Reliability of Brachial Artery Flow-Mediated Dilatation Measurement Using Ultrasound

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Summary

Objective: To determine the reliability of the baseline (BBAB) and post occlusion (POBAD) brachial artery diameters, brachial artery flow-mediated dilatation (BAFMD) measurements, and to quantify the standard error of these measurements (TEM).

Methods: Internal consistency (2 measurements on the same day) was determined in 10 volunteers, whereas stability (2 measurements on separate days) was determined in 13 volunteers. All the volunteers were apparently healthy and all were nonsmokers. The brachial artery images were obtained using a two dimensional Doppler ultrasound instrument equipped with a 14 MHz transducer. The distances between the intima-lumen interfaces were measured before and after the blood flow had been stopped by means of a cuff on the arm for five minutes. BAFMD was considered as the percent change of POBAD in relation to BBAD.

Results: ANOVA did not identify any significant differences between the measurements taken on the same and separate days. For BAFMD, the intraclass correlation coefficients between the measurements taken on the same and separate days were: R = 0.7001 and R = 0.8420, respectively ($p < 0.05$). The coefficients of variation were 5.8% and 12.4% and the relative ETMs were 13.8% and 14.9%, respectively, for the measurements taken on the same and separate days. Analysis of the Bland-Altman graphs indicated that the variables did not present heteroscedastic errors.

Conclusion: The BBAD, POBAD and BAFMD measurements using the manual ultrasound technique were highly reliable for both the same and separate day measurements and therefore can be used to diagnose and monitor endothelial function. (Arq Bras Cardiol 2007;89(3):160-167)

Key words: Internal consistency, stability, typical error of measurement, vascular reactivity, endothelial function.

Introduction

Brachial artery flow-mediated dilatation (BAFMD) is an indicator of endothelial function that can be obtained using the noninvasive ultrasound (US) technique. The technique, initially described by Celermajer et al[1] and recommended by the International Brachial Artery Reactivity Task Force[2], is based on the percent change of the brachial artery diameter caused by reactive hyperemia, which is observed using high resolution transducers – usually 7 MHz or higher. BAFMD values greater than 10% in males and 15% in females present high sensitivity to identify coronary artery disease[3] in both genders.

Chequer et al[4] reported significant correlation between BAFMD and intima media thickness (IMT), also an early indicator of atherosclerosis (Spearman r: -0.315; P = 0.042).

Endothelial function is affected by chronic conditions such as obesity, diabetes, hypercholesterolemia, smoking and hypertension[5], and, acutely, by sleeplessness, mental stress, hormonal changes, physical exercise, menstrual cycle and diet[3]. Vascular reactivity changes caused by these conditions can be observed by BAFMD[5] and affect its reliability as well.

The reliability of BAFMD also appears to be affected by procedural interferences such as the cuff placement site[6], occlusion duration[7], transducer resolution[8], sonographer's experience and intra-individual variability[9].

Among the studies that investigated BAFMD, only a few calculated the intraclass correlation coefficient (ICC)[9,10]. Variation coefficients (VC) reported in literature, were extremely inconsistent, with a mean of 1.8% for same and separate day measurements[11] and values as high as 84% between the separate day measurements[12]. It should be noted that different measurement techniques were used in the available studies.

The studies that investigated the reliability of the same and separate day measurements of the baseline (BBAD) and of the post occlusion brachial artery diameter (POBAD) and BAFMD are shown in Table 1.

To determine reliability, the ICC calculation and distribution of the absolute differences between the repeated measurements in Bland-Altman graphs have been shown to be more appropriate than an isolated CV[13]. Very

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Table 1 - Summary of studies on the reliability of the baseline and post occlusion brachial artery diameters and BAFMD

