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Abstract

Background: Ultrasound has been used for noninvasive assessments of endothelial function in both clinical and athletic settings and to identify changes in muscle blood flow in response to exercise, nutritional supplementation, and occlusion. The purposes of the present study were to examine the reliability and relative contributions of arterial cross-sectional area and time-averaged flow velocity to predict muscle blood flow as a result of fatiguing exercise in men and women. Methods: Eighteen healthy men and 18 healthy women performed 50 consecutive eccentric repetitions of the elbow flexors at 60% of their pretest eccentric peak torque at a velocity of 180° s\(^{-1}\). Test-retest reliability and stepwise linear regression analyses were performed to determine the ability of arterial cross-sectional area and time-averaged flow velocity to predict brachial artery muscle blood flow for the men, women, and combined sample. Results: There was no systematic test versus retest mean differences \((P > 0.05)\) for any of the ultrasound determined variables. The two-variable regression models significantly improved the ability to predict muscle blood flow and were associated with smaller standard error of the estimates (3.7%–10.1% vs. 16.8%–37.0% of the mean baseline muscle blood flow values) compared to the one-variable models. Conclusions: The findings of the present study supported the use of ultrasound for reliable assessments of arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and muscle blood flow from the brachial artery in men and women. Furthermore, time-averaged flow velocity was a more powerful predictor of muscle blood flow than arterial cross-sectional area.

Keywords: Blood flow, eccentric, muscle fatigue, sex, ultrasound

INTRODUCTION

Ultrasound has been used for noninvasive assessments of endothelial function in both clinical\(^{1,2}\) and athletic settings.\(^{3}\) For example, in clinical settings, ultrasound-based assessments of endothelial function from flow-mediated dilation have been used to identify the early onset of hypertension, atherosclerosis, and precursors of heart failure.\(^{1,2}\) Flow-mediated dilation involves the occlusion of an artery that induces the release of the potent vasodilator nitric oxide and subsequently, increases arterial blood flow. The changes in arterial blood flow (hyperemic response) is quantified using ultrasound determined flow-mediated dilation to provide an index of endothelial function.\(^{2}\)

In athletic settings, ultrasound has been used to identify changes in arterial blood flow in response to exercise,\(^{3}\) nutritional supplementation,\(^{4}\) and occlusion.\(^{5}\) Furthermore, ultrasound has been used to provide insight regarding sex- and age-specific arterial blood flow responses to a variety of exercise conditions. For example, women experienced greater increases in femoral artery blood flow than men as a result of maximal effort leg extension muscle actions performed to exhaustion.\(^{13}\) In addition, women experienced greater absolute and relative changes in brachial artery diameter than men after 3 min of blood flow occlusion.\(^{15}\) Compared to young adults, nitrate supplementation enhanced the vasodilatory capabilities of the brachial artery in older adults during hypoxic submaximal forearm flexion muscle actions.\(^{4}\) Thus, ultrasound has been applied in a variety of settings and has implications for monitoring health and tracking exercise- or nutrient-induced changes in arterial responses.
changes in endothelial function and blood flow in both men and women and in young and older adults.

Endothelial function is assessed by changes in arterial blood flow, arterial diameter, arterial cross-sectional area, and time-averaged flow velocity. Arterial blood flow is calculated as the product of arterial cross-sectional area and time-averaged flow velocity. Arterial cross-sectional area can be calculated from arterial diameter measured by ultrasound, and flow velocity is typically collected over a period of three cardiac cycles and is expressed as the time-averaged flow velocity. There are concerns, however, associated with ultrasound measurements that may limit its application. While a recent study has demonstrated reliability among commercially available ultrasound systems for the assessment of arterial blood flow, there are limitations associated with exercise that may adversely affect arterial blood flow analysis. For example, ultrasound-based assessments of arterial blood flow require the assumption that arterial cross-sectional area and time-averaged flow velocity are derived while the artery is stationary. Thus, changes in arterial blood flow measured before versus after exercise may provide insight regarding the effects of exercise on arterial blood flow. The extent that arterial cross-sectional area or time-averaged flow velocity contributes to the estimation of arterial blood flow from the brachial artery as a result of-fatiguing exercise, however, has not been determined. This is of particular interest as the determination of time-averaged flow velocity may not be practical under all conditions due to time limitations. For example, the time-averaged flow velocity is typically obtained over three cardiac cycles compared to arterial-cross sectional area that can be derived nearly instantaneously. Thus, it may be advantageous to measure arterial cross-sectional area that can be obtained more easily as a surrogate of muscle blood flow. Therefore, the primary purpose of the present study was to examine the relative contributions of arterial cross-sectional area and time-averaged flow velocity to predict brachial artery blood flow as a result of-fatiguing exercise in men and women. Secondary purposes were to examine the test-retest reliability of brachial artery blood flow, arterial diameter, arterial cross-sectional area, and time-averaged flow velocity assessed on separate days. Based on a previous investigation, we hypothesized that both arterial cross-sectional area and time-averaged flow velocity would be significantly correlated with brachial artery blood flow, but time-averaged flow velocity would be a more powerful predictor of brachial artery blood flow than arterial cross-sectional area. Furthermore, the ultrasound measurements of arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow would be reliable for both the men and women.

