

Clinical Study

Repeatability of Peripheral Artery Tonometry in Female Subjects

Andrew J. Degnan,¹ Nandini Shah,¹ David M. Carty,¹ John R. Petrie,¹
Christian Delles,¹ and Markus P. Schneider^{1,2}

¹ BHF Glasgow Cardiovascular Research Centre, Institute of Cardiovascular & Medical Sciences, University of Glasgow,
126 University Place, Glasgow G12 8TA, Scotland, UK

² Department of Nephrology and Hypertension, University of Erlangen-Nuremberg, Ulmenweg 18 (Internistisches Zentrum),
91054 Erlangen, Germany

Correspondence should be addressed to Markus P. Schneider; markus.schneider@uk-erlangen.de

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Background. Peripheral arterial tonometry (PAT) is a novel, non-invasive and operator-independent method for simultaneous assessment of endothelial function and arterial stiffness. We examined the repeatability of PAT in females and the influence of the estrous cycle. **Methods.** In 14 healthy female and five healthy male control subjects, PAT was performed on three separate occasions with 10 days between visits. Reactive hyperemia index (RHI), a measure of endothelial function, and peripheral augmentation index (AIx), a measure of arterial stiffness, were determined with the EndoPAT-2000 system. Intraclass correlation coefficient (ICC) was calculated as a measure of repeatability. **Results.** In both female and male groups, RHI and AIx did not differ between the three measurements (all n.s. by 1-way ANOVA). In females, reanalyzing the data after taking phase of estrous cycle into account had no effect on the results. Repeatability for RHI and AIx in females (ICC for RHI = 0.43, ICC for AIx = 0.78) was similar to that in male subjects (ICC for RHI = 0.42, ICC for AIx = 0.63). **Conclusions.** PAT measurements were not affected by the estrous cycle in females, and repeatability was comparable to that in males. This should facilitate inclusion of female subjects into vascular function studies using PAT.

1. Background

Endothelial dysfunction is an important mechanism in the initiation and progression of atherosclerotic lesions. By using forearm plethysmography with intra-arterial administration of vasoactive agents, impaired endothelium-dependent vasodilation has been demonstrated in patients with a variety of conditions such as arterial hypertension [1, 2], hypercholesterolemia [3], and diabetes mellitus [4]. Of potential interest for clinical decision making, impaired endothelial function is an independent predictor of future cardiovascular (CV) events [5–7].

Ultrasound-based assessment of flow-mediated vasodilation (FMD) of the brachial artery is a noninvasive alternative method, also predicting future CV events [8, 9]. However, FMD testing requires substantial training. It has been recommended that at least 100 supervised training scans should be performed prior to scanning patients independently, and

100 scans per year are required to maintain competency [10]. Although the prognostic value of endothelial function testing has been recognized, guidelines do not currently recommend testing routinely, as current methods are invasive, laborious, and/or time consuming [11].

Peripheral arterial tonometry (PAT) is a novel method that promises to overcome some of these issues, as PAT measurements are noninvasive and operator independent. Of note, PAT measures the peripheral augmentation index (AIx), which is an established marker of arterial wave reflection, simultaneously with endothelial function. Normally, the measurement of both endothelial function and arterial wave reflection requires separate equipment, which makes PAT particularly interesting for the clinical environment and for clinical research studies.

Female hormones could potentially affect endothelial function and arterial stiffness measurements, which would have significant implications for the clinical use and for

clinical trials involving PAT measurements. We therefore studied repeatability of endothelial function and arterial stiffness measurements by PAT before and after taking the phase of estrous cycle into account.

2. Methods

2.1. Study Participants. We recruited 19 healthy volunteers (14 female, 5 male) between June and September 2011. Subjects were staff or students at the University of Glasgow. All subjects were free from clinically apparent cardiovascular disease and were nonsmokers. No subjects were on any medications. All subjects fasted for at least 4 hours before the study and refrained from coffee or caffeine products since the previous night. All subjects provided written informed consent, and the Ethics Committee of the University of Glasgow approved the study.

2.2. PAT Procedure. PAT was recorded using the EndoPAT-2000 device (Itamar Medical, Caesarea, Israel) in accordance with the manufacturer's recommendations and in line with the published literature [12]. In brief, the system consists of pneumatic finger probes that assess changes of digital pulse volume amplitude accompanying pulse waves. A blood pressure cuff is placed on the nondominant arm (study arm), while the other arm acts as a control. PAT probes are placed on the index fingers of each hand for continuous recording of the PAT signal. After a 10 min resting period, the blood pressure cuff is inflated to suprasystolic pressures for five minutes. The cuff is then released, and PAT recording continues for a further five minutes to register the reactive hyperemia response.