Reference	n	Number of measurements	Occlusion location	Method (transducer)	Realiability (CV, ICC or diff ± SD)
Celermajer et al	21 (6 smokers, 3 CAD)	2 to 3 (M-line) separate days	Arm	Manual (7.0 MHz)	BAFMD: CV = 2-3 %
Mannion et al[6]	27 males & females	2 same day	Forearm	On-line recording (7.5 MHz)	BBAD: dif ± SD = 0.07 ± 0.07 mm POBAD: dif ± SD = 0.07 ± 0.50 mm
			Arm		BBAD: dif ± SD = 0.08 ± 0.06 mm POBAD: dif ± SD = 0.14 ± NR mm
Herrington et al[8]	30 males & females	2 (M-line) separate days	Forearm	Various scans (13 MHz)	BBAD: CV = 12.7 % POBAD: CV = 11.4 % BAFMD: CV = 26.3 %
	127 males & females	2 (M-line) separate days	Forearm	Various scans (7.5 MHz)	BBAD: CV= 7.0 % POBAD: CV = 7.0 % BAFMD: CV = 45.3 %
Welsch et al[9]	26 males & females	2 separate days	Forearm	Video (7.5 MHz)	BAFMD: ICC = 0.92 ($P < 0.05$)
Malik et al[10]	20	2 (M-line) separate days	Arm	Video (7.5 MHz)	BAFMD: CV = 41 %; ICC = 0.10 (P = NR) $[CV = \|x_1-x_2\|\cdot\sqrt{2}/(x_1+x_2)\cdot 100]$
De Roos et al[12]	21 males	4 separate days	Forearm	Video (8-14 MHz)	BBAD: CV = 6.5 % POBAD: CV = 6.8 % BAFMD: CV = 84 %
Hijmering et al[16]	103 males & females	2 same & separate days	10 cm distal to measurement location	Radiofrequency (7.5 MHz; software)	Same day: BBAD: CV = 1.1 % Separate days: BBAD: CV = 3.6-3.8 %
Woodman et al[17]	24 males & females	2 separate days	Forearm	Manual; Video (12 MHz)	BBAD: CV = 1.4 % BAFMD: CV = 24.8 %
				Software (12 MHz)	BBAD: CV = 0.36 % BAFMD: CV = 6.7 %
West et al[18]	18 Db 2	3 separate days	Forearm	Software (10 MHz)	BAFMD: CV = 29.7%
Liang et al[19]	30 males & females	2 separate days	Arm	Video (10 MHz)	BAFMD: CV = 10.3%
Berry et al[23]	16 males & females	2 (M-line) separate days	Arm & Forearm	Video (7-10 MHz)	BBAD: CV = 2.7 %
Šejda et al[25]	18 males & females	2 separate days	Forearm	Video (7 MHz)	BAFMD: CV = 4.6 e 5.5 % (2 evaluators)
Avest et al[26]	19 males & females	2 separate days	Forearm	Software (7.5 MHz)	SD of mean differences = 4.9 %
De Roos et al [27]	13 males & females	2 to 6 separate days	Forearm	Video (7.5 MHz)	BBAD: CV = 4.8 % POBAD: CV = 5.2 % BAFMD: CV = 50 %

Original Article

Table 1 - continuation

Present study*	10 males & females	2 same day	Arm	Manual (14 MHz)	BBAD: CV = 0.6 %; ICC = 0.9676
					POBAD: CV = 0.8 %; ICC = 0.9469
					BAFMD: CV = 5.8 %; ICC = 0.7001
	13 males & females	2 separate days			BBAD: CV = 1.8 %; ICC = 0.9484
					POBAD: CV = 2.3 %; ICC = 0.9480
					BAFMD: CV = 12.4 %; ICC = 0.8420

*M-line - line between the media and adventitia artery layers; CAD - coronary arterial disease; CV - coefficient of variation; ICC - intraclass correlation coefficient; diff ± SD - mean difference and standard deviation; BBAD - baseline brachial artery diameters; POBAD - post occlusion brachial artery diameters; BAFMD - brachial artery flow-mediated dilatation; * P < 0,05 to all ICC; NR - not reported.*

little information is available on brachial artery BAFMD diameter measurements.

The objective of this study was to determine the internal consistency (same day variability) and stability (separate day variability) of the BBAD, POBAD and BAFMD measurements, as well as to quantify the typical error of the measurement (TEM) associated with these measurements.

