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INNOVATIVE METHODOLOGY

Reliability and agreement of human renal and segmental artery hemodynamics measured using Doppler ultrasound

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Chapman CL, Johnson BD, Hostler D, Lema PC, Schlader ZJ. Reliability and agreement of human renal and segmental artery hemodynamics measured using Doppler ultrasound. *J Appl Physiol* 128: 627–636, 2020. First published February 6, 2020; doi:10.1152/japplphysiol.00813.2019.—To optimize study design and data interpretation, there is a need to understand the reliability of Doppler ultrasound-derived measures of blood velocity (BV) measured in the renal and segmental arteries. Thus, this study tested the following two hypotheses: *1*) renal and segmental artery BV measured over the current standard of three cardiac cycles have good agreement with measurements over nine cardiac cycles (*study 1*); and *2*) renal and segmental artery BV measurements have relatively poor day-to-day reliability (*study 2*). In *study 1*, there was excellent agreement between measurements over three and nine cardiac cycles for BV in both the renal and segmental arteries, as evidenced by BV measurements that were not statistically different ($P \geq 0.68$), were highly consistent ($r \geq 0.99$, $P < 0.01$), had a coefficient of variation $\leq 2.5 \pm 1.8\%$, and 97% (renal artery) and 92% (segmental artery) of the individual differences fell within the 95% limits of agreement. In *study 2*, there was relatively good day-to-day reliability in renal artery BV as evidenced by no differences between three separate days ($P \geq 0.30$), an intraclass correlation coefficient (ICC) of 0.92 (0.78, 0.98), and $7.4 \pm 5.5\%$ coefficient of variation. The day-to-day reliability was relatively poor in the segmental artery with an ICC of 0.77 (0.41, 0.93) and $9.0 \pm 5.6\%$ coefficient of variation. These findings support measuring renal and segmental artery hemodynamics over three cardiac cycles and the utility in reporting renal BV across days. However, because of the variation across days, hemodynamic responses in the segmental arteries should be reported as changes from baseline when making comparisons across multiple days.

NEW & NOTEWORTHY The present study indicates that Doppler ultrasound-derived measures of renal and segmental artery hemodynamics over three cardiac cycles have excellent agreement with those over nine cardiac cycles. These findings support the current practice of measuring renal and segmental artery blood velocity over three cardiac cycles. This study also demonstrates that there is excellent day-to-day reliability for measures of renal artery blood velocity, which supports reporting absolute values of renal artery blood velocity across days. However, it was also found that the day-to-day reliability of segmental artery measurements is relatively poor. Thus, to account for this variability, we suggest that segmental artery hemodynamics be compared as relative changes from baseline across separate days.

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renal blood flow; renal blood velocity; renal vascular resistance; repeatability; reproducibility

INTRODUCTION

The kidneys are highly vascularized organs where precise regulation of blood flow is critical to performing numerous homeostatic functions, including the regulation of body fluids/electrolytes, blood pressure, and blood pH (3). There is great interest in quantifying renal hemodynamics in diseased states (e.g., kidney disease, hypertension), following kidney transplantation or surgery, and when studying the response of the renal vasculature to physiological perturbations (6, 15, 16, 18, 42, 46, 48). Doppler ultrasound is commonly used in both clinical and research settings to assess renal hemodynamics in humans because of its noninvasive nature and the ability to quickly render and capture real-time images (3, 6, 7, 9, 15, 18, 35, 46). This feature allows the sonographer to measure acute vascular responses (i.e., within seconds) in similar regions of the kidney within and between subjects, a vital component that increases scientific rigor (3).

The main limitation of using Doppler ultrasound is that the technique cannot directly quantify volumetric renal or segmental blood flow. This is because the diameter of the vessel walls of the renal vasculature cannot be accurately measured because of the depth of the kidneys in the human body and the associated reduced resolution of Doppler imaging with increasing tissue depth. Thus, Doppler ultrasound in the context of the renal vasculature is only able to quantify blood velocity (3). As a result, use of Doppler ultrasound to quantify hemodynamics in the renal and segmental arteries operates under the assumption that vessel diameter is not changing during physiologically induced changes in blood flow. This assumption is based on evidence that, during infusion of vasoconstrictor (adenosine) and vasodilator (isosorbide dinitrate, papaverine, dopamine, and fenoldopam) agents, changes in renal blood flow are dependent on changes in blood velocity and not changes in the diameter of the renal artery (26, 27). This is further supported by the fact that the renal and segmental arteries are conduit, and not resistance, vessels (12). Therefore, changes in Doppler ultrasound-derived indexes of renal blood velocity are often interpreted as changes in renal blood flow (9–13, 17, 18, 24, 30–37, 39, 43, 46, 51).

There are several ways in which measurement error can be introduced when measuring blood velocity. For instance, the location of the sample volume relative to the size of the vessel (i.e., if it is not in the center of the vessel or if the sample volume is not in the same segment within a vessel) and the insonation location and angle of the transducer relative to the renal vasculature can all introduce measurement error and increase the variability of the blood velocity measurement. This methodological error is potentially further confounded by the fact that kidney function, and presumably blood flow, is modulated by a multitude of factors, including circadian rhythms (50), age (47), sex (25), race (40), diet (38), hydration (1), and exercise (41). Therefore, even if strict controls are in place to reduce confounding variables, the repeatability of renal blood velocity measurements across different days may not be ideal. With this background, there is a need to understand the reliability of renal blood velocity measurements in the renal and segmental arteries. This understanding will improve the interpretation of previously presented data and will aid study design and data interpretation efforts of future studies.

The current practice to measure blood velocity in the renal and segmental arteries is to capture data over two to four cardiac cycles (9, 17, 18, 30–37, 39, 46, 51). This relatively small sample window is often used due to the effect of the respiratory cycle on changes in the insonation angle of the artery in respect to the abdominal wall (35). Even during relatively short-duration (1- to 2-min) physiological stressors, two to four cardiac cycles is a small sample window. However, the kidneys are uniquely sensitive to sympathetic activation, as demonstrated by findings that renal blood velocity decreases by ~14% within 10 s of the start of a sympathetic stressor (i.e., handgrip exercise; see Ref. 31). Thus, obtaining Doppler ultrasound measurements over two to four cardiac cycles is likely necessary when probing the dynamic control of the renal vasculature to sympathetic stimuli. However, it remains unknown if measuring blood velocity in the renal vasculature over two to four cardiac cycles has good agreement compared with measuring blood velocity over a longer measurement period (e.g., 9 cardiac cycles). In this context, we tested the following two hypotheses: *1*) renal and segmental artery blood velocity measurements over three cardiac cycles have good agreement with blood velocity measured over nine cardiac cycles (*study 1*), and *2*) renal and segmental artery blood velocity measurements have relatively poor reliability when measuring across different days (*study 2*).

