Reliability of autonomic and vascular components of baroreflex sensitivity in adolescents

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ABSTRACT

Improvements in the autonomic and vascular systems are implicated in cardiovascular disease risk reduction. Baroreflex sensitivity (BRS) is composed of vascular and autonomic components. This study aimed to investigate between- and within-day reliability of BRS and its autonomic and vascular determinants in adolescents. Thirteen male adolescents (14.1 ±0.5 y) participated in this study. For between-day reliability, participants completed four experimental visits separated by a minimum of 48-h. For within-day reliability, participants repeated BRS assessments three times in the morning with one hour between the measures. BRS was evaluated using the cross-spectral gain (LFgain) between blood pressure and heart rate interval. BRS was further divided into: 1) vascular component using arterial compliance (AC); and 2) autonomic component measured as LFgain divided by AC (LFgain/AC). LFgain, AC, and LFgain/AC presented between-day coefficient of variation (CV) of 20, 17, and 20%, respectively. Similarly, variables associated with blood pressure control such as, cardiac output, mean arterial pressure, heart rate and total peripheral resistance presented CVs ranging from 6 to 15%. Within-day reliability was poorer compared to between-day for LFgain (25%), AC (27%), and LFgain/AC (34%), as well as all hemodynamic variables (CVs from 11-22%, except heart rate with presented CV of 6%). The present study indicates suitable between- and within-reliability of BRS and its autonomic and vascular determinants, as well as hemodynamic variables associated with BRS, in adolescents.

Keywords: youth, cardiovascular disease, blood pressure, between-day, within-day

INTRODUCTION

Atherosclerosis starts in childhood and traditional cardiovascular disease (CVD) risk factors in this age group are associated with atherosclerotic progression in adolescence (Berenson, *et al.* 1998) and adulthood (Raitakari, *et al.* 2003). Improvements in traditional CVD risk factors following an intervention such as exercise, however, only partially explains CVD risk reduction with the existence of ~40% risk factor gap (Joyner, *et al.* 2009). The American Heart Association recognizes that exploring novel CVD risk factors in youth will further contribute to the pathophysiological understanding and CVD management in this population (Balagopal, *et al.* 2011). As improvements in autonomic and vascular functions were found following an exercise intervention with no changes in traditional CVD risk factors in adolescents (Bond, *et al.* 2015), this highlights the importance of these systems as a target for interventions designed to modify CVD risk.

The interplay between the vascular and autonomic systems can be assessed by measuring baroreflex sensitivity (BRS). BRS is the ability to regulate blood pressure (BP) and can be non-invasively assessed using spectral methods (Persson, *et al.* 2001). Specifically, oscillations in BP at a low frequency (0.05-0.15 Hz) are known to cause oscillations in inter-beat intervals (i.e. RR intervals) in the same frequency (Robbe, *et al.* 1987). In this scenario, BRS is the gain of the cross-spectrum (LFgain) between blood pressure and RR intervals expressed in $ms \cdot mmHg^{-1}$. Using common carotid (CCA) ultrasound images, BRS gain can be further divided into its vascular and autonomic components (Taylor, *et al.* 2014; Tzeng 2012). The underlying theory is that carotid distensibility is a surrogate of arterial wall stretching and baroreceptors stimuli (Bonyhay, *et al.* 1996; Hunt, *et al.* 2001). It is then possible to quantify and express changes in CCA diameter per unit of

pressure (i.e. vascular determinant in $\mu m \cdot mmHg^{-1}$), and changes in RR per unit of CCA diameter (i.e. autonomic determinant in $ms \cdot \mu m^{-1}$).

Separating the determinants of BRS can provide non-invasive mechanistic insight of physiological changes in the vascular and autonomic systems in children and adolescents. For instance, it has been suggested that throughout adolescence, the LFgain is maintained via improvements in the autonomic branch (Lenard*, et al.* 2004). While this study provided valuable insights on the maturation of vascular and autonomic systems, there is a dearth of information about test-retest reliability of BRS and its autonomic and vascular determinants. This lack of information is problematic, as reliability is necessary in informing sample size calculations and in the interpretation of results of interventions designed to modify CVD risk. In children, LFgain has been shown to have substantial absolute (i.e. coefficient of variation – CV <20%) and relative (i.e. intraclass coefficient of correlation (ICC) between 0.6-0.8) between-day reliability (Dietrich*, et al.* 2010). Less is known about within-day reliability, with one study including participants with a large age range (7-27 years old) showing a CV of 21.1% (Rudiger*, et al.* 2001). However, mixing adults and children in the same sample can limit the findings due to the known differences in BRS components between the groups (Lenard*, et al.* 2004). Additionally, no study has investigated the relative and absolute reliability of the autonomic and vascular BRS components in youth.