Methods

Sample - Ten men and women participated in the study to determine internal consistency, and thirteen men and women were used to determine stability. All participants were apparently healthy and all were nonsmokers (33.2 ± 12.1 years; 79.6 ± 20.7 kg; 172.1 ± 9.6 cm). All volunteers were briefed on the experimental procedures and informed that no risks were associated with the study, in accordance with institutional guidelines and Resolution nº 96/196 of the National Health Board.

Experimental procedure - To determine internal consistency, the subjects were submitted to two tests on the same day, with a minimum interval of 1.5 hours between the tests. To determine measurement stability, two tests were conducted with a minimum interval of three days between the tests (mean = 7.2 ± 5.3 days). All tests were conducted at the same time – between 12:30p.m. and 2:30p.m.

In both cases, the subjects fasted for at least three hours before the test and did not perform physical exercise on the day of the measurement.

Brachial artery images - The images were obtained using a two dimensional color spectral Doppler ultrasound equipped with a 14MHz linear transducer (Toshiba Nemium®, Japan).

For the test, each subject laid down comfortably in the supine position with the right arm slightly abducted. After locating the brachial artery, the transducer was placed on the anteromedial face of the right arm, perpendicular to the centerline of the arm, 5-10 cm above the antecubital fossa, over the artery. The Doppler was used to confirm satisfactory placement and artery pulse quality.

BBAD and POBAD were manually measured between the intima-lumen interfaces at the end of the diastole. After the BBAD measurement, the transducer contact location on the skin was marked so that the POBAD measurement would be taken at the same site. The occlusion was maintained for 5 minutes, using a cuff on the arm to apply pressure slightly above the systolic artery pressure, which was confirmed by the lack of pulse on the Doppler. POBAD was measured 60 to 90 seconds after the blood flow was released. A typical image obtained from the tests is shown in Figure 1.

All tests were performed by the same evaluator, who was not aware of the values observed during the retesting.

BAFMD was calculated as a percentage of the brachial artery post occlusion diameter increase in relation to the baseline values {[BAFMD = (POBAD – BBAD)/BBAD] x 100 %}

Statistical analysis - The Shapiro-Wilk test was used to analyze the normality of the measurement distribution. The correlation between the test and retest values obtained on the same and separate days was determined using the intraclass correlation coefficient (ICC). An ANOVA with repeated measures was used to identify differences between the values obtained during the tests and retests.

The Pearson correlation coefficient, between the mean values for the test and retest results, and the difference between each pair of measurements, was determined to evaluate if the data presented an heteroscedastic error. This coefficient was also used to determine the relationship between BBAD and BAFMD.

The coefficients of variation (CV) was established for every subject by dividing the standard deviation for each pair of measurements by their mean values (CV = [(SD/mean)*100] Next, the mean CV was calculated using the mean of the individual VCs.

Statistical significance was established as $p < 0.05$. All analyses were conducted using the statistic software package, SPSS 11.0. (SPSS Inc., Chicago, USA)

The method suggested by Bland and Altman[14] was used to determine the degree of concordance between the pairs of