METHODS

Participants

Twelve healthy adults (three women) participated in this study. Subject characteristics were age 23 ± 2 yr, height 176 ± 8 cm, weight 77.9 ± 14.5 kg, and body mass index 25.0 ± 3.2 kg/m^2. All subjects reported to be free from any known cardiovascular, renal, metabolic, neurological, or gastrointestinal diseases and were physically active nonsmokers. Female subjects self-reported to be normally menstruating and were not pregnant at any point during the study, which was confirmed via a urine pregnancy test before each trial. Additionally, female subjects were not on birth control and were tested during the first 10 days of their self-identified menstruation. Subjects visited the laboratory on four separate occasions: a screening visit and three experimental trials. Experimental trials were separated by at least 7 days. This study was approved by the Institutional Review Board at the University at Buffalo in accordance with the Declaration of Helsinki. Informed written consent was obtained from all subjects before their participation in this study.

Nonultrasound Instrumentation and Measurements

Height and nude body weight were measured using a stadiometer and scale (Satorius, Bohemia, NY). All blood pressure measurements were measured manually in duplicate by the same member of the research team.

Doppler Ultrasound Technique and Approach

General methodology. Renal artery and segmental artery blood velocities were obtained via Doppler ultrasound (GE Vivid iQ, Chicago, IL) and a phased-array transducer (2.5–3.5 MHz) using the coronal approach with subjects in the left lateral recumbent position (9, 46; Fig. 1). The coronal approach allowed for measurements in both the distal segment of the renal artery (i.e., immediately before blood enters the kidney) and the segmental arteries in the kidney. Using this approach, the transducer was placed in a lower rib interspace (i.e., coronal approach) where the liver acts as an acoustic window and the vasculature of the kidneys is more consistently imaged because of the absence of superficial bowel gas (14). The anatomy of the kidney was identified due to the more hyperechoic nature of the renal capsule and renal pelvis and the comparatively more hypoechoic nature of the renal parenchyma (Fig. 2*A*). Furthermore, clear branching of the renal vasculature was observed within the renal pelvis to delineate the segmental arteries. Thus, measurements of blood velocity from both the renal and segmental arteries were achieved. Additionally, renal artery blood flow is in parallel to the Doppler beam in the coronal approach, which optimized velocity measurements by eliminating the need to correct for the insonation angle (21). The following sections detail the methodological approach used to measure renal and segmental artery blood velocity.

Optimization of renal ultrasound imaging. The renal presets for the transducer (M4S-RS; General Electric Healthcare, Chicago, IL) were

Fig. 1. Experimental set-up. The coronal approach is used with the transducer placed in the lower rib interspace.

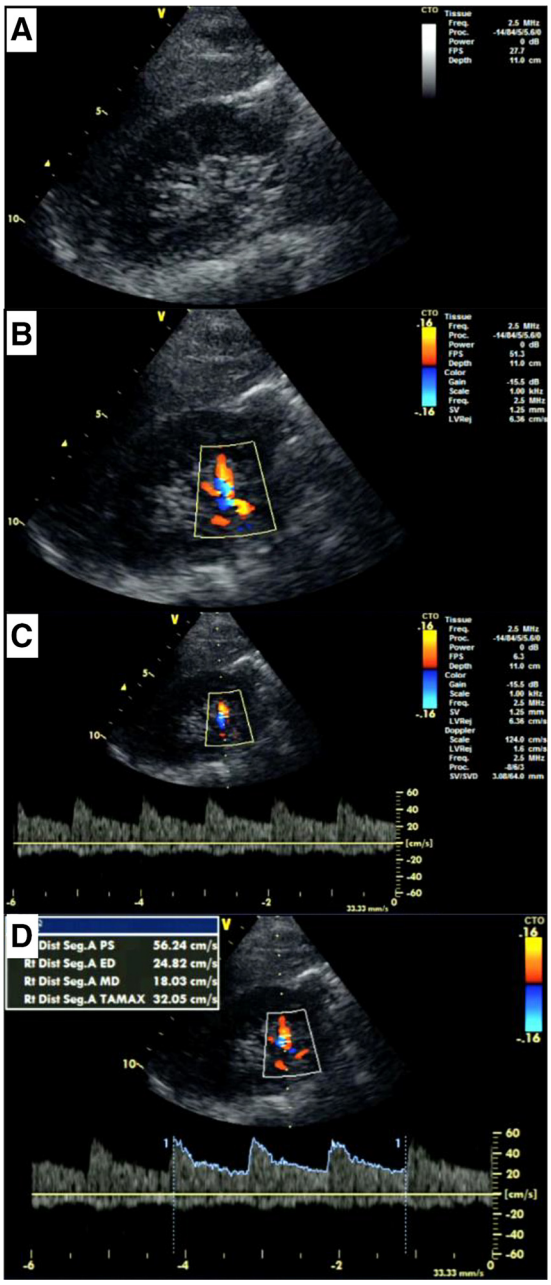

Fig. 2. Doppler ultrasound imaging of the kidney. *A*: grayscale; *B*: color flow mode; *C*: pulsed wave velocity mode; *D*: segmental artery blood velocity envelope is traced over three cardiac cycles, and values for peak systolic velocity (PS), end-diastolic velocity (ED), and time-averaged maximum velocity (i.e., mean blood velocity, TAMAX) are presented.

optimized as follows. Per the recommendations by Galgano et al., grayscale settings were optimized first to facilitate imaging in other Doppler modes (20). The 2D gain setting was adjusted so that the renal parenchyma was slightly hypoechoic to the liver, and the renal pelvis was hyperechoic to the renal parenchyma and liver (Fig. 2*A*). Because increasing depth reduces both resolution and frame rate, the depth was adjusted to the minimum setting that allowed for complete visualization of the renal vasculature (20). Additionally, the focal zone was adjusted to the depth of the renal artery (18). Color Doppler mode was then optimized following the adjustments made to the grayscale settings (Fig. 2*B*). Per the methods of Galgano et al., the color gain was increased until the appearance of random color specks and was then subsequently reduced until these random specks of color from nonvascular structures disappeared (20). After this, pulsed wave mode was optimized (Fig. 2*C*). The sample volume was set to ~5–6 mm for the renal artery and ~3–4 mm for the segmental artery. The velocity scale was adjusted so that peak systolic velocity could be captured (20).