The aim of this study was to assess between- and within-day reliability of BRS and its autonomic and vascular determinants in adolescents. In addition, as BRS ultimately regulates BP via changes in cardiac output ($\dot{Q}$; the product of heart rate (HR and stroke volume (SV)), mean arterial pressure (MAP) and total peripheral resistance (TPR), the

within- and between-day reliability of these hemodynamic outcomes will also be investigated.

METHODS

Participants

Thirteen male adolescents (14.0±0.5 years old) volunteered to take part in this study Participants, with assistance from their parents/guardians, completed a health questionnaire before participation and were free of conditions, such as asthma, congenital heart disease, hypertension, amongst others that could alter autonomic and vascular functions. All procedures conducted in the present investigation were approved by institutional Ethics Committee and assent and consent forms were obtained from adolescents and their parents/guardians, respectively. Two weeks before starting the experimental visits, participants were then familiarized to the BRS protocol. In this same visit, participants had their stature, body mass, skinfolds (to estimate body composition) and maximum oxygen uptake (VO_2max) measured. VO_2max was obtained and verified breath-by-breath (Cortex Metalyzer III B, Leipzig, Germany) using a combined incremental-supramaximal treadmill protocol (Barker*, et al.* 2014). Pubertal status for the sample was determined by self-assessment of secondary sexual characteristics (Morris*, et al.* 1980).

Experimental Design

To establish between-day reliability, participants completed four experimental visits separated by a minimum of 48-h, and with no longer than 2 weeks in between. For each visit, participants were driven to the laboratory following a 12-h overnight fast, and all measurements were performed between 8 and 9 am. For within-day reliability, in one of the four visits participants were randomly asked to complete the BRS protocol three times

with a one-hour interval in each measurement. Participants were instructed to avoid extraneous exercise and to wear accelerometers (GeneActive, UK) in the 48-h preceding testing. Accelerometer data were treated using freely available spreadsheets (www.geneactive.org), using 60 seconds epoch. Moderate-to-vigorous physical activity (MVPA) was obtained using population specific cut-offs (Phillips*, et al.* 2013). Participants were also asked to complete food diaries in the 48-h before reporting to the laboratory. From food diaries, total kilocalories and relative contribution from lipids, carbohydrate and protein were analysed (CompEat Pro, Nutrition Systems). Additionally, in the 48-h preceding visits 2-4 participants were instructed under parental supervision to keep a similar diet to the 48-h preceding visit 1.

Baroreflex sensitivity protocol

A finger pressure device (Finometer PRO, Netherlands) and a three-led ECG were fitted and the BRS protocol started after a 10-min supine rest in a temperature (21-24°C) and light controlled room. The BRS protocol consisted of: 1) measurement of brachial BP to calibrate Finometer BP assessment (Guelen*, et al.* 2008), which has been validated in paediatric groups (Tanaka*, et al.* 1994); 2) after BP calibration, CCA ultrasound images were recorded for 15 cardiac cycles; and 3) following CCA images, participants were instructed to pace breathing frequency at 12 cycles per minute for 5-min. This breathing frequency is known to increase autonomic modulation of heart rate in adolescents (Williams*, et al.* 2002), and also shifts breathing frequency above the LF range, as suggested when examining spontaneous BRS (Bothova*, et al.* 2010; Tzeng*, et al.* 2009). This 5-min period was used to calculate LFgain. All BRS measurement procedures were completed within ~ 20 min.