**Methods**

**Subjects**

Eighteen men (mean age ± standard deviation [SD] = 23.2 ± 3.0 years; body mass = 85.4 ± 12.1 kg; height = 179.6 ± 8.2 cm; resistance training = 6.9 ± 2.8 h/week) and 18 women (mean age ± SD = 22.3 ± 1.7 years; body mass = 64.1 ± 8.3 kg; height = 167.5 ± 5.9 cm; resistance training = 6.3 ± 3.5 h/week) volunteered to participate in this investigation. The subjects had no known cardiovascular, pulmonary, metabolic, muscular, and/or coronary heart disease, or regularly used prescription medication. In addition, all subjects had been actively participating in resistance training for at least the past 6 months. The subjects visited the laboratory on two occasions separated by at least 72 h within a 2-week period and performed the testing procedures at the same time of day. Subjects were instructed to avoid performing upper body exercise 48 h before the testing visit. The study was approved by the University Institutional Review Board for Human Subjects and all subjects completed a health history questionnaire and signed a written informed consent before testing.

**Procedures**

**Visit one**

The first laboratory visit was used to determine visit one baseline values for arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow for the ultrasound reliability assessments as well as to familiarize the subjects with the strength testing protocols. Ultrasound measurements were assessed before the warm-up (after the subjects laid quietly for 10 min) using an ultrasound-imaging device (GE Logiq e, USA). Arterial diameter was derived from ultrasound images of the brachial artery (proximal to the antecubital fossa) that were captured using brightness mode (B-mode). Specifically, a perpendicular line that extended from the top to the bottom of the artery wall was drawn, and this distance was measured to determine arterial diameter [Figure 1]. Once the brachial artery was located using ultrasound, the location of the ultrasound probe was superficially marked with a permanent marker and anatomical landmarks within the ultrasound image were recorded to allow for consistent replacement of the ultrasound probe.

**Figure 1:** Displays the determination of arterial cross-sectional area from arterial diameter and time-averaged flow velocity obtained over three cardiac cycles.
Arterial blood flow was assessed using pulsed wave Doppler sampled at a repetition frequency of 8MHz obtained and a multifrequency linear-array probe (12 L-Rs; 5–13 MHz; 38.4 mm field-of-view). All ultrasound measurements were performed at an insonation angle of 60° to the brachial artery, and time-averaged flow velocity was determined over a period of three cardiac cycles [Figure 1].[10,11] Brachial artery blood flow was derived using Equation 1.[6] All measurements were taken while the subjects were lying in the supine position on the isokinetic dynamometer with both their arms and legs supported. Great care was taken to ensure that consistent, minimal pressure was applied with the probe to limit compression of the artery. To enhance acoustic coupling and reduce near-field artifacts, a generous amount of water-soluble transmission gel was applied to the skin before each measurement.

Arterial blood flow = time-averaged flow velocity × π (arterial diameter ÷ 2)² × 60 (1)

where arterial cross-sectional area was calculated as: (arterial diameter ÷ 2)²

During visit one, the subjects also performed submaximal and maximal eccentric isokinetic (on a calibrated Cybex 6000) muscle actions of the dominant forearm flexors at 180°·s⁻¹. To familiarize the subjects with the fatiguing protocol, the subjects practiced performing eccentric isokinetic muscle actions at a velocity of 180°·s⁻¹ and at an intensity that corresponded to 60% of their eccentric peak torque which was visually tracked using real-time torque displayed on a computer monitor.