Subject familiarization with the breath holding protocol. Previous work has indicated that respiration has an effect on renal blood velocity measurements by moving the renal artery with respect to the abdominal wall (35) and by slightly increasing the variation between successive measurements at higher breathing rates (49). However, a breath hold of 20–40 s following moderate expiration does not appear to have an effect on renal blood velocity measurements (49). Thus, in the present study, we adopted a non-Valsalva midexhalation breath hold technique lasting no more than 5–10 s (9, 46) to reduce the potential confounding effects of increased sympathetic activity associated with breath holding (8). In the present study, subjects were familiarized with the breath-holding protocol and were instructed to take a normal inhalation before the breath hold began. Additionally, the same verbal script was used throughout the study protocol in which subjects were instructed, "When you are ready, exhale half-way and hold your breath." Subjects were also instructed by the sonographer to resume breathing immediately after storing the velocity waveform image for future analysis. This process was repeated at least six times (three for the renal artery and three for a segmental artery) following confirmation of the transducer location (see below) to ensure proper collection of blood velocity waveforms.

Control of renal and segmental artery blood velocity measurement location. Based on the judgment of an experienced sonographer, the optimal transducer location was determined by the resolution of the renal vasculature and the ability to repeat measurements with at least six breath holds. The location was marked by tracing the perimeter of the transducer with indelible ink. Blood velocity in the renal and segmental arteries of the right kidney were measured with the focal zone set to the depth of the target artery. Additionally, the insonation angle was $<60°$ (21). The same segmental arteries were used within a given subject. This documented approach yielded a same-day within-subject test-retest coefficient of variation for blood velocity measurements of $3.9 \pm 0.8\%$ in the renal artery and $3.9 \pm 1.2\%$ in the segmental artery for the same sonographer (Chapman; see Ref. 9). For subsequent visits, subjects were instructed to keep the transducer location viable by retracing the location with the permanent marker provided to them.

Experimental Protocol

Subjects arrived at the laboratory having refrained from strenuous physical activity (e.g., exercise), caffeine, and alcohol for 12 h. Additionally, subjects were instructed to finish eating 2 h before the start of the study in an effort to minimize the amount of bowel gas that may interfere with imaging (21). After adherence to the dietary controls were confirmed via the food/beverage log, a euhydrated state was confirmed via urine specific gravity <1.020 (45). Subjects were asked to record their fluid and food intake for the 24 h before the start of the study and to replicate these intakes for the subsequent visits. There were no differences in subject fluid and food intake across days (Table 1). Subjects then laid in the left lateral recumbent position and were familiarized with the experimental protocols described above. After confirming the transducer location and familiarizing the subjects with the breath-hold protocol, subjects rested for 20 min. After this, manual brachial artery blood pressure was measured in duplicate. Renal and segmental artery blood velocity measurements were then taken over three cardiac cycles. Following this, subjects were to catch their breath, and then measurements resumed. This process was repeated a total of three times over ~30 s, yielding renal and segmental artery blood velocity measurements over three, six, and nine cardiac cycles. Renal artery blood velocity measurements were always taken

Table 1. *Twenty-four-hour fluid and beverage intake before experimental trial*

	Day 1	*Day 2*	*Day 3*
Fluid volume, mL	4,002 (1,044)	3,948 (1,223)	3,772 (857)
Total energy, kcal	2,491 (600)	2,498 (992)	2,398 (760)
Fat, g	96 (38)	89 (44)	85 (39)
Protein, g	111 (41)	120 (43)	120 (56)
Carbohydrate, g	298 (100)	298 (132)	284 (111)
Sodium, mg	3,219 (1,590)	3,286 (2,001)	3,111 (1,771)

Values expressed as means (SD). Data were analyzed using a one-way ANOVA.

first, followed by segmental artery velocity measurements. Subjects returned to the laboratory on three different days (all visits were separated by at least 7 days) to repeat the same protocol.

Data and Statistical Analyses

Food and beverage intake logs were analyzed for fluid volume and the amount of total energy, fat, protein, carbohydrate, sugar, and sodium using online software (myfitnesspal; Under Armour, Baltimore, MD). Mean arterial pressure was calculated as one-third pulse pressure plus diastolic pressure. Mean renal and segmental artery blood velocity were measured using the timed-average blood maximum velocity (Fig. 2*C*) and were averaged over three, six, and nine cardiac cycles. Time-averaged maximum blood velocity was used to calculate mean blood velocities because the sample volume cannot be accurately fitted to the vessel diameter (4). Segmental artery blood velocity was averaged across three segmental arteries in $n = 11$ and across two segmental arteries in $n = 1$. Vascular resistance in the renal and segmental arteries was calculated as mean arterial pressure divided by blood velocity, and vascular conductance was calculated as blood velocity divided by mean arterial pressure. Given the prevalence of studies that have reported renal and/or segmental artery blood velocity and vascular resistance (9–13, 17, 18, 24, 30–37, 39, 43, 46) or conductance (51), all analyses were completed on measures of blood velocity, vascular resistance, and vascular conductance.

Fluid and beverage intakes were analyzed using a one-way ANOVA. Per the recommendations of Atkinson and Nevill, a battery of statistical tests was used to assess the reliability of renal and segmental artery blood velocity measurements (2). The purpose of *study 1* was to determine the agreement (i.e., variability between measurement techniques; see Ref. 2) of renal and segmental hemodynamics measured over three cardiac cycles compared with those obtained when measured over six and nine cardiac cycles. Renal and segmental artery hemodynamics were obtained in $n = 12$. In *study 1*, a one-way ANOVA with post hoc Tukey pairwise comparisons were used to analyze differences across blood velocity and vascular resistance over three, six, and nine cardiac cycles. The coefficients of variation were calculated across three, six, and nine cardiac cycles in both renal and segmental arteries to provide an index of the relative differences of blood velocity and vascular resistance/conductance. The reliability of blood velocity and vascular resistance/conductance in the renal and segmental arteries across three, six, and nine cardiac cycles was assessed using a two-way mixed-effects model intraclass correlation where people effects are random and measures effects are fixed (23, 28). Type A intraclass correlation coefficients with an absolute agreement definition were used. Intraclass correlation coefficients were interpreted from the 95% confidence intervals of the mean as recommended by Koo and Li (23), where "values less than 0.5 are indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability." Bland-Altman plots were created by calculating mean bias and limits of agreement, where the difference between measurements (e.g., between three and nine cardiac cycles) was to be between the limits of agreement with 95% probability if the measurements are considered to be reliable (5, 19).