Baroreflex sensitivity analysis

BRS analysis procedures in the present study were performed accordingly to previous validated methods (Chirico*, et al.* 2015; Lenard*, et al.* 2004; Robbe*, et al.* 1987; Saul*, et al.* 1991). ECG and BP were recorded simultaneously at 1000 Hz (PowerLab, ADInstruments). RR intervals and systolic blood pressure (SBP) data were extracted and saved for later analysis. Ectopic beats were automatically identified and linear interpolation with a low filter was applied when <3% error was present (Kubios v3.0) (Tarvainen*, et al.* 2014). SBP trace was visually checked and errors manually replaced by linear interpolation using adjacent SBP. Integrated gain (LFgain) of BRS was determined from the final five minutes of the BRS protocol. For this purpose, beat-to-beat RR interval and brachial reconstructed SBP were interpolated at 2 Hz and a Fast-Fourier Transformation was applied to obtain the power spectrum in the low frequency (LF: 0.04-0.15 Hz) band. A cross-spectral transfer function was then applied and the mean cross-spectrum (LFgain) in the range where the coherence was > 0.5 was expressed as the baroreflex gain in ms·mmHg^{-1}. This index was chosen due to its established validity compared to BRS assessment using vasoactive drugs (Di Rienzo*, et al.* 2001; Persson*, et al.* 2001).

Vascular and autonomic determinants

CCA images were recorded ~ 2 cm distal from the carotid bulb using a high-resolution (~ 13 MHz) linear array transducer (Apogee, 1000, SIUI, China). The images were obtained over 15 cardiac cycles recorded at 15 frames per second. Subsequently CCA images were analysed using validated wall tracking software (Carotid Analyzer - Medical Imaging Applications LLC) (Mancini*, et al.* 2004) for determination of diastolic lumen diameter (DLD) and systolic lumen diameter (SLD). The average of 3-7 cardiac cycles with clear definitions of the near and far walls were used. During the 15 cardiac cycles, beat-to-beat

brachial reconstructed BP (Guelen*, et al.* 2008) was averaged and used to determine pulse pressure (PP). The vascular components of BRS were determined according to previously published literature as follows (Laurent*, et al.* 2006):

$$\text{Arterial strain (\%)} = \Delta D/DLD$$

Where ΔD is SLD minus DLD;

$$\text{Arterial compliance – AC } (\mu m \cdot mmHg^{-1}) = \Delta D/PP$$

Where PP is the obtained pulse pressure;

$$\text{Arterial distensibility – AD } (mmHg^{-1} \times 10^{-3}) = \Delta CSA/PP \cdot CSAmin$$

Where CSA in the cross sectional CCA artery calculated as CSA = $\pi r2$ being r = diameter/2 and ΔCSA the maximal CSA minus minimal CSA (CSAmin).

During the BRS protocol, beat-to-beat $\dot{Q}$ was obtained from the Finometer and SV was calculated as $\dot{Q}$ divided by the HR from the ECG trace. TPR was calculated as MAP divided by $\dot{Q}$. Hemodynamic variables ($\dot{Q}$, HR, SV, MAP and TPR) were averaged over the same 15 cardiac cycles used for analysis of the CCA outcomes and saved for later analysis.

The autonomic and vascular determinants of BRS were determined according to previous study (Lenard*, et al.* 2004). Briefly, AC was considered as the vascular component of the BRS and expressed as $\mu m \cdot mmHg^{-1}$. To calculate the autonomic determinant, LFgain was divided by the AC and expressed as LFgain/AC in $ms \cdot \mu m^{-1}$.

Statistical analyses

Data are presented as mean and standard deviation (SD) unless otherwise stated. Differences between MVPA and food diary outcomes were compared using ANOVA with repeated measures. Sphericity was tested using Mauchly's test and when violated

Greenhouse-Geisser correction was applied. SPSS version 22 was used for analyses, and an alpha level of 0.05 was considered significant.

Following recommendations by Hopkins (2000), between- and within-day reliability were calculated as: 1) systematic error as changes in mean and tested using repeated measures ANOVA with least significance differences post hoc comparisons; 2) absolute reliability assessed as random error calculated as the within-subject variation expressed in absolute (typical error (TE)) and standardised (%CV) units; and 3) relative reliability calculated as test-retest correlation using Pearson's correlation. Data were log transformed and analysed using freely available spreadsheets (http://sportsci.org/resource/stats/).

RESULTS

Between-day reliability

Participant characteristics are presented in Table 1. From the 13 initial participants, two were not included in the CCA analysis due to technical issues with the ultrasound, and another did not complete one of the visits, for reasons unrelated to the study. The final number of participants included was 10. For the BRS measures, in addition to the participant excluded for not completing the visit, another was excluded due to errors being >3% in the ECG data. The number of participants included in the BRS between-day reliability was therefore 11.