Visit two

During the second laboratory visit, visit two baseline values for arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow were determined using the same procedures as visit one. Following the determination of baseline ultrasound measurements, the subjects performed a warm-up consisting of 30 (three sets of 10 repetitions separated by 60-s of rest) submaximal (approximately 50% effort) eccentric-concentric muscle actions of the dominant forearm flexors at 180°·s⁻¹ on the isokinetic dynamometer. Following the warm up, the subjects rested for 5 min and then performed two pretest maximal eccentric peak torque trials at 180°·s⁻¹, and the highest peak torque of the two trials was selected as the pretest eccentric peak torque. The eccentric muscle actions were performed through a 90° of motion (90°-0° of flexion at the elbow, where 0° corresponds to full extension). After the determination of pretest eccentric peak torque, the subjects performed 50 consecutive eccentric repetitions at 60% of their pretest eccentric peak torque at a velocity of 180°·s⁻¹. Real-time torque was displayed on a computer monitor, and each eccentric contraction was followed by a passive concentric muscle action that was assisted by the investigator. To measure the effect of the fatiguing protocol, eccentric peak torque trials were also performed immediately after (posttest) completing the fatiguing protocol using the same procedures as the pretest. In addition, ultrasound measurements were determined immediately after (posttest = 57 ± 8 s) the fatiguing protocol.

Data analysis

Test-retest reliability

Test-retest reliability for brachial artery blood flow, arterial diameter, arterial cross-sectional area, and time-averaged flow velocity were assessed from visit one and visit two. Repeated measures ANOVAs were used to assess systematic error, and model 2,[12] was used to calculate intraclass correlation coefficients (ICCs), standard errors of measurement (SEMs), and minimal difference (MD) needed to consider a change as real.[13] Furthermore, 95% confidence intervals were constructed for each ICC (ICC95%).

Statistical analyses

2 (Sex [men, women]) × T (Pretest, Posttest) mixed factorial ANOVAs were used to examine normalized (to pretest) eccentric peak torque, arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow as a result of the fatiguing protocol for the men and women. Separate stepwise linear regression analyses were performed to determine the ability of arterial cross-sectional area and time-averaged flow velocity to predict brachial artery blood flow for the men, women, and combined sample at baseline and after exercise. The correlation for each model as well as zero-order correlations, R² change, standardized and unstandardized Beta coefficients, and standard error of the estimates (SEEs) was calculated for each regression analyses. The F-test was used to examine if the increment in proportion of variance accounted for in the two-variable model (arterial cross-sectional area and time-averaged flow velocity) versus one-variable model (arterial cross-sectional area or time-averaged flow velocity) was significant. In addition, simple-linear regression analyses were performed on change scores (baseline to posttest differences at visit two) between arterial cross-sectional area and brachial artery blood flow as well as time-averaged flow velocity and brachial artery blood flow. All statistical analyses were performed using IBM SPSS version. 21 (Armonk, NY, USA) and an alpha of p ≤ 0.05 considered statistically significant for all comparisons.

Results

Pretest to posttest responses

There was no significant (P = 0.171–0.954) Sex × Time interactions for normalized eccentric peak torque, arterial diameter, arterial cross-sectional area, time-averaged flow velocity, or brachial artery blood flow. There were, however, significant (P < 0.001) main effects for Time (collapsed across Sex) for eccentric PT (decreased 18.9%), arterial diameter (increased 14.6%), arterial cross-sectional area (increased 30.6%), time-averaged flow velocity (increased 87.8%), and brachial artery blood flow (increased 149.7%).

Brachial artery blood flow, arterial diameter, arterial cross-sectional area, and time-averaged flow Velocity.
The means (±SD) for brachial artery blood flow, arterial diameter, arterial cross-sectional area, and time-averaged flow velocity for visits one and two are displayed in Table 1. There were no systematic mean differences from visit one to visit two (P > 0.05) for any of the variables and the ICC, ICC95%, SEM, and MD values are listed in Table 2.