The purpose of *study 2* was to determine the day-to-day reliability (i.e., stability variability; see Ref. 2) in renal and segmental hemodynamic measures across three different days. Hemodynamics were obtained in $n = 11$ in the renal artery (with one subject excluded due to acoustic shadowing of the kidney) and $n = 12$ in the segmental artery. First, a one-way ANOVA with post hoc Tukey pairwise comparisons was used to analyze differences across blood velocity and vascular resistance/conductance across three separate days. Coefficients of variation were calculated across *days 1*, *2*, and *3* in both renal and segmental arteries to index relative differences in blood velocity and vascular resistance/conductance. The reliability of blood velocity and vascular resistance/conductance in the renal and segmental arteries across days was analyzed and interpreted using intraclass correlations as described above. The ANOVAs and Bland-Altman plots were analyzed using GraphPad Prism (version 8; GraphPad Prism, La Jolla, CA). Intraclass correlations were analyzed using SPSS (version 25; IBM, Armonk, NY).

RESULTS

Study 1: Agreement of Measuring Renal and Segmental Hemodynamics Over Three Cardiac Cycles with Nine Cardiac Cycles

Mean arterial pressure was 86 ± 6 mmHg. There were no differences in blood velocity ($P \geq 0.68$), vascular resistance ($P \geq 0.85$), or vascular conductance ($P \geq 0.80$) in the renal artery across cardiac cycles (Fig. 3). Additionally, there were no differences in blood velocity ($P \geq 0.72$), vascular resistance ($P \geq 0.91$), or vascular conductance ($P \geq 0.72$) in the segmental artery (Fig. 3). Intraclass correlations revealed that blood velocity, vascular resistance, and vascular conductance in the renal and segmental arteries were highly consistent between measurements over cardiac cycle duration ($r \geq 0.99$, Table 2). Additionally, the coefficient of variation between measurements of three versus nine cardiac cycles was low for blood velocity (≤2.5 ± 1.8%), vascular resistance (≤2.6 ± 1.7%), and vascular conductance (≤2.6 ± 1.8%) in the renal and segmental arteries (Table 3). In the renal artery, the mean bias and range of limits of agreement (min, max) for three-to-nine cardiac cycles was 0.24 (−3.17, 2.70) cm/s for blood velocity, 0.01 (−0.23, 0.26) $mmHg \cdot cm^{-1} \cdot s^{-1}$ for vascular resistance, and 0.0 (−0.04, 0.03) $cm \cdot s^{-1} \cdot mmHg^{-1}$ for vascular conductance, with 97% (35 out of 36) of the individual differences falling within the limits of agreement (Fig. 4, *D–F*). In the segmental artery, the mean bias and range of limits of agreement (min, max) for three-to-nine cardiac cycles was 0.18 (−1.39, 1.75) cm/s for blood velocity, −0.01 (−0.39, 0.36) $mmHg \cdot cm^{-1} \cdot s^{-1}$ for vascular resistance, and 0.0 (−0.02, 0.02), with 92% (33 out of 36) of the individual differences falling within the limits of agreement (Fig. 5, *D–F*).

Study 2: Day-to-Day Reliability of Measuring Renal and Segmental Hemodynamics Over Three Cardiac Cycles Across Three Separate Days

Based on the excellent absolute reliability of measuring renal and segmental hemodynamics over three cardiac cycles compared with nine cardiac cycles found in *study 1*, and because this measurement period aligns with previously established literature, the day-to-day reliability of these measures

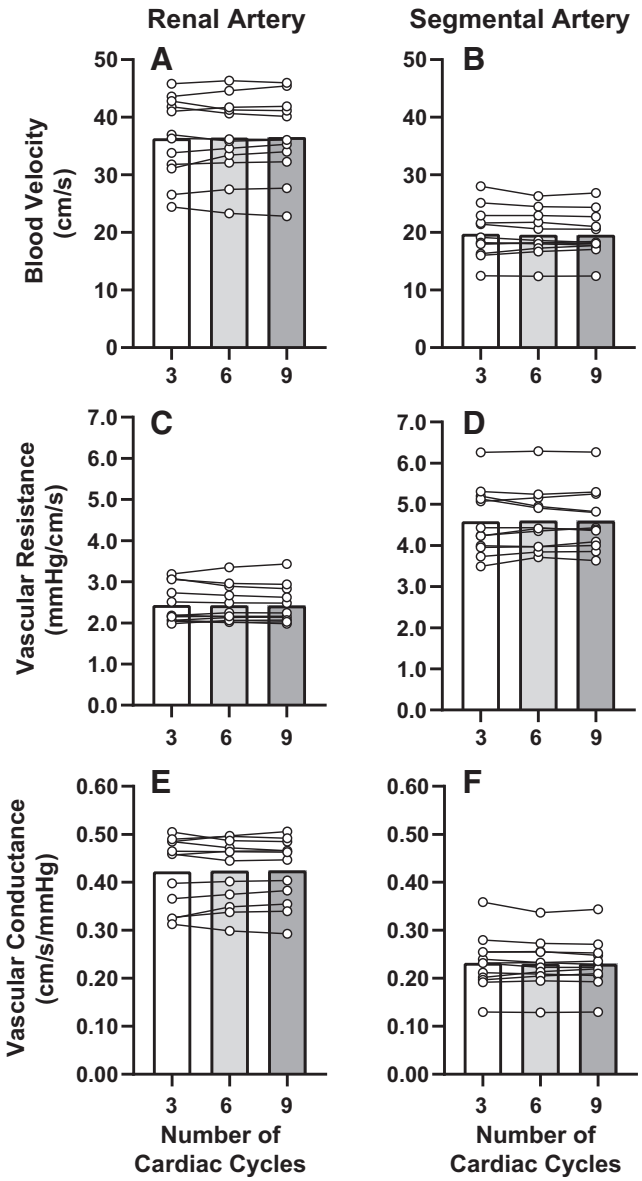

Fig. 3. Blood velocity (*A* and *B*), vascular resistance (*C* and *D*), and vascular conductance (*E* and *F*) measured via Doppler ultrasound in the renal ($n = 12$) and segmental ($n = 12$) arteries across 3, 6, or 9 cardiac cycles. Data were analyzed using a one-way ANOVA and revealed no significant differences between cardiac cycles in any measure ($P \geq 0.69$). Data are presented as means (SD) with individual values.