Physical activity and diet records are presented in Table 2. There were no differences for MVPA in the 48-h preceding the experimental visits. For this analysis, however, just 7 participants had repeated data in the 4 visits. Similarly, energy intake and the proportion of the energy derived from carbohydrate, lipid and protein were not different between visits (all $P>0.05$).

Between-day reliability data are described in Table 3. There was no significant mean bias between the visits for any of the variables (all $P>0.05$). All variables had an absolute reliability between 2-20%, with the most reliable measurement being vessel diameter (DLD 2.4% and SLD 2.3%). All variables presented a relative reliability ranging between $r=0.50$ and $r=0.91$, except for PP ($r=0.37$).

Within-day reliability

Participant characteristics are presented in Table 1. From the 13 participants, one participant was excluded from the CCA analysis due to technical issues with the ultrasound. One participant excluded from BRS due to errors >3% in the ECG trace. The number of participants included in the within-day reliability was 12 (Table 1).

Within-day reliability statistics are presented in Table 4. Systematic error was identified for DLD ($P=0.02$) and LSD ($P=0.04$) at 120-min post compared to baseline. Similarly, LFgain was higher at 120-min compared to 60-min ($P=0.03$). All variables had an absolute reliability between 2-34%, with the most reliable measures being vessel diameters (DLD 2.3% and SLD 2.2%) and HR (CV of 6%). All variables presented relative reliability ranging between $r=0.50$ and $r=0.89$, except for MAP ($r=0.42$).

DISCUSSION

This is the first study to investigate between- and within-day reliability of BRS assessment and its autonomic and vascular determinants, as well as the reliability of hemodynamic variables associated with BRS, in adolescents. The key findings of the present investigation were: 1) BRS and its autonomic and vascular determinants presented between-day CVs <20%; 2) vessel diameter presented the best between- and within-day reliability; 3) within-day BRS reliability was poorer compared to between-days; and 4) hemodynamic variables presented between- and within-day CVs <20%.

Between-day reliability

No between-days systematic error was observed for BRS and its autonomic and vascular components. In the present study, participants completed a habituation to the protocol in the weeks before the start of the study. This may have precluded a possible learning effect and caused no systematic changes in the BRS and its autonomic and vascular determinants. The present investigation conducted in a sample of healthy adolescents, showed poorer reliability (20% CV and r=0.63) of the LFgain compared to adults (CV of 5.4% and ICC of 0.76) (Maestri*, et al.* 2009; Reynolds*, et al.* 2016). However, our reliability results are similar to that observed in 11-y olds of a CV of 13.8% and ICC of 0.49 for the LFgain (Dietrich*, et al.* 2010). This highlights the importance of population-specific studies investigating the reliability of BRS assessment.

The observed CVs <20% contain biological and technical variability which might be augmented if important sources of errors before and during BRS assessment are not controlled. For instance, aiming to decrease biological variability participants were asked to keep a similar diet and physical activity in the days preceding data collection, and report to the laboratory at the same time of the day following an overnight fast. This was done because prior physical activity and diet can alter autonomic and vascular functions (Al Haddad*, et al.* 2009). Similarly, aiming to decrease technical errors, breathing frequency was kept outside LF range to increase reliability of BRS and autonomic modulation (Davies*, et al.* 1999; Pinna*, et al.* 2007), and participants were familiarized to this procedure before the experiment. Additionally, all data trace was free of >3% errors and all analysis performed by the same researches. The present study indicates that BRS assessed with LFgain presents acceptable between-day reliability in adolescents;

however, the above important factors before and during the measurements should be controlled or the error is likely to be larger.

The present investigation is the first to calculate the magnitude of systematic and random error in the measurements of the autonomic and vascular BRS determinants in adolescents. The measures of the vascular determinant used were AC and AD, as previously reported in this population (Lenard*, et al.* 2004). AC and AD measures presented CVs of 16.8 and 17.2%, without any systematic error between visits (Table 3). The reliability observed for AC and AD measures reflect small between-day variation in vessel diameters, and the main source of errors in AC and AD calculation derived from PP measures. These results indicate that factors affecting PP should be minimised when designing studies to further improve reliability. For instance, due to hydrostatic pressure Finometer readings of PP at the finger level exacerbate the differences between systolic and diastolic pressure (Imholz*, et al.* 1998). To minimize this, participants were asked to keep their hands at the heart level during BRS protocol. The autonomic determinant measured using LFgain/AC presented an absolute and relative reliability of 19.8% CV and r=0.87, and did not systematically change between-days. Despite being calculated with a series of other measurements, this is the first study to demonstrate that LFgain/AC is a robust index that can be reliably used to investigate autonomic determinant of BRS in adolescents.