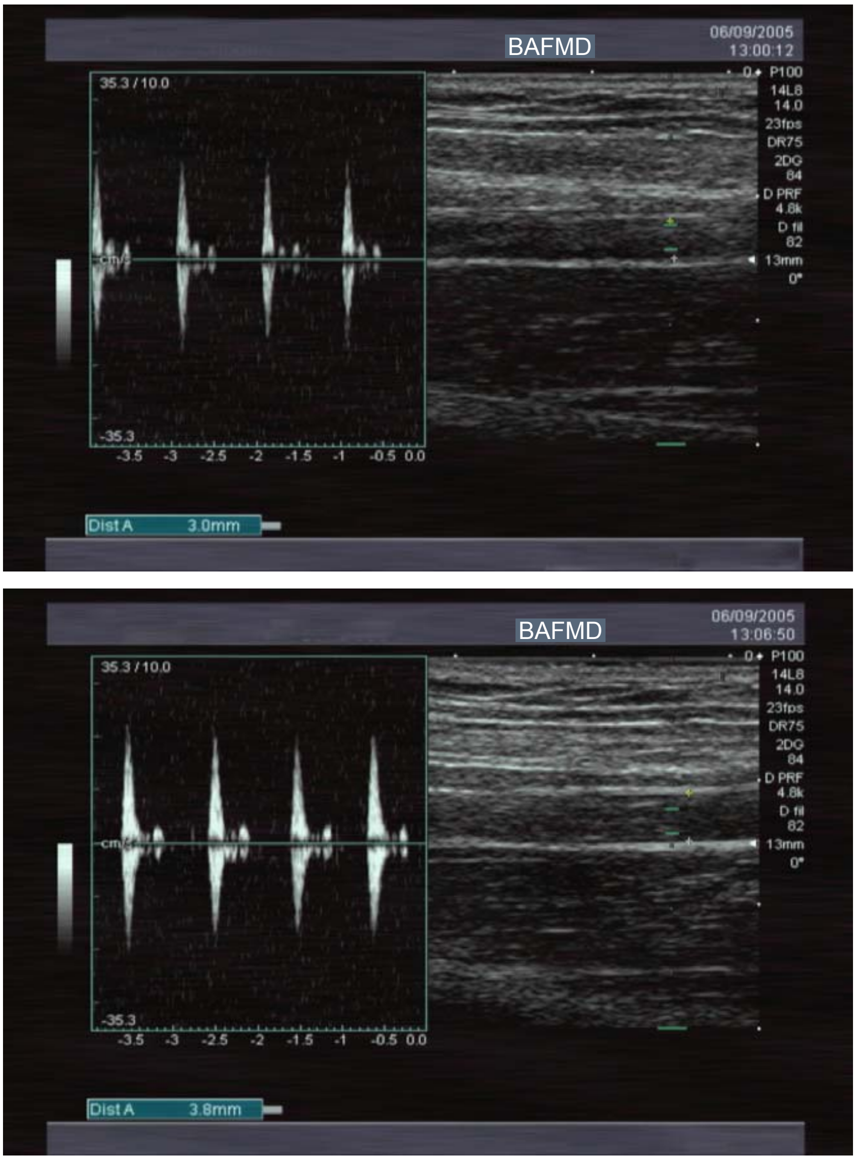

Fig. 1 - *Typical example of baseline (above) and post reactive hyperemia (below) brachial artery ultrasound images.*

measurements obtained on the same and separate days. The coefficient of repeatability (CR) was obtained by multiplying the standard deviation (SD) of the differences between the test and retest by 1.96.

TEM was established as the result of the ratio between the SD of the differences obtained from the pairs of same day measurements and the square root of 2, ($TEM = SD/\sqrt{2}$) as suggested by Hopkins[15].

Results

The Shapiro-Wilk test indicated that the BBAD measurement values presented normal distribution for the tests conducted on the same and separate days. However, the BAFMD values only presented normal distribution for the measurements taken on separate days ($p = 0.001$).

The ANOVA results did not identify any significant differences between the pairs of measurements obtained on the same or separate days for any of the variables. The highest CVs were found in the separate day values for both measurements. In relation to ICC, all were statistically significant, however the ICC for the same day measurements was lower than the ICC for the separate day measurements of BAFMD (0.7001 *versus* 0.8420, respectively) (Tables 2 and 3).

None of the Pearson correlations between the mean values obtained during the tests and retests or the differences between each pair of measurements were statistically significant, indicating that the variables did not present heteroscedastic errors. For the same and separate days BBAD

Table 2 - Decriptive values, mean variation coefficient (VC) and intraclass correlation coefficient (ICC) of the brachial artery baseline (BBAD) and post occlusion (POBAD) diameters taken on the same day and brachial artery flow-mediated dilatation (BAFMD).

Variable	Mean ± SD	VC	ICC	
			R	P
BBAD				
Measurement 1	3.4 ± 0.3 mm	0.6%	0.9676	0.0000
Measurement 2	3.6 ± 0.3 mm			
POBAD				
Measurement 1	4.1 ± 0.3 mm	0.8%	0.9469	0.0000
Measurement 2	4.2 ± 0.4 mm			
BAFMD				
Measurement 1	19.9 ± 5.0%	5.8%	0.7001	0.0082
Measurement 2	19.6 ± 4.8%			

SD - standard deviation.