over three cardiac cycles were analyzed. There were no differences in mean arterial pressure ($P \geq 0.44$) on *day 1* (87 ± 6 mmHg), *day 2* (85 ± 5 mmHg), or *day 3* (85 ± 7 mmHg). Additionally, there were no differences in blood velocity ($P \geq 0.30$), vascular resistance ($P \geq 0.27$), or vascular conductance ($P \geq 0.46$) in the renal artery across measurements over 3 days (Fig. 6). Additionally, there were no differences in blood velocity ($P \geq 0.35$), vascular resistance ($P \geq 0.20$), or vascular conductance ($P \geq 0.15$) in the segmental artery, across measurements over the 3 days (Fig. 6). The coefficients of variation for blood velocity, vascular resistance, and vascular conductance in the renal and segmental artery across days were ≤9.9 ± 5.8% (Table 4). Across days in the renal artery, there was good to excellent reliability for blood velocity, and moderate to excellent reliability for vascular resistance and conductance (Table 5). However, in the segmental artery, blood velocity measurements had moderate to excellent reliability across days, and vascular resistance and conductance measurements had poor to excellent reliability across days (Table 5). Thus, these data indicate that the day-to-day reliability for measurements of renal artery hemodynamics is relatively good, whereas the reliability in segmental artery hemodynamic measurements is rather poor.

Table 2. *Reliability of renal and segmental artery hemodynamics across cardiac cycle duration*

	Renal Artery	Segmental Artery
Blood velocity	0.996 (0.989, 0.999)	0.996 (0.989, 0.999)
Vascular resistance	0.993 (0.982, 0.998)	0.994 (0.985, 0.998)
Vascular conductance	0.995 (0.986, 0.998)	0.996 (0.990, 0.999)

Values are expressed as the coefficients of average measures with 95% confidence intervals. Blood velocity, vascular resistance, and vascular conductance in the renal ($n = 12$) and segmental ($n = 12$) arteries were measured across 3, 6, and 9 cardiac cycles with the same sonographer. The reliability of these measurements was analyzed with intraclass correlation coefficients using a two-way mixed-effects model where people effects are random and measures effects are fixed. Type A intraclass correlation coefficients with an absolute agreement definition were used.

DISCUSSION

In support of our first hypothesis, there is excellent agreement in renal and segmental artery measures of blood velocity, vascular resistance, and vascular conductance between measurement periods over three cardiac cycles compared with those data obtained over six and nine cardiac cycles. These findings support the use of Doppler ultrasound-based measures of renal and segmental artery hemodynamics over three cardiac cycles when practical limitations preclude use of longer measurement periods, such as during acute sympathetic stressors (e.g., the cold pressor test). In contrast to our second hypothesis, there is relatively good day-to-day reliability in measures of blood velocity and vascular resistance/conductance in the renal artery across separate days. However, in support of our second hypothesis, there is relatively poor day-to-day reliability in measures of blood velocity and vascular resistance/conductance in the segmental artery across days. Therefore, it is likely more appropriate to report segmental artery hemodynamics between days as a function of the absolute changes from baseline values before the physiological stressor. However, our data support that it may be appropriate to compare

Table 3. *Coefficients of variation*

	3 versus 6	3 versus 9	6 versus 9
Renal artery			
Blood velocity, %	2.0 (1.3)	2.5 (1.8)	0.7 (0.5)
Vascular resistance, %	2.0 (1.2)	2.6 (1.7)	0.8 (0.6)
Vascular conductance, %	2.0 (1.3)	2.6 (1.8)	0.7 (0.5)
Segmental artery			
Blood velocity, %	1.7 (1.5)	2.4 (1.7)	1.0 (0.7)
Vascular resistance, %	1.7 (1.4)	2.3 (1.6)	1.1 (0.7)
Vascular conductance, %	1.7 (1.5)	2.4 (1.7)	1.0 (0.7)

Values expressed as means (SD). Coefficient of variation (%) for blood velocity, vascular resistance, and vascular conductance in the renal and segmental arteries between measurements over 3, 6, and 9 cardiac cycles.