Within-day reliability

Notably all parameters (except vessel size) presented poorer within-day compared to between-day reliability. LDD, LSD and LFgain presented systematic changes 2-h after the initial measurement suggesting circadian changes are present. To our knowledge the current study is the first to report this observation in healthy adolescents. This is in

accordance with previous adult literature suggesting an increase in BRS and its autonomic and vascular determinants throughout day (Taylor*, et al.* 2011). The mechanisms underlying circadian changes are beyond the scope of the present investigation, but might involve a heightened sympathetic tone and vascular constriction in the early morning compared to late morning (Panza*, et al.* 1991). This might also explain the increased carotid diameter observed 120-min post compared to baseline. Similarly, random errors were exacerbated in the within-day protocol for all measures with the BRS autonomic component presenting CV of 34% and r=0.80. This arises from a sum of factors, such as PP, AC, and LFgain, which were altered between the time assessments. These results highlight that a control group is essential when changes throughout day are investigated (i.e. the effects of exercise or diet intervention on acute BRS changes), and that time of the day should be strictly controlled in between-days protocols.

Reliability of hemodynamic outcomes

BRS assessment and interpretation can be influenced by a diversity of factors. Specifically, BRS is the ability to adjust MAP by triggering a series of mechanisms to modulate $\dot{Q}$ and TPR (Persson 1996). Poor reliability of MAP, $\dot{Q}$ and TPR therefore would hamper BRS interpretation. In the present investigation MAP, $\dot{Q}$, TPR, SV and HR presented CVs <15% between-days and <21% within-days. The main sources of error in these measurements would be technical and biological variations between days which would affect the observed CV. As all variables (except HR) are determined from finger plethysmography, technical errors can derive from positioning of the cuff, cuff size, and movements during the calibration, as well as possible differences in finger temperature between-days (Imholz*, et al.* 1998). In the current study, aiming to decrease technical errors cuff placement were performed by the same researcher, with adequate cuff size and

participants were thoroughly instructed to stay as quite as possible during BRS protocol and Finometer calibration. Additionally, room temperature was maintained in a narrow range between- and within-days.

Limitations

The present sample comprised only boys, and therefore studies involving girls are needed. There are considerable technical skills required to operate the ultrasound, as well as data processing, which might hamper the application of the BRS protocol. The autonomic gain calculated as the ratio between LFgain and AC although theoretically sound and previously used in this population (Lenard*, et al.* 2004), has not been validated. One alternative would be the use of methods with infusion of vasoactive drugs to test the neural component, however such methods raise ethical concerns for use in a pediatric population. Similarly, CCA measures were used with no information about aorta distensibility (Klassen*, et al.* 2016). Finally, we acknowledge that for AC and DC measurements it is desirable to assess PP at the carotid site, however, others have suggested that BP derived from Finometer is a valid measure of intra-arterial pressure (Guelen*, et al.* 2008), and our present results are comparable to methods measuring PP at the carotid site (Lenard*, et al.* 2004).

Practical applications

The current study provides practical information to aid interpretation of interventions, and in sample size calculation for future trials. Sample size can be calculated considering between-subject variation (i.e. pooled standard deviation) and the observed CV for each outcome. Applying the principle of Cohen's effect sizes of 0.2 (small), 0.5 (moderate), and 0.8 (large) (Cohen 1977), and using Hopkins between and within variation formulas (available at http://sportsci.org/resource/stats/ssdetermine.html#long), the number of

participants needed to achieve statistical power of 0.80 at an alpha level 0.05 in a randomized controlled trial investigating changes in LFgain with a control and an experimental group will be 423, 63, and 22 per group, respectively. For AC, the number of participants needed is 537, 80, and 29 and for LFgain/AC the number of participants needed will be 191, 27, and 9. Finally, the calculated sample sizes should be inflated by 20% considering possible data loss due to errors in the ECG and BP trace, as well as in images acquisition.