Simple linear regression and stepwise regression analyses

There were significant one- and two-variable models for the prediction of brachial artery blood flow for the men (r = 0.549–0.996), women (r = 0.737–0.988), and combined sample (r = 0.719–0.981) [Tables 3 and 4]. The two-variable models, however, significantly improved the ability to predict brachial artery blood flow at baseline and after exercise. In addition, the two-variable models were associated with smaller SEEs [Table 4].

The correlations for changes in arterial cross-sectional area versus changes in brachial artery blood flow and changes in time-averaged flow velocity versus changes in brachial artery blood flow are displayed in Figures 2 and 3. In general, changes in time-averaged flow velocity were more highly correlated with changes in brachial artery blood flow than changes in arterial cross-sectional area.

DISCUSSION

The baseline arterial diameter and brachial artery blood flow responses [Table 1] were consistent with baseline values for young men and women reported in the previous studies.[1,5,14–17] For example, previous investigations have reported baseline values of 0.29–0.46 cm for arterial diameter[6,15,16] and 91–214 mL/min for brachial artery blood flow[6,14,17] in men and women assessed from the brachial artery. On average, the fatiguing eccentric intervention resulted in 15%, 31%, 88%, and 150% increases in arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow, respectively, for the combined sample of men and women [Table 1]. Approximately, the same magnitude of percent increases also occurred for each of these parameters for the separate samples of men and women.

Reliability

The results of the present study indicated that ultrasound-based assessments of baseline arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow measured three to 14 days apart during visits one and two were highly reliable [Table 2]. There were no significant mean differences between visits for any of the baseline variables for the men, women, or combined sample. Thus, there was no evidence of systematic error for any of the ultrasound-based variables in the present study.[13] In addition, the ICC values ranged from 0.734–0.959 which in the current study reflects the ability of the ultrasound-based variables to differentiate between individuals.[13] The ICC is unitless, a relative measure of reliability, and affected by the homogeneity of the samples.[13] The SEM, however, is expressed in the units of measure of the variable of interest, is an absolute index of reliability, and unaffected by between-subject variability of the sample.[13] In the present study, the SEM values ranged from 0.010–0.026 cm, 0.006–0.015 cm², 3.34–4.41 cm/s, and 28.76–34.18 mL/min for arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow, respectively. These values represent absolute errors ranges equal to 2.38–8.24, 4.44–17.86, 15.87–20.52, and 19.03%–26.72% of the respective, mean values [Table 1].

### Table 1: The means (±SD) for baseline and posttest arterial diameter, arterial cross-sectional area, time averaged flow velocity, and brachial artery blood flow assessed during visits one and two

| Variables | Visit one | Visit two |
|-----------|-----------|-----------|
|           | Baseline  | Baseline  | Posttest |
| Men (n=18) |           |           |          |
| Arterial Diameter (cm) | 0.418±0.034 | 0.422±0.031 | 0.473±0.040 |
| Arterial Cross-Sectional Area (cm²) | 0.141±0.023 | 0.138±0.021 | 0.177±0.029 |
| Time Averaged Flow Velocity (cm·s⁻¹) | 21.60±7.08 | 21.38±7.37 | 42.50±17.35 |
| Brachial Artery Blood Flow (mL·min⁻¹) | 180.61±59.03 | 178.57±69.83 | 459.83±217.75 |
| Women (n=18) |           |           |          |
| Arterial Diameter (cm) | 0.319±0.049 | 0.321±0.053 | 0.378±0.049 |
| Arterial Cross-Sectional Area (cm²) | 0.082±0.024 | 0.084±0.025 | 0.114±0.029 |
| Time Averaged Flow Velocity (cm·s⁻¹) | 20.88±7.38 | 21.24±8.17 | 37.55±12.18 |
| Brachial Artery Blood Flow (mL·min⁻¹) | 103.53±47.60 | 111.76±59.53 | 265.30±132.54 |
| Men and Women (n=36) |           |           |          |
| Arterial Diameter (cm) | 0.371±0.067 | 0.371±0.064 | 0.425±0.065 |
| Arterial Cross-Sectional Area (cm²) | 0.112±0.038 | 0.111±0.036 | 0.145±0.043 |
| Time Averaged Flow Velocity (cm·s⁻¹) | 21.24±7.14 | 21.31±7.67 | 40.02±14.98 |
| Brachial Artery Blood Flow (mL·min⁻¹) | 142.07±65.7 | 145.16±72.37 | 362.53±203.21 |