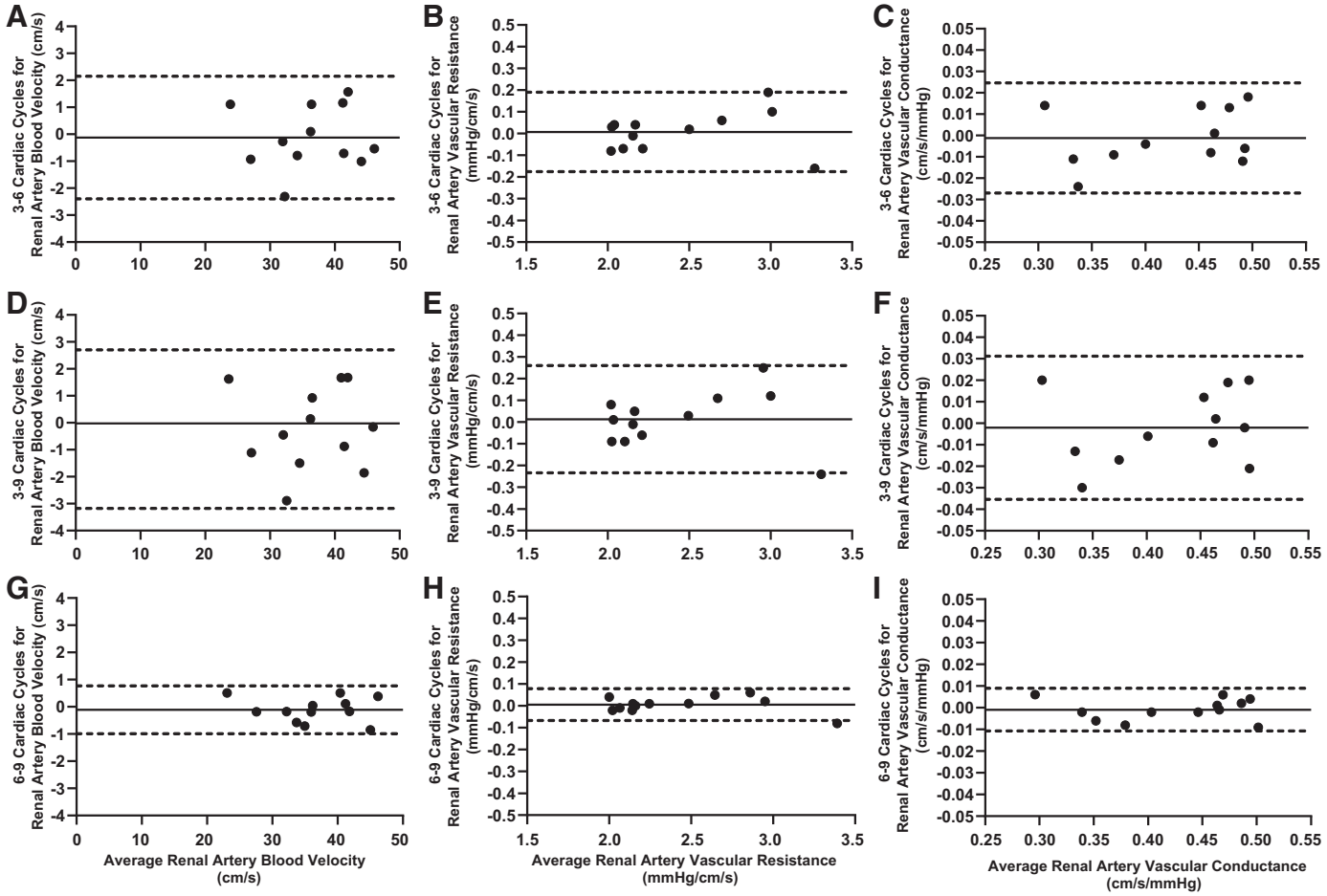

Fig. 4. Bland-Altman plot comparing the average blood velocity, vascular resistance, or vascular conductance in the renal artery (*x*-axis) with the difference in respective value between number of cardiac cycles over which the measurement was taken (*y*-axis) of 3–6 (*A–C*), 3–9 (*D–F*), and 6–9 (*G–I*) cardiac cycles. The solid bold line represents the mean bias, and the dashed lines represent the 95% limits of agreement. $n = 12$.

absolute values of renal artery hemodynamics across days given the relatively good day-to-day reliability in these measures across days.

Agreement Between Shorter- and Longer-Duration Measures of Renal and Segmental Artery Hemodynamics

The findings from our study suggest that measuring renal and segmental artery blood velocity over three cardiac cycles is an appropriate approach for quantifying hemodynamics in these arteries compared with sample windows of up to nine cardiac cycles. This is important because there may be practical limitations that may preclude the ability to measure blood velocity over nine cardiac cycles. For instance, respiration can modify the blood velocity measurement by changing the angle of insonation of the vessel to the transducer and by moving the vessel with respect to the abdominal wall. Thus, the duration of the blood velocity measurement may be limited by the ability of the subject to hold their breath (35). Additionally, long-duration breath holding (i.e., 30 s) increases sympathetic activity (8) and may add a confounding sympathetic stimulus to experiments investigating the renal vascular response to sympathetic activation. To overcome this, our laboratory has adopted a non-Valsalva midexhalation breath hold for no more than 5–10 s (9). Thus, the practical limitations of this breath-holding technique require that blood velocity measurements be performed over three cardiac cycles. Additionally, the renal vasculature responds rapidly to sympathetic activation as evidenced by 14% reductions in renal artery blood velocity within 6–10 s of handgrip exercise (31). Therefore, it may be necessary to measure changes in hemodynamics over three cardiac cycles to quantify the initial response of the renal vasculature to the sympathetic stimulus. In these instances, traditional clearance-based techniques (e.g., *para*-aminohippurate clearance, MRI) are unable to quantify changes in renal hemodynamics over such short durations. Thus, Doppler ultrasound is an appealing technology to noninvasively quantify these rapid and dynamic changes in renal hemodynamics. Collectively, our findings, which demonstrate that measuring three cardiac cycles has excellent agreement with those data collected over six and nine cardiac cycles, provide support for quantifying rapid changes in renal hemodynamics over three cardiac cycles.

Day-to-Day Reliability of Measuring Renal and Segmental Artery Hemodynamics

In *study 2*, renal and segmental artery hemodynamics were measured on three different days where there were no differences in pretrial dietary and fluid intakes. Our data indicate that