CONCLUSION

There was acceptable (i.e. CV<20%) between-day reliability of BRS and its autonomic and vascular determinants in male adolescents. Similarly, all components of the BP equation, namely MAP, $\dot{Q}$, HR, SV and TPR, presented adequate between-day reliability. CCA diameter was the most reliable variable in the present study and the main source of error in the arterial distensibility and compliance coefficients was PP. Within-day reliability was poorer compared to between-days for all BRS and hemodynamic measurements, possibly due to circadian rhythm. The present results will help future research for sample size calculation and clinical interpretation of findings of interventional studies. Our results also highlight that a control group is essential when changes throughout day are investigated due to the observed diurnal variation.

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Conflict of interests: None

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Table 1: Participants characteristics

	Between day reliability		
	All (n=13)	CCA (n=10)	BRS (n=11)
Age (y)	14.0±0.5	14.1±0.3	14.1±0.4
Stature (cm)	162.2±10.5	163.6±10.8	162.4±10.9
Body Mass (kg)	46.6±13.2	52.1±14.2	49.9±14.1
Body Fat (%)	12±4.7	12.7±4.8	12±4.8
VO_2max ($mL \cdot kg \cdot min^{-1}$)	50.1±5.2	52.1±3	50.1±5.3
Stage of maturation	2=3	2=2	2=3
	3=1	3=1	3=0
	4=8	4=6	4=7
	5=1	5=1	5=1
	Within day reliability		
	All (n=13)	CCA (n=12)	BRS (n=12)
Age (y)	14.0±0.5	14±0.4	14±0.5
Stature (cm)	162.2±10.5	161.7±10.8	161.7±10.7
Body Mass (kg)	46.6±13.2	50.4±13.5	49.2±13.7
Body Fat (%)	12±4.7	12.5±4.6	11.7±4.7
VO_2max ($mL \cdot kg \cdot min^{-1}$)	50.1±5.2	50.9±5.3	50.6±5.3
Stage of maturation	2=3	2=3	2=3
	3=1	3=1	3=0
	4=8	4=7	4=8
	5=1	5=1	5=1

CCA: Common carotid artery. BRS: Baroreflex sensitivity.

Table 2: Average physical activity and food consumption in the 48-h preceding the experimental visits

		Day 1	Day 2	Day 3	Day 4	*P*
n=7	MVPA (min·day^{-1})	116.1±56.1	99.8±51.3	126.1±29.7	132.2±75.1	0.46
n=12	Total kcal (kcal·day^{-1})	2025±177	2150±178	1944±134	1975±114	0.68
n=12	Carbohydrate (%)	51±2	50±2	50±2	51±2	0.72
n=12	Lipids (%)	32±2	34±2	32±2	31±1	0.34
n=12	Protein (%)	16±1	15±1	17±1	17±1	0.67

MVPA: moderate-to-vigorous physical activity

Table 3: Between-day reliability of BRS gain and its autonomic and vascular determinants

		Day 1	Day 2	Day 3	Day 4	*P* value ANOVA	*r*	CV	TE
n=10	DLD (μm)	5288.0±278.5	5260.0±300.4	5190.0±313.6	5262.0±420.8	0.39	0.91	2.4	127.0
n=10	SLD (μm)	6133.0±308.4	6129.0±337.5	6087.0±320.3	6115.0±448.4	0.78	0.90	2.3	143.5
n=10	Delta diameter (μm)	845.0±126.8	869.0±128.3	897.0±144.6	853.0±154.3	0.32	0.80	7.7	63.0
n=10	Diastolic CSA (mm)	22.0±2.3	21.8±2.5	21.2±2.6	21.9±3.4	0.40	0.91	4.9	1.1
n=10	Systolic CSA (mm)	29.6±3.0	29.6±3.3	29.2±3.1	29.5±4.5	0.78	0.89	4.7	1.4
n=10	Delta CSA (mm)	7.6±1.3	7.8±1.4	7.9±1.4	7.6±1.6	0.62	0.81	8.7	0.64
n=10	Arterial Strain (%)	16.0±2.6	16.6±2.5	17.4±3.2	16.3±3.4	0.17	0.84	8.0	1.3
n=10	AC ($\mu m \cdot mmHg^{-1}$)	18.9±4.5	20.0±3.6	19.3±3.9	19.7±5.0	0.85	0.50	16.8	3.1
n=10	AD (10^{-3}/mmHg)	7.7±1.8	8.3±1.5	8.1±1.9	8.2±2.7	0.80	0.60	17.2	1.3
n=11	LFgain ($ms \cdot mmHg^{-1}$)	23.6±5.7	21.4±5.9	21.0±5.4	21.1±6.8	0.34	0.63	20.4	3.9
n=9	LFgain/AC ($ms \cdot \mu m^{-1}$)	1.32±0.49	1.13±0.35	0.96±0.52	1.21±0.45	0.11	0.87	19.8	0.2
n=11	HR ($beats \cdot min^{-1}$)	66±9	66±5	66±8	67±6	0.84	0.83	5.7	4
n=11	$\dot{Q}$ ($L \cdot min^{-1}$)	3.0±0.8	3.2±0.7	3.0±0.6	3.0±0.7	0.41	0.82	11.6	0.3
n=11	SV (mL)	46.6±13.8	48.1±11.6	45.3±9.0	44.8±11.8	0.27	0.87	10.2	4.2