Table 3 - Descriptive values, mean variation coefficient (VC) and intraclass correlation coefficient (ICC) of the brachial artery baseline (BBAD) and post occlusion (POBAD) diameters taken on separate days and brachial artery flow-mediated dilatation (BAFMD).

Variable	Mean ± SD	VC	ICC	
			R	P
BBAD				
Day 1	3.5 ± 0.6 mm	1.8%	0.9484	0.0000
Day 2	3.6 ± 0.7 mm			
POBAD				
Day 1	4.0 ± 0.6 mm	2.3%	0.9480	0.0000
Day 2	4.2 ± 0.7 mm			
BAFMD				
Day 1	16.5 ± 6.8%	12.4%	0.8420	0.0001
Day 2	17.3 ± 5.7%			

SD - standard deviation.

measurements, the respective correlations found were: r = 0.180 and r = -0.517, and for BAFMD, r = 0.540 and r = 0.322 ($p > 0.05$).

No significant correlation was found between BBAD and BAFMD for the same day measurements (r = -0.247; P = 0.491) or the separate day measurements (r = -0.457; p = 0.116).

The Bland-Altman graphs demonstrating the degree of concordance between the pairs of measurements obtained on the same and separate days with their respective CRs are shown in Figure 2. The limits of agreement and the mean values for the differences between the tests and retests are shown in Table 4. For each of the same day measurement variables, one subject did not fall within the limits of concordance. The same trend was seen for BBAD and POBAD for the separate day measurements; however, in the case of BAFMD two subjects did not fall within the concordance limits.

TEMs for BBAD, POBAD and BAFMD were, respectively, 0.05 mm (1.4%), 0.07 mm (1.8%) and 2.7% (13.8%) for the same day tests. For the separate day measurements the TEMs were 0.13 mm (3.5%) for BBAD; 0.11 mm (2.7%) for POBAD; and 2.5% (14.9 %) for BAFMD.

Discussion

Little variability was observed for the same and separate day BBAD measurements. For the same day measurements, Mannion et al[6] observed a mean test- retest difference similar to the present study (0.07 ± 0.07 mm *versus* -0.030 ± 0.067 mm), and Hijmering et al[16] found a 1.1% CV. In relation to measurement stability, other authors found CVs ranging from 0.36%[17] to 12.7%[8].

Little information is available in relation to the reliability of the POBAD measurement. In regard to internal consistency, previous results indicate mean test – retest differences greater than those found in the present study (0.14 mm – SD not reported[6] versus -0.030 ± 0.106 mm) as well as higher CVs for measurement stability (CV = 5.2%[18] to 11.4%[8] versus 2.3%).

The BAFMD values obtained in the present study were greater than those described in literature for healthy individuals using blood flow occlusion in the arm (9.8%[6] and 13.1%[19] versus 16.5% to 19.9% in the present study). In accordance with results of a recent meta analysis[20], the technical aspects that have the greatest influence on the measurement differences observed are the cuff placement site and occlusion duration.