There were significant (P<0.05) increases from baseline to posttest (collapsed across Sex) for arterial diameter, arterial cross-sectional area, time averaged flow velocity, and brachial artery blood flow. There were no significant differences for any group or variable from visit one baseline to visit two baseline.
addition, the MD for arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow ranged from 0.03–0.08 cm, 0.02–0.04 cm², 9.97–13.16 cm/s, and 85.82–102.00 mL/min. The magnitude of the SEM values indicated there was low day-to-day variability for arterial diameter and arterial cross-sectional area, but greater day-to-day variability for time-averaged flow velocity and arterial blood flow. In conjunction with these findings, the MD values indicated that small changes in arterial diameter and arterial cross-sectional area would be required for a changed to be considered a “real” (increased sensitivity), while large changes in time-averaged flow velocity and arterial blood flow would be required to identify a significant change (decreased sensitivity). [13] Thus, there may be limitations when assessing changes in ultrasound-based assessments of time-averaged flow velocity or arterial blood flow when the expected magnitude of change is small (i.e., <25% of baseline values). However, all ultrasound-based measurements (arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow) could be reliably assessed from the brachial artery in both men and women. Collectively, the findings of the present study supported the use of ultrasound for the reliable assessments of arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow from the brachial artery in both men and women.

### One-variable models for predicting brachial artery blood flow

There were significant one-variable models for predicting baseline brachial artery blood flow from arterial cross-sectional area and time-averaged flow velocity for the men, women, and combined sample [Table 3]. For both arterial cross-sectional area and time-averaged flow velocity, however, the SEE values were greater than or equal to 16.8% (range = 16.8%–37.0%) of the respective mean values. Furthermore, there were significant one-variable models for predicting fatigue-induced changes in arterial cross-sectional area and changes in brachial artery blood flow for the men and combined sample, but not for the women [Figure 2a-c]. The fatigue-induced changes in time-averaged flow velocity were significantly correlated with changes in brachial artery blood flow for the men, women, and combined sample [Figure 3a-c]. Thus, time-averaged

### Table 2: Reliability data for arterial diameter, arterial cross-sectional area, time averaged flow velocity, and brachial artery blood flow assessed at baseline from visit one versus visit two

| Variables                              | P    | ICC  | ICC 95% | SEM  | MD   | Grand Mean |
|----------------------------------------|------|------|---------|------|------|------------|
| Arterial Diameter (cm)                 | 0.233| 0.955| 0.881-0.983 | 0.010| 0.03 | 0.420      |
| Arterial Cross-Sectional Area (cm²)    | 0.194| 0.959| 0.892-0.985 | 0.006| 0.02 | 0.140      |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.893| 0.734| 0.270-0.901 | 4.41 | 13.16| 21.492     |
| Brachial Artery Blood Flow (mL·min⁻¹) | 0.860| 0.845| 0.580-0.942 | 34.18| 102.00| 179.589    |
| Arterial Diameter (cm)                 | 0.634| 0.838| 0.564-0.939 | 0.026| 0.08 | 0.321      |
| Arterial Cross-Sectional Area (cm²)    | 0.588| 0.822| 0.522-0.933 | 0.015| 0.04 | 0.083      |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.751| 0.903| 0.740-0.964 | 3.34 | 9.97 | 21.059     |
| Brachial Artery Blood Flow (mL·min⁻¹) | 0.403| 0.836| 0.568-0.938 | 28.76| 85.82| 107.642    |
| Arterial Diameter (cm)                 | 0.954| 0.950| 0.903-0.975 | 0.021| 0.06 | 0.371      |
| Arterial Cross-Sectional Area (cm²)    | 0.869| 0.957| 0.915-0.978 | 0.011| 0.03 | 0.111      |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.942| 0.827| 0.660-0.912 | 4.06 | 11.66| 21.275     |
| Brachial Artery Blood Flow (mL·min⁻¹) | 0.678| 0.888| 0.780-0.943 | 31.35| 90.00| 143.616    |

P (ANOVA for systematic error), intraclass correlation coefficient (ICC), ICC 95% confidence interval (ICC 95%), standard error of the measurement (SEM), and minimal difference (MD) values.

