Measurement of the Luminal Diameter of Peripheral Arterial Vasculature in Yorkshire×Landrace Swine by Using Ultrasonography and Angiography

To select animals of appropriate size for preclinical studies of cardiovascular devices, reference knowledge of the cardiovascular anatomy relative to body weight is crucial. We measured the luminal diameters of the arteries (carotid, femoral, and iliac arteries) that are the common access vessels for endovascular and vascular procedures in Yorkshire×Landrace swine. Measurements were performed by using both ultrasound and angiographic methods and were correlated with body weight. Results showed no statistically significant difference between the left and right vessels in the diameters of the carotid, femoral, and iliac arteries. The diameters of the measured arteries showed high correlation with animal weight in pigs that weighed less than 70 kg.

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Preclinical testing using large animal models is an essential step in translating novel endovascular technology into the clinical treatment of cardiovascular diseases.⁶ Due to their anatomic and physiologic similarities to humans, swine are one of the most commonly used large animals model for translational research and procedural training.^{7,8} Indepth documentation of the anatomy of the cardiovascular system, including the diameters of arteries, is important for selecting animals of the ideal size for the specific testing to be done. Given both the animal welfare and economic costs and the ethical imperative to reduce total animal numbers used in experimentation, the number of animals used for preclinical testing needs to be decreased to a minimum. Because of limited research funds, often only small numbers of prototype profiles or sizes of devices can be fabricated and therefore selecting animals with appropriately sized artery dimensions is essential. Furthermore, for some sophisticated endovascular devices, a lower threshold of artery size for device access is required. Therefore, a reliable way of predicting and selecting swine arterial diameter will improve both the planning and execution of the research studies and reduce waste of both animals and resources.

Little information is available on swine peripheral artery dimensions. One study reported angiographic measured diameters and segment lengths of the iliofemoral arteries of Yorkshire crossbred swine³ and showed that diameters and segment lengths correlate with animal weight. A second study compared the diameter ratios and bifurcation angles of the iliofemoral arteries in mixed-Landrace crossbred swine and Yucatan minipigs with those of the human carotid arteries by using angiographic images, with results suggesting that both Landrace and Yucatan iliofemoral arteries are suitable animal models for human carotid interventions.¹ However, angiography is an invasive procedure and is unsuitable for preprocedural screening and selection of animals. Furthermore, experimental manipulations required to perform angiography (e.g., passing guidewires and catheters) can induce vasoconstriction,² leading to inaccurate measurement of the arteries. Therefore, due to its noninvasive nature, ultrasound imaging of arteries has distinct advantages over angiography as an alternative way of measuring vessel diameter. In this study, we performed ultrasonography to measure the size of superficially located carotid and femoral arteries of swine and angiography to evaluate the deeper lying iliac arteries.

Materials and Methods

Animals. All animal procedures adhered to the guidelines of the National Advisory Committee for Laboratory Animal Research and were approved by the National University of Singapore IACUC. A total of 38 SPF female Yorkshire×Landrace swine (Sus scrofa domesticus; weight, 33.4 to 99.6 kg) specifically bred for research purposes were obtained from the National Large Animal Research Facility of Singapore and used in this study. All swine were housed individually and received standard care according to the standard operating procedures of the Comparative Medicine Department at the National University of Singapore, including Teklad pig diet T8753.15 (Envigo, Indianapolis, IL), room temperature of 22 to 23 °C, 12:12-h light:dark cycle, and unlimited water from an automatic watering system. This facility has been AAALAC-accredited since 2010. All swine were given 5-d acclimation period and underwent physical examination prior to surgery to ensure that they were free of

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Table 1. Diameters of the carotid and femoral arteries as measured by ultrasonography