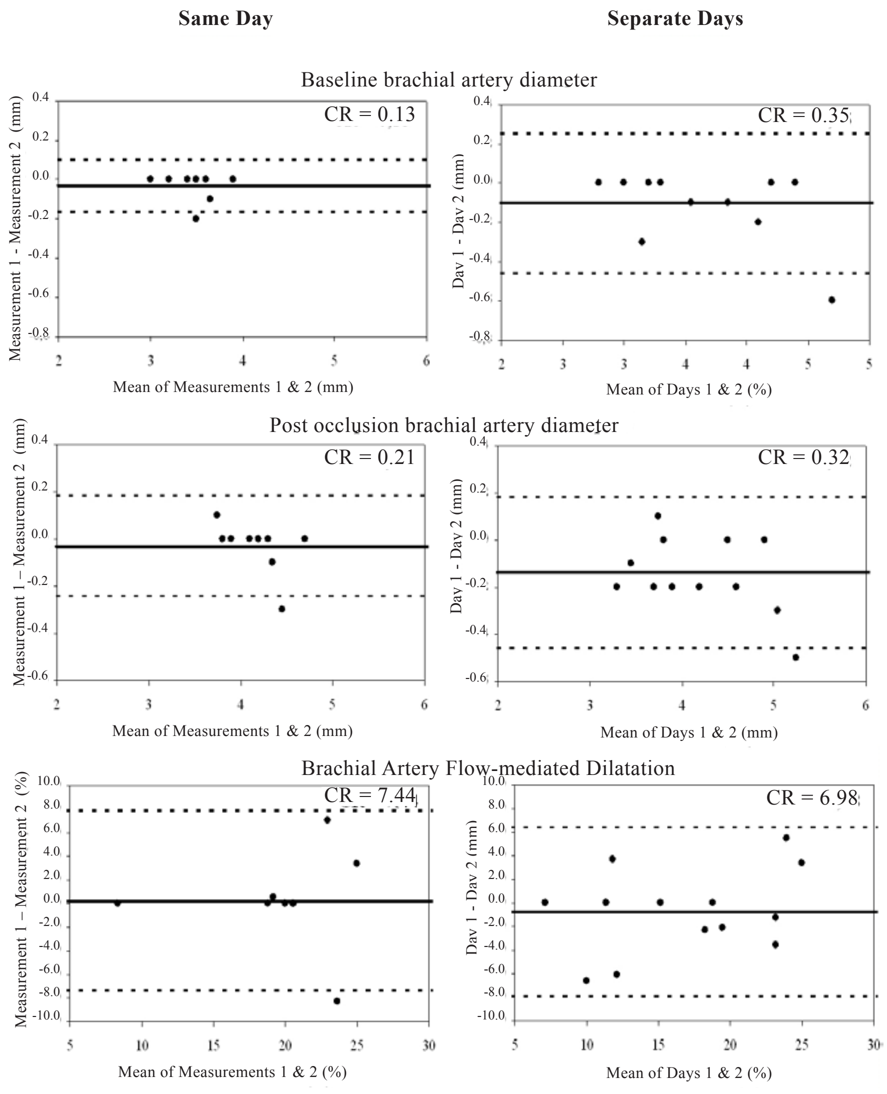

Fig. 2 - *Bland-Altman analysis showing the absolute and mean differences between the test and retest. Graphs on the left - Same day measurements. Graphs on the right - Separate day measurements; CR - coefficient of repeatability.*

Table 4 - Results of the Bland-Altman analysis (mean of the test-retest differences and concordance limits) of the brachial artery baseline (BBAD) and post occlusion (POBAD) diameters and brachial artery flow-mediated dilatation (BAFMD).

Variable	Mean ± SD of the differences	Concordance limits
BBAD		
Same day	-0.030 ± 0.067 mm	-0.165 – 0.105 mm
Separate days	0.100 ± 0.178 mm	-0.456 – 0.256 mm
POBAD		
Same day	-0.030 ± 0.106 mm	-0.242 – 0.182 mm
Separate days	-0.138 ± 0.161 mm	-0.460 – 0.183 mm
BAFMD		
Same day	0.255 ± 3.79 %	-7.333 – 7.843 %
Separate days	0.803 ± 2.52 %	-4.244 – 5.850 %

SD - standard deviation.

The reliability of the BAFMD measurement has also revealed a great deal of discrepancy in the various studies (CV ranging from 1.8%[11] to 84%[12] – Table 1). The CV values observed in the present study were 5.8% and 12.4% for the same and separate day measurements, respectively. These values are higher than those found in biochemical analyses of important variables for clinical diagnosis and monitoring such as blood concentrations of total cholesterol and high density lipoprotein (HDL) cholesterol. Pereira et al[21] found VC of 3.0% for the separate day measurements of each of these variables.

The BAFMD measurement, using the noninvasive US technique, originally predicted the onset of reactive hyperemia as a result of the blood flow occlusion caused by positioning the cuff on the arm[1]. Currently, blood occlusion on the forearm or wrist is more popular since it appears to express dilatation promoted exclusively by nitric oxide (NO)[22]. Using distal occlusion, the BAFMD values are lower than those observed after proximal arm occlusion[6] (6.8 ± 3.8% versus 9.8 ± 5.7%, respectively.) However, the cuff placement site does not appear to affect BAFMD reliability[6].