PAT data are analyzed using proprietary software in an operator-independent manner. The ratio of the average amplitude of the PAT signal over a one-minute period starting one minute after cuff deflation (maximum pulse amplitude) divided by the average amplitude of the PAT signal over a 3.5-minute period before cuff inflation (baseline pulse amplitude) is calculated. PAT ratios from the study arm are then normalized to the control arm to correct for systemic factors, such as room temperature, resulting in the RHI. All recordings of our study were performed in the same room by the same two investigators (Andrew J. Degnan, Nandini Shah).

2.3. Statistical Analysis. Statistical analyses were performed with SPSS version 18. We used 1-way analysis of variance (ANOVA) to compare the data obtained on the three separate study days. In females, this was repeated after reallocating the data according to the phase of the oestrous cycle at the time the particular measurement had been performed.

To determine within-subject agreement of measurements, we calculated the intraclass correlation coefficient (ICC) derived from variance analysis. We used the two-way random effects, single-measure model for absolute agreement between measurements, where $ICC = ((\text{variance between subjects}) - (\text{variance residual subjects})) / ((\text{variance between subjects}) + ((k - 1) \times (\text{variance residual subjects})) + (k \times (\text{variance between measurements} - \text{variance residual}$

TABLE 1: Clinical parameters of study participants.

Parameter (mean \pm SD)	Females ($N = 14$)	Males ($N = 5$)	P value
Age (years)	24 \pm 8	21 \pm 1	0.42
Height (cm)	167 \pm 8	178 \pm 4	0.01
Weight (kg)	74 \pm 19	67 \pm 5	0.43
BMI (kg/m ²)	26 \pm 6	21 \pm 1	0.01
SBP (mmHg)	118 \pm 10	130 \pm 9	0.03
DBP (mmHg)	73 \pm 9	69 \pm 7	0.38
HR (bpm)	69 \pm 8	70 \pm 11	0.83

subjects)/ n), k is the number of measurements, and n is the total number of subjects. We also reported the average coefficient of variation (CV) of the measurements, derived from the average of individual standard deviations/average of individual means.

3. Results

3.1. Subject Characteristics. Clinical parameters are shown in Table 1. We included 14 female subjects and a smaller group of five male subjects as controls (Table 1). All subjects were clinically free from CV disease and were nonsmokers.

3.2. Repeatability. The measurements of vascular function were scheduled consecutively in both males and females, that is regardless of the phase of the estrous cycle in the female study participants. The first measurement was performed within the first 10 days of enrolment. The second and third measurements then followed with another 10 days between each measurement.

In the females, there was no difference in RHI between the measurements on the three study days ($P = 0.30$ by 1-way ANOVA, Figure 1(a)). We then reallocated the data into three new groups, taking into account whether the individual measurement fell into the initial, the middle, or the final phase of the estrous cycle. Even after taking phase of estrous cycle into account, RHI was similar between these new groups ($P = 0.32$ by 1-way ANOVA, Figure 1(b)), suggesting that phase of the estrous cycle did not affect the results. We then examined repeatability of the RHI measurements by calculating the intraclass correlation coefficient (ICC) and the coefficient of variation (CV). The ICC for RHI was 0.46 (95% CI 0.13–0.76, $P = 0.003$), and $CV = 20\%$.

Further, there was no difference in AIX between the three study days in the females ($P = 0.23$ by 1-way ANOVA, Figure 2(a)). Again, reanalyzing the data after taking phase of the estrous cycle into account did not change this result ($P = 0.94$ by 1-way ANOVA, Figure 2(b)). As regards repeatability, the ICC for AIX was 0.78 (95% CI 0.55–0.92, $P < 0.001$), and $CV = 45\%$.

In a smaller control group of male subjects ($n = 5$), RHI was not different between the three measurements ($P = 0.31$ by 1-way ANOVA). As regards repeatability, the ICC for RHI was 0.42 (95% CI 0.12–0.71, $P = 0.003$), and $CV = 27\%$. Finally, there was no difference in AIX between the three

