Current smokers, n (%)	7 (36.8)
Heart disease, n (%)	1 (5.3)
Stroke, n (%)	3 (15.8)
History of neoplasm, n (%)	3 (15.8)
Drug therapy	
Aspirin, n (%)	17 (.89.5)
Statin, n (%)	17 (89.5)
Angiotensin-converting enzyme inhibitor or receptor blocker, n (%)	12 (63.2)
Diuretics, n (%)	9 (47.4)
Dihydropyridine calcium channel antagonist, n (%)	6 (31.6)
Oral hypoglycemic, n (%)	5 (26.3)

Continuous variables are expressed as mean ± SD. Obesity defined as body mass index ≥ 30 kg/m^2. Hypertension, dyslipidemia and diabetes mellitus were self-reported by the subjects.

Table 2. Test duration, total walked distance, raw heart rate (HR) and relative heart rate recovery (HRR) indices measured in test and retest treadmill exercise tests in patients with intermittent claudication (n=19).

Variable	Test 1	Test 2	Difference	P-value
Test				
Test duration, s	780 ± 425	793 445	-13 ± 86	0.520
Total walked distance, m	656 ± 318	665 ± 338	-8 ± 84	0.666
Raw HR indices, bpm				
Peak HR	126.4 ± 14.2	122.5 ± 13.1	3.9 ± 8.3	0.056
HR30s	117.8 ± 15.9	114.6 ± 15.6	3.2 ± 7.6	0.083
HR60s	104.0 ±12.7	100.1 ± 13.1	3.9 ± 8.4	0.057
HR120s	93.9 ± 13.4	89.1 ± 13.9	4.8 ± 9.1	0.032*
HR180s[a]	90.3 ± 10.9	87.8 ± 12.0	2.5 ± 9.0	0.253
HR240s	88.8 ± 11.9	85.2 ± 11.2	3.6 ± 8.8	0.089
HR300s	86.6 ± 11.5	83.7 ± 10.3	2.9 ± 7.6	0.114
Relative HRR indices, bpm				
HRR30s	8.5 ± 6.8	7.8 ± 5.6	0.7 ± 5.3	0.579
HRR60s	22.4 ± 7.1	22.4 ± 7.8	0.0 ± 4.2	1.000
HRR120s	32.5 ± 7.9	33.4 ± 9.3	-0.9 ± 5.7	0.476
HRR180s[a]	36.2 ± 9.8	34.9 ± 8.1	1.2 ± 4.7	0.289
HRR240s	37.5 ± 9.0	37.3 ± 9.0	0.3 ± 5.6	0.839
HRR300s	39.8 ± 9.3	38.8 ± 8.8	1.0 ± 4.8	0.374

Values are mean ± SD. HR = heart rate; HRR = heart rate recovery. [a]n=18, due to technical issues. *$p < 0.05$

Table 3. Reproducibility data of raw heart rate (HR) and relative heart rate recovery (HRR) indices after treadmill exercise tests in patients with intermittent claudication (n=19).

Variable	ICC	TE (bpm)	CV (%)	MDD (bpm)
Raw HR indices				
Peak HR	0.898	5.9	4.7	16.3
HR30s	0.938	5.4	4.6	14.9
HR60s	0.883	5.9	5.8	16.4
HR120s	0.875	6.4	7.0	17.8
HR180s[a]	0.820	6.3	7.1	17.6
HR240s	0.831	6.2	7.2	17.3
HR300s	0.853	5.4	6.3	14.9
Relative HRR indices				
HRR30s	0.781	3.7	45.6	10.4
HRR60s	0.915	3.0	13.2	8.2
HRR120s	0.879	4.0	12.2	11.1
HRR180s[a]	0.926	3.3	9.4	9.3
HRR240s	0.831	3.9	10.5	10.9
HRR300s	0.925	3.4	8.6	9.4

Values are mean ± SD. ICC = intraclass correlation coefficient; TE = typical error; MDD = minimal detectable difference; CV = coefficient of variation. [a]n=18, due to technical issues

Figure Legends

Figure 1 – Bland & Altman plots (systematic bias ± limits of agreement) for individual values of raw heart rate (HR) indices measured in test and retest treadmill exercise in patients with intermittent claudication.