Weight group	Diameter (mm; mean ± 1 SD)					
	Left carotid	Right carotid	Left femoral	Right remoral		
< 40 kg	5.135 ± 0.705	4.874 ± 0.603	4.580	4.685		
	<i>n</i> = 2	<i>n</i> = 2	n = 1	n = 1		
40 to 49 kg	4.993 ± 0.448	5.186 ± 0.709	4.396 ± 0.368	4.181 ± 0.212		
	n = 14	<i>n</i> = 16	<i>n</i> = 6	<i>n</i> = 5		
50 to 59 kg	5.405 ± 0.535	5.738 ± 0.508	5.546 ± 0.310	5.614 ± 0.414		
	<i>n</i> = 10	<i>n</i> = 9	<i>n</i> = 3	<i>n</i> = 3		
60 to 69 kg	5.605 ± 0.468	5.994 ± 0.72	5.337 ± 0.405	5.466 ± 0.494		
	<i>n</i> = 8	<i>n</i> = 11	n = 7	<i>n</i> = 6		
≥70 kg		6	5.789 ± 0.606	5.935 ± 0.47		
	n = 0	n = 1	<i>n</i> = 6	n = 4		

Table	2. Mean	diameters	s of the ca	rotid and	d femoral	arteries	measured
by ulti	asonog	raphy in d	ifferent b	ody weig	ght group	s	

Diameter (mm; mean ± SD)				
Weight group	Carotid	Femoral		
<40 kg	5.004 ± 0.556	4.633 ± 0.074		
	n = 4	<i>n</i> = 2		
40 to 49 kg	5.096 ± 0.6	4.298 ± 0.314		
	<i>n</i> = 30	<i>n</i> = 11		
50 to 59 kg	5.563 ± 0.536	5.58 ± 0.329		
	<i>n</i> = 19	<i>n</i> = 6		
60 to 69 kg	5.83 ± 0.642	5.396 ± 0.433		
	<i>n</i> = 19	<i>n</i> = 13		
≥70 kg	6	5.847 ± 0.532		
	n = 1	n = 10		

clinical signs of disease. Baseline kidney and liver enzymes parameters and CBC counts were recorded.

All 38 swine underwent subsequent testing of cardiovascular devices under parallel IACUC-approved protocols. Soon after stable anesthesia was obtained, ultrasonography and angiography of the carotid, iliac, and femoral arteries for the purposes of the current study were performed prior to any other manipulations. All measurements of arterial lumen diameters were performed in naïve vessels that had not been manipulated previously.

Medications and anesthesia. To avoid acute arterial thrombosis during experimental procedures, swine were premedicated orally with 300 mg clopidrogel (Ceruvin, Ranbaxy Kedah, Malaysia) 1 d before procedures. Premedication and induction of anesthesia were achieved by using a mixture of ketamine (10 mg/kg IM; Ceva Animal Health, Glenorie, New South Wales, Australia), atropine (0.044 mg/kg IM; Atrosite, Troy Laboratories, Glendenning, New South Wales, Australia), and midazolam (0.6 mg/kg IM; Dormicum, Hoffmann-La Roche, Basel, Switzerland) followed by an intravenous bolus of thiopental (3 to 5 mg/kg, injected to effect; Inresa, Freiburg, Germany) prior to intubation. Pigs were ventilated, and anesthesia was maintained through inhalation of isoflurane (Attane, Piramal Critical Care, PA) in a mixture of medical-grade air and oxygen (2:1 ratio; FiO_{γ} , 30% to 40%) for the duration of the procedure. During measurements, maintenance fluid (0.9% NaCl) was administered at 4 mL/kg/h, invasive blood pressure was monitored (average mean arterial pressure, 60 to 80 mm Hg; LifeWindow Lite, Digicare Animal Health, Boynton Beach, FL), and body temperature (range, 36.0 to 37.8 °C) was recorded by using a rectal temperature probe.

Ultrasonography of the carotid and femoral arteries. All 38 swine underwent ultrasound assessment of carotid and femoral artery diameter in ventrodorsal recumbency prior to other scheduled experimental procedures. Ultrasonography was performed by using a linear-array transducer (L15-7io; EPIQ 7, Philips Ultrasound, Bothell, WA). Only the diameters of the common carotid arteries were measured, because in swine the bifurcation of carotid artery is situated distally near the jaw bone, a location that is difficult to examine by ultrasound. Furthermore, the internal carotid artery of swine typically is too small for vascular manipulation and is quite short prior to dividing into a plexus of vessels (rete mirabile). The diameter of the common carotid artery was measured at the midneck region. On 3 transverse views of the artery, measurements of the image freeze-framed at maximal systolic expansion⁴ were made by using the ellipse measuring tool, which is a program function of the EPIQ7 Philips Ultrasound machine. For femoral artery measurement, the probe was slid along the longitudinal scan path of the artery from just below the bifurcation of the profunda femoral artery (proximal femoral measurement) to just above the bifurcation of the circumflex femoral artery (distal femoral measurement), with the midfemoral measurement sited halfway between these outer points. For all measurements, the transducer was positioned perpendicular to the long axis of the artery. The diameters of the 3 transverse views of each artery were averaged to give a single mean diameter for subsequent analysis.