### Table 3: Simple linear regression models for arterial cross-sectional area and time averaged flow velocity to predict brachial artery blood flow for the men (n=18), women (n=18), and combined sample (men and women, n=36) at visit two baseline and after exercise (posttest change)

| Variables                              | Correlation (r) | P    | SEE (mL/min) |
|----------------------------------------|-----------------|------|--------------|
| Arterial Cross-Sectional Area (cm²)    | 0.549           | 0.018| 60.1         |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.909           | <0.001| 30.0         |
| Arterial Cross-Sectional Area (cm²)    | 0.649           | 0.004| 146.5        |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.923           | <0.001| 74.2         |
| Arterial Cross-Sectional Area (cm²)    | 0.737           | <0.001| 41.4         |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.891           | <0.001| 27.8         |
| Arterial Cross-Sectional Area (cm²)    | 0.336           | 0.172| 112.0        |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.925           | <0.001| 45.1         |
| Arterial Cross-Sectional Area (cm²)    | 0.737           | <0.001| 41.4         |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.891           | <0.001| 27.8         |
| Arterial Cross-Sectional Area (cm²)    | 0.579           | <0.001| 137.5        |
| Time Averaged Flow Velocity (cm·s⁻¹)  | 0.894           | <0.001| 75.6         |

One-variable model

Standard error of the estimate (SEE)
Table 4: Stepwise linear regression analyses for the two-variable model (time averaged flow velocity and arterial cross-sectional area) to predict brachial artery blood flow for the men (n=18), women (n=18), and combined sample (men and women, n=36) at visit two baseline and after exercise (posttest change)

| Blood flow | Two-variable model | Model R | Unstandardized Beta | Standardized Beta | $R^2$ | $F$ change | P | SEE (mL·min$^{-1}$) |
|------------|--------------------|---------|---------------------|------------------|------|------------|---|-------------------|
| Men Baseline | Time Averaged Flow Velocity (cm·s$^{-1}$) | 0.992 | 7.98 | 0.842 | 0.826 | < 0.001 | 6.58 |
| Arterial Cross-Sectional Area (cm$^2$) | 1380.98 | 0.413 | 0.166 | < 0.001 |
| Constant | -182.65 | |
| Men Posttest Change | Time Averaged Flow Velocity (cm·s$^{-1}$) | 0.968 | 10.74 | 0.792 | 0.852 | < 0.001 | 35.7 |
| Arterial Cross-Sectional Area (cm$^2$) | 2728.29 | 0.365 | 0.116 | < 0.001 |
| Constant | -50.71 | |
| Women Baseline | Time Averaged Flow Velocity (cm·s$^{-1}$) | 0.976 | 5.19 | 0.713 | 0.794 | < 0.001 | 9.76 |
| Arterial Cross-Sectional Area (cm$^2$) | 1087.68 | 0.462 | 0.182 | < 0.001 |
| Constant | -90.06 | |
| Women Posttest Change | Time Averaged Flow Velocity (cm·s$^{-1}$) | 0.911 | 8.29 | 0.899 | 0.856 | < 0.001 | 36.6 |
| Arterial Cross-Sectional Area (cm$^2$) | 1537.75 | 0.236 | 0.055 | 0.008 |
| Constant | -27.26 | |
| Men and Women Baseline | Time Averaged Flow Velocity (cm·s$^{-1}$) | 0.962 | 6.41 | 0.680 | 0.629 | < 0.001 | 14.59 |
| Arterial Cross-Sectional Area (cm$^2$) | 1193.15 | 0.588 | 0.332 | < 0.001 |
| Constant | -124.04 | |
| Men and Women Posttest Change | Time Averaged Flow Velocity (cm·s$^{-1}$) | 0.911 | 9.98 | 0.793 | 0.799 | < 0.001 | 50.9 |
| Arterial Cross-Sectional Area (cm$^2$) | 2666.66 | 0.350 | 0.112 | < 0.001 |
| Constant | -60.41 | |

Standard error of the estimate (SEE)