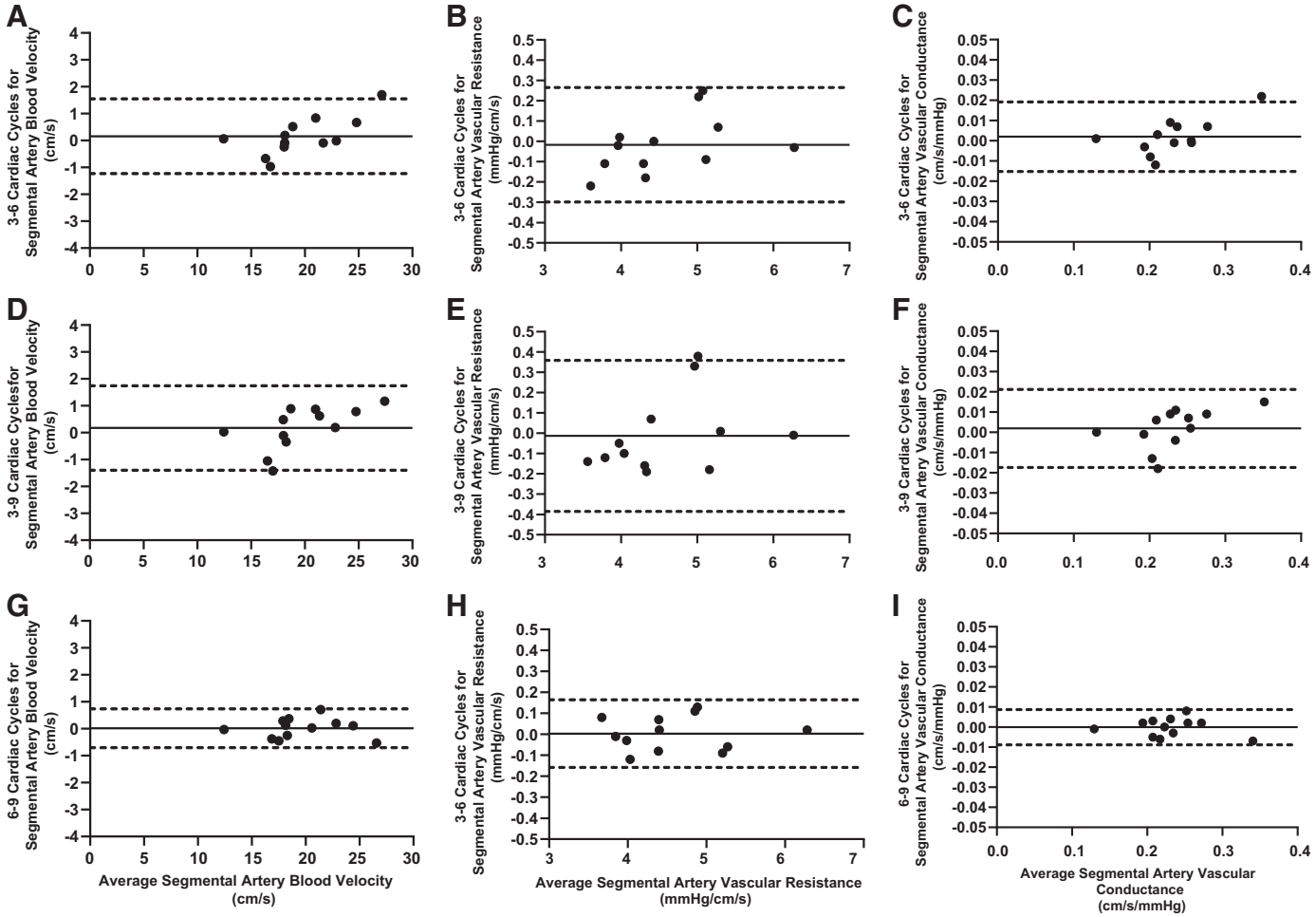

Fig. 5. Bland-Altman plot comparing the average blood velocity, vascular resistance, or vascular conductance in the segmental artery (*x*-axis) with the difference in respective value between number of cardiac cycles over which the measurement was taken (*y*-axis) of 3–6 (*A–C*), 3–9 (*D–F*), and 6–9 (*G–I*) cardiac cycles. The solid bold line represents the mean bias, and the dashed lines represent the 95% limits of agreement. $n = 12$.

measuring renal artery hemodynamics across multiple days has good reliability and supports the use of reporting absolute values of renal artery blood velocity. Importantly, we speculate that the reliability of renal artery hemodynamics will be worse without strict experimental controls. This is supported by the incredible variability in renal function (and presumably renal blood flow) associated with circadian rhythms (50), age (47), sex (25), race (40), diet (38), hydration (1), and exercise (41). However, it remains unknown if there is still good day-to-day reliability for renal artery hemodynamics when stringent experimental controls are not in place.

In contrast to the data obtained from the renal artery, segmental artery hemodynamics demonstrated relatively poor day-to-day reliability. It is unclear whether these finding are the result of the measurement technique or physiological reasons. The nature of measuring segmental artery hemodynamics requires measurements to be obtained in smaller vessels, where slight deviations from the exact location within the artery (i.e., proximal, middle, or distal segments) could result in greater variability within each measurement. Thus, it is possible that our findings could be explained by slight deviations of the sample volume location or angle of insonation of the transducer when measuring segmental artery hemodynamics. However, the within-day coefficient of variation for measuring blood velocity in the segmental artery was relatively low at $3.9 \pm 1.2\%$, which suggests that either repeating the exact location of the sample volume within the middle portion of the segmental artery does not have a large effect on blood velocity measurements and/or suggests that we are able to successfully reproduce the location of the sample volume within a precise window with the current technique. Thus, we conclude that our findings of poor day-to-day reliability of segmental artery hemodynamics across days is physiological in nature and may be contributed to by the aforementioned variability in renal function. It is not clear why the day-to-day reliability was not consistent between the renal and segmental arteries. However, it may be that the day-to-day variability is higher in arteries located anatomically closer to the resistance vessels because of the smaller vessel size, which may increase the chance that the sample volume is not in the exact same location as previous measures. To account for this variability across days, we suggest that studies measuring the segmental artery hemodynamic response to a given physiological stressor across multiple days present data as a change from preperturbation to limit the confounding influence of poor day-to-day reliability of the Doppler ultrasound measurement technique.

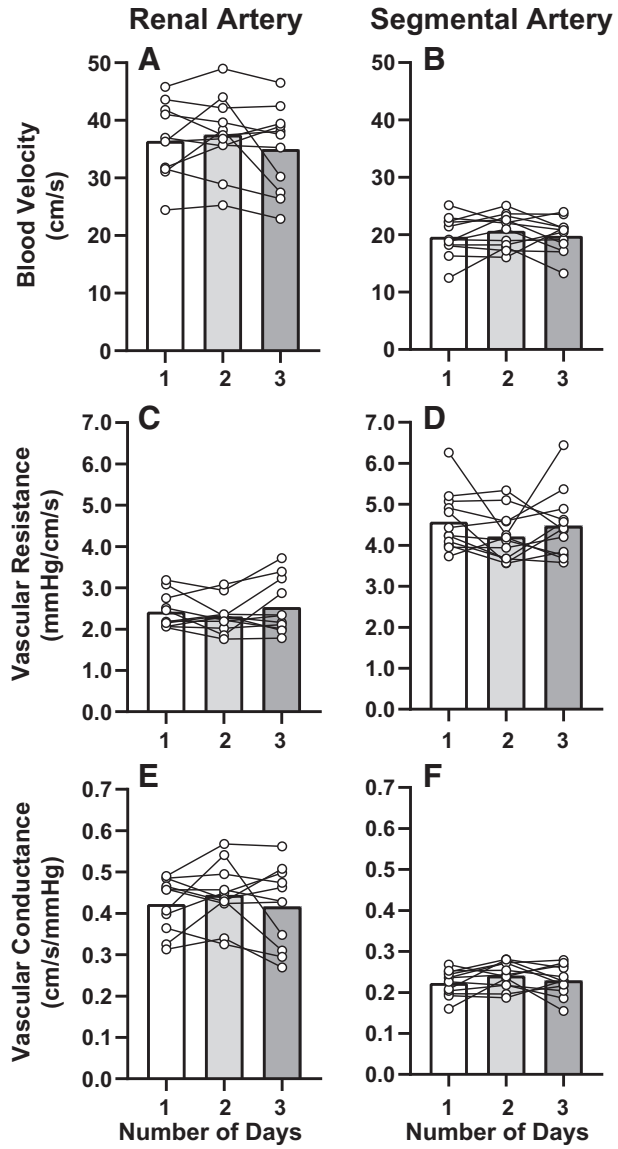

Fig. 6. Blood velocity (*A* and *B*), vascular resistance (*C* and *D*), and vascular conductance (*E* and *F*) measured via Doppler ultrasound in the renal ($n = 11$) and segmental ($n = 12$) arteries measured across 3 separate days with ≥7 days separating each trial. Data were analyzed using a one-way ANOVA and revealed no significant differences between cardiac cycles in any measure ($P \geq 0.20$). Data are presented as means (SD) with individual values.