Angiographic measurement of the iliac and femoral arteries. In addition to ultrasonography, 11 of the 38 swine underwent angiography of the iliofemoral arteries for subsequent stent placement, allowing angiographic measurement of the diameter of the proximal, mid, and distal iliac arteries as well as femoral arteries. Vascular access was achieved through surgical cutdown to one of the carotid arteries and placement of a 7-French sheath. Intravenous heparin (100 IU/kg/h; Leo, Ballerup, Denmark) for anticoagulation and diltiazem (5 mg; Herbesser, Mitsubishi Tanabe Pharma, Tokyo, Japan) to prevent vasospasm were administered prior to sheath insertion. A 7-French guide catheter was then passed to the distal abdominal aorta to perform angiography (Cios Alpha multifunctional C-arm system, Siemens Healthcare, Henkestr, Germany) of the iliac and femoral arteries by using bolus injection of half-strength contrast agent (Omnipaque 350, GE Healthcare AS, Oslo, Norway). Before each measurement, a calibration process was performed by using the diameter of the guide catheter as reference. Measurement of artery diameter was taken from angiograms obtained by using the highest magnification setting of the imaging system.

Repeated measurements. To assess intraobserver and interobserver variability, repeated ultrasound imaging and

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Figure 1. Overlaying boxplots and scatterplots of the diameters of the (A) carotid and (B) arteries femoral as measured by ultrasonography. Box limits show the 25th and 75th percentiles, the internal line marks the median, and vertical lines with error bars show the 10th and 90th percentiles. Significant differences between groups (Holm–Sidak test) are indicated as *, P < 0.05; †, P < 0.01; and ‡ P < 0.001.



Figure 2. Quadratic regression analysis of diameter of the (A) carotid and (B) femoral arteries as measured by ultrasonography.

measurement of arteries of the first 7 swine was undertaken by 2 experienced operators independently (blinded to the findings of the other operator), with each operator imaging and measuring each artery twice.

Calculations and statistical analysis. Swine were divided into 5 groups according to their body weight at the time of the procedure, namely less than 40 kg, 40 to 49.9 kg, 50 to 59.9 kg, 60 to 69.9 kg, and greater than or equal to 70 kg. Vessel diameters are reported as mean \pm 1 SD. Differences between the means of more than 2 groups were determined through ANOVA followed by the Holm–Sidak test for pairwise multiple comparison. The independent 2-sample *t* test was used to determine significant differences in mean diameter between 2 groups. Nonparametric tests, including the Wilcoxon signed-rank and Mann–Whitney rank sum tests, were used for nonnormally distributed sample groups. Quadratic regression analysis was used to find the correlation between weight and diameter and was determined by using the Pearson correlation; \pm 0.75 to \pm 1, strong correlation;

 \pm 0.25 to \pm 0.75, moderate correlation; and 0 to \pm 0.25, minimal correlation. Statistical significance was assumed at a *P* value of less than 0.05. Statistical analyses were performed by using SigmaPlot (version 11.0, Systat Software, San Jose, CA).

Results

Analysis of vessel diameter as measured by ultrasonography showed no significant difference between the left and right vessels for both the carotid and femoral arteries of our Yorkshire×Landrace swine (Table 1). Therefore, the diameters for both sides were combined for further analysis.

The relationship between the luminal diameter of the carotid or femoral artery and body weight is summarized in Table 2 Overlaying boxplots and scatterplots of the diameters of the carotid and femoral arteries as measured through ultrasonography (data for left and right vessels combined) are shown in Figure 1. For both the carotid and femoral arteries, one-way ANOVA revealed significant (P < 0.001) differences in mean artery diameter among the various weight categories.