Figure 2: (a-c) Displays the correlations between the changes from baseline to posttest at visit two in arterial cross-sectional area and brachial artery blood flow for the men (a), women (b), and combined sample (men and women; c). In addition, for each correlation, the associated $r^2$ value and P value have been provided.
flow velocity was more highly correlated with brachial artery blood flow than was arterial cross-sectional area. For both time-averaged flow velocity and arterial cross-sectional area, however, the SEE values were too large for accurate predictions of baseline or fatigue-induced increases in brachial artery blood flow. Therefore, although time-averaged flow velocity was highly related to the changes in brachial artery muscle blood flow, neither time-averaged flow velocity or arterial cross-sectional area provide a meaningful measurement of brachial artery muscle blood flow when used separately.

**Stepwise regression for predicting brachial artery blood flow from arterial cross-sectional area and time-averaged flow velocity**

In the present study, both arterial cross-sectional area and time-averaged flow velocity contributed significantly \((p < 0.05)\) to the stepwise regression models to predict baseline and fatigue-induced changes in brachial artery blood flow from the men, women, and combined sample [Table 4]. Together, these variables accounted for 96.2%–99.2% of the variance in baseline brachial artery blood flow and 91.1%–96.8% of the variance in the changes in brachial artery blood flow. For the men, women, and combined sample, time-averaged flow velocity \((r^2 = 0.629–0.856)\) was the most powerful predictor of baseline and fatigue-induced changes in brachial artery blood flow, while arterial cross-sectional area contributed an additional 5.5%–33.2% of the variance [Table 4]. These findings were similar to those of Parker et al.\(^{[3]}\) who reported that the addition of arterial diameter to time-averaged flow velocity accounted for only 2%–14% of the variance in femoral artery blood flow during incremental cycle ergometry. These analyses indicated that together arterial cross-sectional area and time-averaged flow velocity accurately estimated baseline and fatigue-induced changes brachial artery blood flow from the brachial artery in the men, women, and combined sample. These findings, in conjunction with the one-variable models, indicated that the accurate assessment of brachial arterial muscle blood flow requires the measurement of both time-averaged flow velocity and arterial cross-sectional area. Thus, it may be imperative that researchers measure both time-averaged flow velocity and arterial cross-sectional area when making inferences on the magnitude of changes in muscle blood flow as a result of exercise and/or supplementation.

In summary, the findings of the present study supported the use of ultrasound for reliable assessments of arterial diameter, arterial cross-sectional area, time-averaged flow velocity, and brachial artery blood flow from the brachial artery in both men and women. Although there were significant one-variable models for predicting baseline as well as fatigue-induced changes in brachial artery blood flow from arterial cross-sectional area and time-averaged flow velocity, the SEE values were too large (16.8%–37.0% of mean values) to make these one-variable models of practical value. The two-variable models for predicting brachial artery blood flow,
however, accounted for 96.2%–99.2% of the variance in baseline brachial artery blood flow and were associated with SEE values that ranged from 3.7%–10.1% of the mean values. For the men, women, and combined sample, time-averaged flow velocity was the most powerful predictor accounting for 62.9%–85.6% of the variance in brachial artery blood flow, while arterial cross-sectional area contributed an additional 5.5%–33.2% of the variance in brachial artery blood flow. Together, these findings demonstrated the contributions of arterial cross-sectional area and time-averaged flow velocity in the assessment of brachial artery blood flow in the men, women, and combined sample.

**Limitations**
In the present study, brachial artery blood flow was derived from ultrasound-based measurements of arterial cross-sectional area and time-averaged flow velocity. The relative contributions of arterial cross-sectional area and time-averaged flow velocity (indirect measures of blood flow) were used to determine brachial arterial blood flow, instead of a direct assessment of brachial artery blood flow. Under these conditions, it is also assumed that the measurement of arterial cross-sectional area is captured while the artery is stationary, and time-averaged flow velocity is collected over the same period. In the present study, however, time-averaged flow velocity was collected within 8 s of arterial cross-sectional area. Finally, under the conditions imposed in the present study, it was assumed that arterial cross-sectional area and time-averaged flow velocity would contribute to the determination of brachial artery blood flow similarly for each subject.

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**Conflicts of interest**
There are no conflicts of interest.

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