Considerations

The findings of the present study are constrained to the methodology applied herein. Thus, our findings of excellent agreement between measures of renal and segmental artery hemodynamics with Doppler ultrasound are confined to using the coronal approach and taking these measures in the distal segment of the right renal artery and the middle portion of the segmental artery in the right kidney. The notable difference of using the coronal approach in the left kidney is that the spleen is used as the acoustic window; however, all other aspects of the approach are similar to that of the right kidney (14). The present study investigated the reliability of renal and segmental artery hemodynamics to a maximum of nine cardiac cycles. The reliability of these measurements over more cardiac cycles is not known. To this point, future investigations should consider the efficacy and reliability of measuring renal and segmental artery hemodynamics during continuous breathing to further explore if the breath hold-technique is necessary for maintaining consistency in the measurements. The findings of the present study are likely constrained to relatively static measurements since, to our knowledge, the reliability of renal and segmental artery blood velocity measurements during dynamic physiological stress has never been assessed. Additionally, it is not known if our findings in the right renal artery are indicative of the agreement between measurement duration and/or the day-to-day reliability across days for Doppler ultrasound measurements in the renal artery of the left kidney, or in the proximal segment of either renal artery using the anterior approach. Whether these findings hold true for different locations within the segmental artery (i.e., proximal, middle, or distal segments) remains unknown. It is also not known if our findings are applicable beyond that of young healthy adults, considering the loss of functional nephrons associated with healthy aging (22) or that some diseased states may narrow the walls of the renal artery (i.e., stenosis; see Ref. 44). Additionally, the subjects in the present study were in a normal weight range. Therefore, these findings may not be representative of those with larger habitus where there is an increased difficulty in obtaining ultrasound measurements in these individuals. Finally, to our knowledge, there has not been a study to investigate the agreement between Doppler ultrasound measures of renal hemodynamics with the more traditionally used *para*-aminohippurate clearance. When examining changes in renal hemodynamics during brief periods of sympathoexcitation (e.g., 2-min cold pressor test), *para*-aminohippurate clearance may not be an appropriate technique because of its dependence on steady-state infusion/excretion. However, the

Table 4. *Coefficients of variation for renal and segmental hemodynamics measured on three different days*

	Renal Artery	Segmental Artery
Blood velocity, %	7.4 (5.5)	9.0 (5.6)
Vascular resistance, %	9.5 (5.8)	9.5 (4.9)
Vascular conductance, %	9.7 (6.3)	9.9 (5.8)

Coefficients of variation (%) across the three separate days were calculated and are expressed as means (SD). Blood velocity, vascular resistance, and vascular conductance in the renal ($n = 11$) and segmental ($n = 12$) arteries were measured in the same subjects on three different days with the same sonographer.

Table 5. *Reliability of renal and segmental artery hemodynamics measured on three different days*

	Renal Artery	Segmental Artery
Blood velocity	0.918 (0.778, 0.976)	0.841 (0.589, 0.950)
Vascular resistance	0.865 (0.639, 0.960)	0.768 (0.413, 0.926)
Vascular conductance	0.853 (0.604, 0.956)	0.771 (0.423, 0.927)

Values expressed as the coefficients of average measures with 95% confidence intervals. Blood velocity, vascular resistance, and vascular conductance in the renal ($n = 11$) and segmental ($n = 12$) arteries were measured in the same subjects on three different days with the same sonographer. The reliability of these measurements was analyzed with intraclass correlation coefficients using a 2-way mixed-effects model where people effects are random and measures effects are fixed. Type A intraclass correlation coefficients with an absolute agreement definition were used.

degree to which Doppler ultrasound measurements of renal and segmental artery hemodynamics represent renal perfusion has not yet been examined. Notably, however, both *para*-aminohippurate clearance and Doppler ultrasound-derived measures of renal vascular resistance during sympathetic activation elicit virtually analogous increases (by ~16–20%; see Refs. 29, 35, and 39). This indirectly supports the use of Doppler ultrasound in the context of renal responses to sympathetic stimuli, but further studies are required.

Perspectives

The findings from the present study provide support for the continued use of Doppler ultrasound measures of renal and segmental artery hemodynamics. This technique is appealing because of its noninvasive nature and the ability to capture dynamic changes in blood velocity responses within seconds, a feature that cannot be obtained via traditional clearance techniques. Thus, there is utility in using Doppler ultrasound to quantify the control of the renal vasculature to acute sympathetic perturbations in clinical and experimental settings. Future work should consider the reliability of this technique across various populations and diseased states and the validity of the technique relative to gold standard measures of renal perfusion (e.g., *para*-aminohippurate clearance, MRI, etc.).

Conclusion

In the present study, we found that there is excellent agreement between Doppler ultrasound-based measures of blood velocity, vascular resistance, and vascular conductance in the renal and segmental arteries over three cardiac cycles compared with nine cardiac cycles. Thus, our findings support the current practice of measuring blood velocity with Doppler ultrasound in renal and segmental arteries across three cardiac cycles. Last, in the present study, we found good day-to-day reliability when measuring renal artery blood velocity, vascular resistance, and vascular conductance across multiple days, but these same measurements had poor day-to-day reliability when measured in the segmental artery. These findings support the use of reporting absolute values of renal artery blood velocity across days and reporting segmental artery blood velocity as comparisons of the change from baseline across days.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.L.C., B.D.J., and Z.J.S. conceived and designed research; C.L.C. and Z.J.S. performed experiments; C.L.C. and Z.J.S. analyzed data; C.L.C., B.D.J., D.H., P.C.L., and Z.J.S. interpreted results of experiments; C.L.C. prepared figures; C.L.C. and Z.J.S. drafted manuscript; C.L.C., B.D.J., D.H., P.C.L., and Z.J.S. edited and revised manuscript; C.L.C., B.D.J., D.H., P.C.L., and Z.J.S. approved final version of manuscript.

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