Table 3. Diameters of iliac and femoral arteries as measured by angiography

		Diameter (mm; mean ± 1 SD)		
Weight group	Left proximal iliac	Left midliac	Left distal iliac	
40 to 49 kg	5.65 ± 0.65	5.35 ± 0.21	4.9 ± 0.36	
	n = 4	n = 4	<i>n</i> = 3	
50 to 59 kg	6.53 ± 0.57	6.67 ± 0.21	6.5 ± 0.000	
	<i>n</i> = 3	<i>n</i> = 3	<i>n</i> = 2	
60 to 69 kg	7.43 ± 1.15	6.9 ± 1.13	6.77 ± 1.31	
	n = 4	n = 4	<i>n</i> = 3	
	Right proximal iliac	Right midiliac	Right distal iliac	
40 to 49 kg	5.73 ± 0.45	5.57 ± 0.4	4.83 ± 0.9	
	<i>n</i> = 3	<i>n</i> = 3	<i>n</i> = 3	
50 to 59 kg	7.33 ± 0.61	7.2 ± 1.18	6.6 ± 0.460	
	<i>n</i> = 3	<i>n</i> = 3	<i>n</i> = 3	
60 to 69 kg	7.35 ± 1.87	6.75 ± 1.4	6.7 ± 0.73	
	n = 4	n = 4	n = 4	
	Left femoral	Right femoral		
40 to 49 kg	3.91 ± 0.32	3.93 ± 0.28		
	n = 4	n = 4		
50 to 59 kg	5.37 ± 0.33	5.98 ± 0.32		
	<i>n</i> = 2	<i>n</i> = 3		
60 to 69 kg	6.39 ± 0.64	5.57 ± 0.24		
	<i>n</i> = 3	<i>n</i> = 2		

Table 4. Diameters of iliac and femoral arteries as measured by angiography

Diameter (mm: mean ± 1 SD)					
Weight group	Proximal iliac	Midiliac	Distal iliac	Femoral	
40 to 49 kg	5.686 ± 0.527	5.443 ± 0.299	4.867 ± 0.615	3.921 ± 0.277	
	n = 7	<i>n</i> = 7	n = 6	<i>n</i> = 8	
50 to 59 kg	6.933 ± 0.686	6.933 ± 0.812	6.56 ± 0.327	5.737 ± 0.439	
	<i>n</i> = 6	n = 6	n = 5	<i>n</i> = 5	
60 to 69 kg	7.388 ± 1.439	6.825 ± 1.18	6.729 ± 0.912	6.063 ± 0.652	
	<i>n</i> = 8	<i>n</i> = 8	<i>n</i> = 7	<i>n</i> = 5	

For carotid artery diameter, the Holm-Sidak test showed significant (P < 0.001) differences between the 40- to 49.9-kg group compared with the 60- to 69.9-kg group. Comparison between the 40- to 49.9-kg and 50- to 59.9-kg groups showed a significant (P = 0.079) trend for wider carotid arteries in larger pigs. Regarding femoral artery diameters, Holm-Sidak comparison showed that the 40- to 49.9-kg group was significantly (P < 0.001) different from all 3 heavier weight groups (50 to 59.9 kg, 60 to 69.9 kg, and greater than or equal to 70 kg). In addition, femoral arteries were significantly narrower in pigs weighing less than 40 kg than in those weighing 50 to 59.9 kg (P < 0.05) or at least 70 kg (P < 0.01). For the femoral artery, a 50-kg body weight appears to be a clear cut point for artery diameter, with all pigs lighter than 50 kg having a femoral artery diameter of less than 5 mm and all but 4 pigs heavier than 50 kg having femoral arteries wider than 5mm. The cut point regarding body weight was not as clear for carotid artery diameter but did show a trend for carotid artery diameter to be greater than 5.5 mm in larger pigs and less than 5.5 mm in smaller pigs.