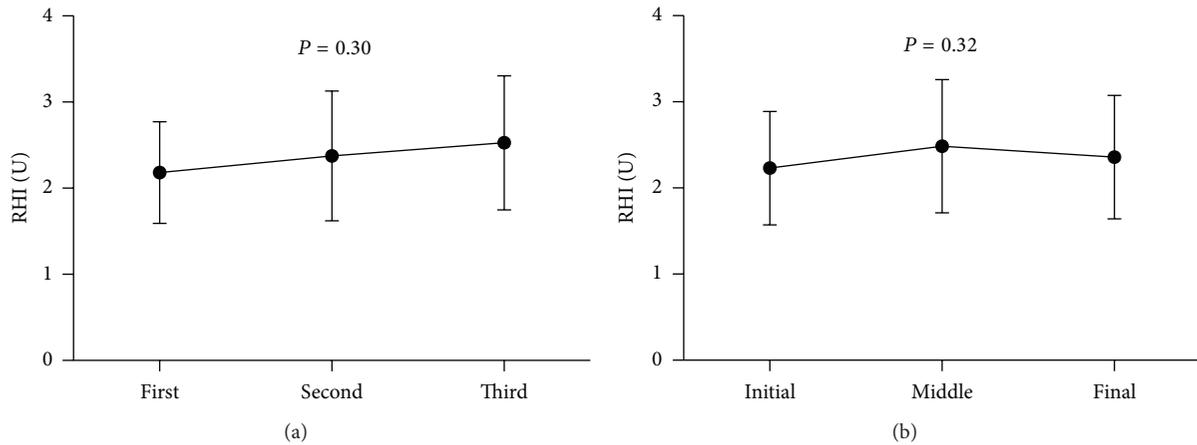


FIGURE 1: Reactive hyperemia index (RHI) at the first, second, and third measurements after study enrolment (a) and after reallocating data according to estrous cycle into the initial, middle, and final phases of the estrous cycle (b) (data given as mean \pm SD).

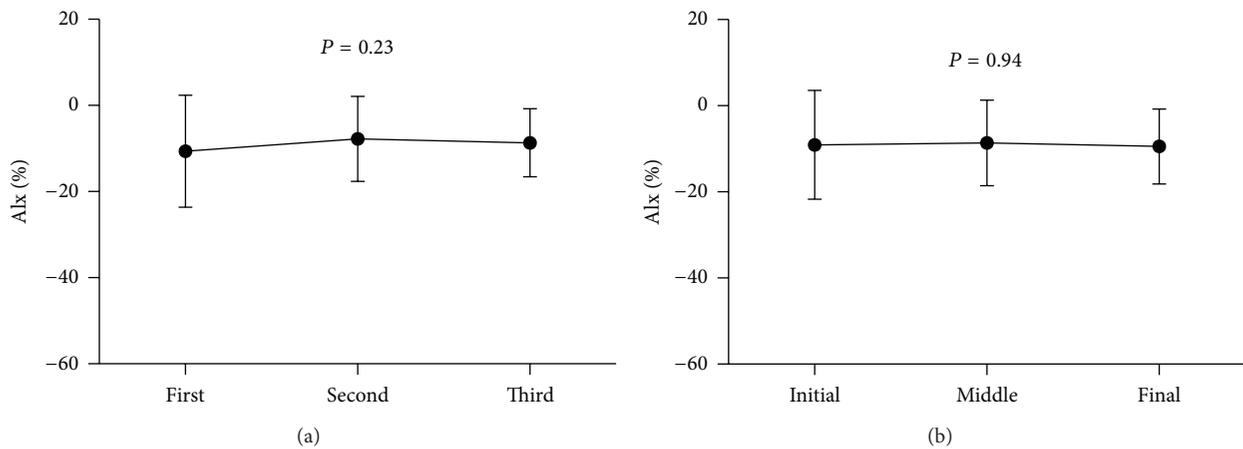


FIGURE 2: Augmentation index (AIx) at the first, second, and third measurements after study enrolment (a) and after reallocating data according to estrous cycle into the initial, middle, and final phases of the estrous cycle (b) (data given as mean \pm SD).

measurements ($P = 0.70$). ICC for AIx measurements was 0.63 (95% CI 0.47–0.81, $P = 0.002$), and CV = 47%.

4. Discussion

The main results of this study were that RHI and AIx measurements by PAT are not significantly affected by phase of estrous cycle in females, and repeatability was comparable to that in males. Thus, measurements in females do not need to take into account phase of the estrous cycle, which is an important aspect of practicability for the clinical setting, and which should facilitate the inclusion of females into studies on vascular function by PAT.

RHI by PAT is novel method for the assessment of endothelial function, based on the measurement of reactive hyperemia-induced changes of digital pulse wave amplitude in the index finger with biosensors. Intraarterial infusion of the nitric oxide synthase (NOS) inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) attenuates the RHI response

by 46%, confirming that RHI is partly NO dependent [13]. Changes of pulse wave amplitude on the arm of occlusion are corrected for any potential systemic changes during RHI measurements by simultaneously recording from the index finger of the nonoccluded arm. In the case of forearm plethysmography, correction for systemic changes has been shown to reduce the variability of measurements (and consequently sample sizes required for clinical studies). Studies investigating the relationship between RHI and FMD have shown modest correlations (ranging from $r = 0.3$ to $r = 0.55$ [14–16]). In the Framingham Heart Study, RHI has been shown to correlate with multiple traditional and metabolic CV risk factors [17]. In the Gutenberg Heart Study comprising 5000 individuals, the risk factors that determined RHI and FMD were largely overlapping (age, sex, body mass index, and hypertension), but some distinct differences were also noted (fasting glucose only a determinant of RHI) [18]. There is also more recent data from the Framingham Study suggesting that FMD and PAT provide independent information, which

is representing different aspects of endothelial function [19]. Finally, RHI has been shown to identify patients with coronary artery disease [12], and in a separate study, RHI has been shown to predict CV events independently of conventional risk factors [20].