Apparently, the most important consideration is the time course between the release of the flow and the POBAD reading, that varies according to the technique used. Berry et al[23] demonstrated that the highest BAFMD values for a forearm occlusion were observed roughly 49 ± 3 seconds after the blood flow release, whereas upper arm occlusions produced peak BAFMD values approximately 71 ± 5 seconds after cuff removal ($p < 0.01$).

According to Doshi et al[22], the fact that forearm occlusion promotes exclusively NO mediated hyperemia is not sufficient to determine the abandon of the upper arm occlusion technique. Since the dilatation provoked by placing the cuff on the forearm is limited, which makes it difficult to identify the discriminatory values to distinguish endothelial dysfunction and modifications induced by diet and/or exercise.

Another important factor is the occlusion duration. Leeson et al[7] demonstrated that the maximum artery responses are obtained after a forearm occlusion period of 4 to 5 minutes and that no additional effects are obtained with longer occlusion timeframes. Bots et al[20] demonstrated that most (~ 51%) of the studies use occlusion durations greater than 4.5 minutes.

Another variable that can affect measurement reliability is the transducer frequency. Herrington et al[8] compared BAFMD stability measured in the M line (interface between the media and adventitia artery layers) and observed significantly better measurement results using 13 MHz transducers (CV = 26.3%) in comparison to 7.5 MHz transducers (CV = 45.3%).

In the present study, the occlusion was maintained for 5 minutes, the POBAD measurement was taken 60 to 90 seconds after releasing the flow and a 14MHz transducer was used, therefore demonstrating that sufficient care was taken to obtain the desired response.

Siber et al[24] demonstrated that BAFMD is proportional to the postischemic hyperemia response; however, the hyperemic stimulus is greater in smaller arteries due to the smaller radius. Therefore, depending on the BBAD, higher BAFMD values can be found, which does not necessarily reflect better artery function. In the present study, no significant correlations were found between BBAD and BAFMD, which agrees with the observations of Šejda et al[25] but disagrees with the results of Herrington et al[8].

Analysis of the Bland-Altman graphs indicated that the study variables did not present heteroscedastic or absolute systematic errors, that is, the differences between the test and retest have no significant relation with the measurement magnitude and do not present systematically positive or negative trends. The mean differences between the test and retest were close to zero for all measurements; however, the limits of agreement for the same and separate day BAFMD values were high (Table 4 and Figure 2) demonstrating that in some subjects the differences between the test and retest were high.

This observation, alongside the awareness of TEM (2.5 percent points in the present study), confirms that clinical significance can only be considered for modifications that are greater than TEM, in order to eliminate interferences caused by evaluator inaccuracy and biological measurement variability when using BAFMD as a dependent variable in surgical studies. However, consideration should be given to the fact that hematological variables that could affect BAFMD measurement variability[5], such as blood lipid concentrations and systemic blood pressure, were not controlled in the present study.

For the most part, the studies available in literature obtain the measurements from VHS tape recordings[10,12,19,23] or semi-automatic measurements using computer programs developed for this purpose[17,26]. These procedures reduce an important source of measurement error, which is the experience of the ultrasonographer[9], making comparison with the results of the present study more difficult.

Woodman et al[17], comparing the reliability of manual and semi-automatic measurements using software, observed significantly better results with the support of a computerized system. The respective CVs for the manual and semi-automatic techniques were: 1.4% and 0.4% for the BBAD measurements ($p < 0.05$) and 24.8% and 6.7% for BAFMD ($p < 0.05$).

However, in a clinical setting, manual records of BBAD and POBAD measurements are more feasible than the measurements that depend on VHS tape recordings or software, as they are quicker and easier to obtain.

In closing, the results described herein, demonstrate that baseline and post occlusion brachial artery diameters and BAFMD measurements using the manual ultrasound technique are highly reliable for both the same and separate day values, which enables the use of BAFMD for endothelial function diagnostic and monitoring purposes. Nevertheless, for the purpose of observing modifications as a result of exercise, medication or diet programs, it is important to only consider of clinical relevance variations higher than the typical error of the measurement. In addition, more comprehensive studies with a larger population including asymptomatic and symptomatic subjects are required in order to establish BAFMD reference values.

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