Regression analyses of carotid and femoral artery diameter to body weight are shown in Figure 2. Curve fitting revealed that quadratic regression gave the curves of best fit (highest *r* value). According to Pearson correlation, carotid artery diameter and body weight were moderately positively correlated, whereas femoral diameter and body weight were strongly positively correlated. For the femoral artery, qualitative description of the curve indicates that, at lower body weights (40 to 65 kg), as weight increases then artery diameter also increases. Thereafter, as pigs continue to grow (greater than 65 kg), the slope of the curve tapers off, with artery diameter plateauing at 5.5 to 6.0 mm. This plateauing was not apparent for the carotid artery–body weight relationship. However, note that the *r* value was lower for the regression of carotid artery diameter against body weight and that we did not measure carotid artery diameter in pigs heavier than 70 kg.

Analysis of angiography-measured iliac and femoral artery diameters showed no significant difference between the left and right vessels (Table 3); we therefore combined these data when we evaluated their relationship to body weight (Table 4. Overlaying boxplots and scatterplots of the diameters of the proximal, mid-, and distal iliac and femoral arteries as measured by angiography are shown in Figure 3. One-way ANOVA for proximal and midiliac artery diameters plotted against body weight revealed significant (P < 0.05) differences. Holm–Sidak analysis showed a significant (P < 0.05) difference in the diameter of the proximal iliac artery between the 40- to 49.9-kg and 60 to 69.9-kg groups. For the midiliac artery diameter, significant (all P < 0.05) differences emerged between the 40- to 49.9-kg and 50- to 59.9-kg groups and between the 40- to 49.9-kg and 60- to

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Figure 3. Overlaying boxplots and scatterplots of the (A) proximal, (B) mid-, and (C) distal iliac artery and (D) femoral artery as measured by angiography. Significant differences between groups (Holm–Sidak test) are indicated as *, P < 0.05; †, P < 0.01; and ‡, P < 0.001.

69.9-kg groups. For both the distal iliac artery and femoral artery diameters, one-way ANOVA revealed a significant (P < 0.001) difference. Holm–Sidak testing showed significant differences in distal iliac artery diameter between the 40- to 49.9-kg and 60- to 69.9-kg groups (P < 0.01) and between the 40- to 49.9-kg and 50- to 59.9-kg groups (P < 0.001). Similarly, significant (all P < 0.001) differences were observed in femoral artery diameter between the 40- to 49.9-kg and 60- to 69.9-kg groups and between the 40- to 49.9-kg and 50- to 59.9-kg groups and between the 40- to 49.9-kg and 50- to 59.9-kg groups.

As seen with the angiography data, 50 kg body weight is a clear cut point for artery diameter, with approximately 90% of the pigs weighing more than 50 kg having an iliac artery diameter of greater than 6 mm, whereas approximately 90% of the smaller pigs had iliac arteries narrower than 6 mm. The same pattern was true of the femoral arteries, with all pigs heavier than 50 kg having femoral artery diameters greater than 5 mm but all pigs lighter than 50 kg with femoral arteries narrower than 5 mm.

Regression analyses of iliac and femoral artery diameters (measured by angiography) to body weight are shown in Figure 4. As seen earlier with the ultrasound data, quadratic regression gave the curves of best fit (highest *r* value). Pearson correlation yielded moderate positive correlation between the iliac artery diameter at all 3 (proximal, mid, and distal) levels measured and

body weight and a strong positive correlation between femoral diameter and body weight. Again, a qualitative description of the curves shows that at lower body weights (less than 60 kg), as weight increases then artery diameter also increases. Thereafter, as pigs continue to grow (60 to 65 kg), the slope of the curve tapers off, with artery diameter plateauing in pigs heavier than 65 kg at approximately 7 mm for the iliac artery and 6 mm for the femoral artery.

Discussion

In our study in Yorkshire×Landrace swine, we measured the luminal diameters of the carotid, femoral, and iliac arteries, which are all common access vessels for endovascular and vascular procedures. Measurements were performed by using both ultrasonographic and angiographic methods, and artery diameters were correlated with body weight. The results showed a high degree of correlation between diameters at the 5 vessel regions and animal weight, when the weight is less than 70 kg. Arterial diameter fits well to quadratic regression, with vessel diameters increasing as pigs grow at lower weights (< 60 kg). However, as pigs continue to grow (beyond 65 kg), then the vessel diameter plateaus.