n=10	PP (mmHg)	46.0±8.2	43.9±6.3	47.0±4.0	44.2±7.3	0.45	0.37	14.7	5.9
n=11	MAP (mmHg)	78.9±5.4	79.6±6.6	80.8±9.9	77.5±7.8	0.55	0.50	7.4	5.6
n=11	TPR (units)	27.8±7.4	26.3±5.8	28.0±5.1	26.9±4.4	0.64	0.63	14.4	3.6

LDD: lumen diastolic diameter; LSD: lumen systolic diameter; PP: pulse pressure; AC: arterial compliance; AD: arterial distensibility.

Table 4: Within-day reliability of BRS gain and its autonomic and vascular determinants

		Baseline	60 min	120 min	*P* value ANOVA	*r*	CV	TE
n=12	DLD (µm)	5220.0±329.9	5269.2±332.6	5360.8±373.0*	**0.038**	0.89	2.3	126.7
n=12	SLD (µm)	6086.7±340.2	6135.0±346.1	6235.0±387.0*	**0.051**	0.87	2.2	134.0
n=12	Delta diameter (µm)	866.7±126.1	865.8±125.8	874.2±138.1	0.93	0.79	7.3	60.3
n=12	Diastolic CSA (mm)	21.5±2.7	21.9±2.8	22.7±3.2*	**0.048**	0.89	4.6	1.12
n=12	Systolic CSA (mm)	29.2±3.3	29.7±3.3	30.7±3.4	0.06	0.88	4.4	1.39
n=12	Delta CSA (mm)	7.7±1.3	7.8±1.3	8.0±1.4	0.56	0.80	8.1	0.61
n=12	Arterial Strain (%)	16.7±2.8	16.5±2.7	16.4±3.0	0.85	0.82	7.9	1.2
n=12	AC ($\mu m \cdot mmHg^{-1}$)	20.0±3.7	20.8±9.5	19.9±4.4	0.82	0.57	25.4	6.0
n=12	AD (10^{-3}/mmHg)	8.3±1.4	8.5±3.7	8.1±1.8	0.77	0.45	26.1	2.5
n=12	LFgain ($ms \cdot mmHg^{-1}$)	21.7±5.8	20.3±7.9	24.4±8.2**	**0.051**	0.74	25.1	4.1
n=11	LFgain/AC ($ms \cdot \mu m^{-1}$)	1.18±0.36	1.26±0.72	1.28±0.53	0.67	0.81	31.4	0.57
n=12	HR ($beats \cdot min^{-1}$)	66±5	65±8	63±6	0.11	0.79	6.0	4
n=12	$\dot{Q}$ ($L \cdot min^{-1}$)	2.9±0.8	2.8±0.8	2.7±0.6	0.56	0.67	19.2	0.4
n=12	SV (mL)	44.1±13.6	43.4±14.8	44.5±12.3	0.90	0.77	17.7	5.8

n=12	PP (mmHg)	44.4±9.0	46.4±14.0	44.9±9.3	0.77	0.63	22.0	7.4
n=12	MAP (mmHg)	78.2±6.7	80.1±7.1	78.8±6.8	0.43	0.83	4.4	3.37
n=12	TPR (units)	28.9±7.5	31.2±8.9	29.6±5.4	0.61	0.53	18.8	6.05

LDD: lumen diastolic diameter; LSD: lumen systolic diameter; PP: pulse pressure; AC: arterial compliance; AD: arterial distensibility.

*P<0.05 compared to baseline. **P<0.05 compared to 60-min.