Figure 2 - Bland & Altman plots (systematic bias ± limits of agreement) for individual values of relative heart rate recovery (HRR) indices measured in test and retest treadmill exercise in patients with intermittent claudication.

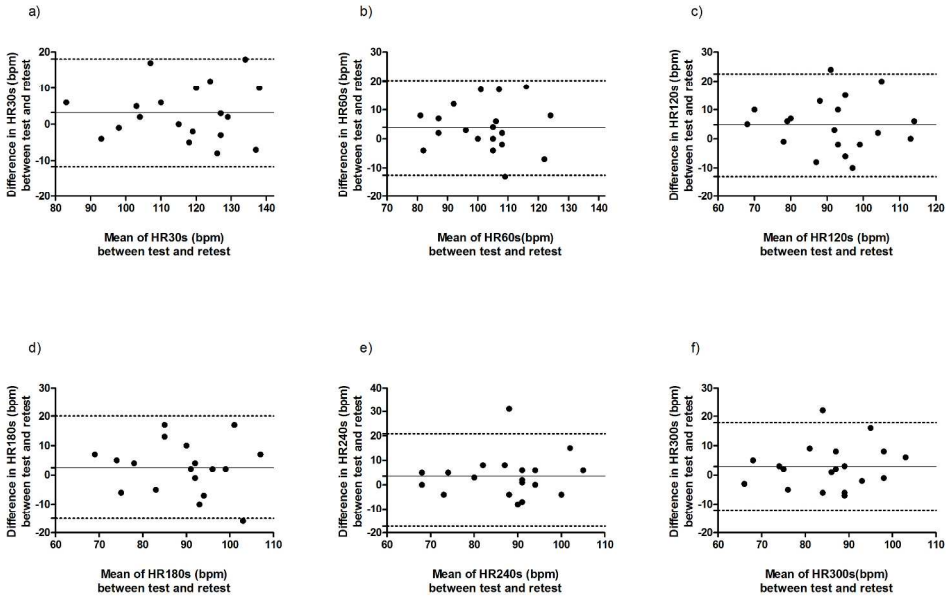

Figure 1. Bland & Altman plots (systematic bias ± limits of agreement) for individual values of raw heart rate (HR) indices measured in test and retest treadmill exercise in patients with intermittent claudication.

268x171mm (300 x 300 DPI)

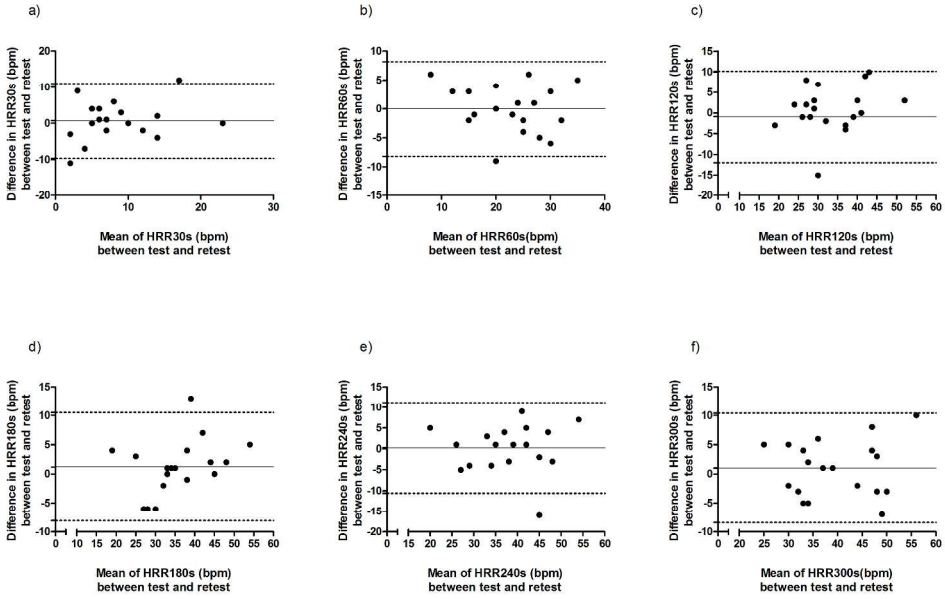

Figure 2. Bland & Altman plots (systematic bias ± limits of agreement) for individual values of relative heart rate recovery (HRR) indices measured in test and retest treadmill exercise in patients with intermittent claudication.

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