Figure 4. Quadratic regression analysis of diameters of the proximal- (A), mid- (B), and distal- (C) iliac artery and femoral artery (D) measured by angiography.

For the evaluation of drug-eluting stents, a preclinical studies consensus group recommended that stents should be appropriately sized for the targeted vessel, with a stent: artery ratio of 1 to 1.2 for the porcine coronary artery model.⁵ This ratio is important because if the tested stent is the same size or smaller than the target vessel, stent migration and incomplete stent-strut apposition might occur.¹⁰ Conversely, the stent should not be aggressively oversized relative to the target vessel, as excessive expansion force exerted onto the vessel is undesirable. Similar requirements for stent-artery ratio apply to the preclinical testing of novel stents in peripheral arteries, such as the carotid and iliofemoral systems. Due to the constraints of custom design, manufacturing, and cost, a complete size range of test stents may not always be available during preclinical testing. Therefore, the ability to preselect animals with appropriate arterial diameters that are compatible with stent size is highly desirable when conducting preclinical animal studies. The results of our current study greatly increase the data available regarding carotid, iliac, and femoral artery diameters across a broad range of pig body weights and provide researchers with an expanded reference for preselecting swine with appropriately sized arteries.

For measurement of artery diameter, angiography is considered the 'gold standard'. However, in animal studies, general anesthesia must be administered before an angiogram can be obtained. Anesthesia may have unpredictable and unwanted effects on vessel size. For example, an anesthetic agent might induce vasodilation, increasing the arterial diameter, or might cause hypotension and vasoconstriction, reducing vessel diameter. Furthermore, endovascular wiring and cannulation at or near the arteries under examination can trigger vessel constriction or vasospasm. Ultrasound examination of arteries enables measurement of artery diameter to be conducted in sedated animals, without the need for deep anesthesia; furthermore, this method is relatively noninvasive. The effects of different sedatives and anesthetic drugs and combinations thereof on arterial vasodilation or vasoconstriction are highly variable and difficult to predict. However, any subsequent interventions, such as deployment of cardiovascular devices, likely require identical anesthetic procedures to those we used in the present study to document vessel luminal diameter measurements. Consequently, the luminal diameters that we measured are likely relevant to subsequent manipulations and interventions, given that they all are likely to require the same methods and levels of anesthesia.

Over the neck and groin area, carotid and femoral arteries are easily imaged through ultrasonography. By contrast, the more deeply seated iliac arteries proved challenging to locate and successfully image by ultrasonography, especially in larger swine. Another potential problem of ultrasonography is that imaging and measurement of vessel size is operator-dependent. ExcesVol 59, No 4 Journal of the American Association for Laboratory Animal Science July 2020

sive pressure of the ultrasound probe may compress the artery to become an oval-shaped structure, such that neither the long nor the short axis accurately represents the true diameter of the vessel. Using the ellipse measuring tool of the machine may resolve this problem. Some sonographers prefer to measure arterial diameter in the transverse and longitudinal views. However, in the longitudinal view, if the transducer is not directly along the middle of the artery, the measured size may not represent the actual diameter. Nonetheless, the longitudinal view allows the operator to review any obvious tapering of the vessel over the examined segment. In this study, we chose to take measurements from the transverse view. In the transverse view, parameters of gain, depth of field, and focus must be adjusted to give a clear image, thereby ensuring more accurate imaging. The diameter of the vessel is then measured from the leading edge of the near wall intima-lumen echo to the far wall lumen-intima interface.9

In conclusion, we present ultrasonography- and angiographyderived data describing the luminal size of the carotid, iliac, and femoral arteries of growing adult Yorkshire×Landrace pigs. These data show that in pigs less than 60 kg, artery diameters increase with increasing body mass. Artery diameters continue to increase but at a slower rate in pigs weighing 60 to 65 kg, whereas beyond 65 kg, artery diameters plateau even though the pigs continue to increase in body weight. This information adds to the previous sparse literature reporting vessel diameters in this important species for translational studies and should contribute to better weight-associated selection of pigs for future studies.

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