The augmentation of central aortic pressure, largely determined by arterial stiffness (pulse wave velocity) and magnitude of peripheral wave reflection (peripheral vasoconstriction), is a powerful and independent predictor of cardiovascular morbidity and mortality [21–23]. Central pressure augmentation index (AIx) is most commonly assessed by recording the peripheral pulse waveform with a tonometric device on the radial artery and by applying a transfer function to derive the central pressure waveform. In contrast to the established Sphygmocor or the Complior devices, PAT can assess endothelial function and AIx simultaneously, which is attractive for the clinical setting and for research studies. A recent study has shown that AIx by PAT closely correlates with the number of CV risk factors [24].

Despite the growing use of the PAT method, there is little information on its repeatability. Moreover, in the few studies available, females have either not been included or have not been analyzed separately from the male subjects. Separate study of females is important because some studies have suggested that the phase of the estrous cycle could affect vascular function, although this is contentious [25, 26]. Liu et al. reported repeatability in a total of 10 male subjects measured on three consecutive days with an ICC between -0.07 to 0.47 [27]. Selamet Tierney et al. studied repeatability of RHI in a larger cohort of 30 healthy adolescents, but only on two separate occasions (no more than 7 days apart). An excellent repeatability with an ICC of 0.78 was found, but separate analyses in males and females were not performed [28]. McCrea et al. reported an ICC of 0.74 for RHI and 0.83 for AIx in 20 healthy subjects on the basis of two measurements at least one week apart. Again, separate analyses in females and the potential influence of estrous cycle were not reported. In the current study, we measured RHI and AIx in 14 young, healthy female subjects and 5 male control subjects on three separate occasions one week apart. Firstly, 1-way ANOVA did not demonstrate a difference between measurements on the three separate occasions in either group. In the females, reallocating the data into three new groups according to the phase of the estrous cycle did not affect this result, suggesting that estrous cycle does not significantly affect RHI or AIx by PAT. As regards repeatability, we found an ICC of 0.43 for RHI and an ICC of 0.78 for AIx measurements in the females, which was similar to the repeatability in the males. It is noteworthy that the ICC for RHI in our males and females was lower than the ICC reported by Selamet Tierney et al. and McCrea et al., who had data from two separate occasions, but more similar to the ICC of -0.07 to 0.47 reported by Liu et al, who measured on three separate occasions. For the estimation of the “true” repeatability, a study design with a greater number of repeated measurements in each subject gives a higher precision in estimating the “true” repeatability than an approach with a greater number of subjects but less repeated measurements. As an example, a study with 15 subjects using two measurements (total of 30

observations) achieves a precision around the “true” within-subject standard deviation of 36%, while a study with only 10 subjects but three measurements (total of 30 observations) achieve a more narrow and therefore better precision around the within-subject standard deviation of 31% [29].

It is noteworthy that the repeatability of AIx by PAT with an ICC of 0.78 was excellent in the current study, which is in keeping with the previous report from McCrea et al., who found an ICC of 0.83 [30]. Although PAT is a novel technique and, as mentioned above, in contrast to the Sphygmocor or the Complior systems, prospective data linking AIx by PAT with morbidity and mortality are not available yet, the excellent repeatability found for AIx seems to be promising.

As a limitation of the current study, sample size of this study was relatively small. However, as outlined above, the accuracy for the determination of measures of repeatability increases with the number of repeated measurements, which is why we have chosen to perform 3 separate measurements of endothelial function rather than performing only 2 measurements with a slightly greater number of subjects. As a second limitation, aside from a thorough physical examination, we did not perform extensive laboratory tests to ascertain that the subjects in this study were free from CV disease, but we believe that this is very likely considering the young age of the subjects included in this study.

In conclusion, estrous cycle did not affect data in females, and repeatability in females was similar to that in males. Thus, PAT measurements of RHI and AIx do not require “timing” to phase of estrous cycle. This enables shorter study durations and also the assessment of interventions with rapid effects on the vasculature, such as documented for statin therapy [31].

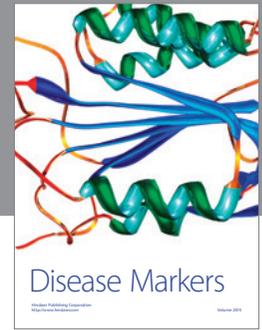
Conflict of Interests

John R. Petrie is the recipient of donated services and equipment for PAT measurements from Itmar Medical for the current study. There is no further conflict of interests to report.

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