

Relationship between brachial and femoral artery endothelial vasomotor function/phenotype in pigs

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Abstract

In humans, the measurement of brachial artery endothelial vasomotor function is used as a surrogate index of systemic endothelial health; however, the applicability of brachial artery findings to other vasculatures needs to be examined. The purpose of the present investigation was to test the following hypotheses: (1) brachial and femoral artery endothelium-dependent/independent relaxation is correlated; (2) endothelial expression of pro-/antiatherogenic proteins is correlated between brachial and femoral arteries; and (3) within vessel, there is a positive correlation between expression of antiatherogenic proteins and endothelium-dependent/independent relaxation, and an inverse correlation between expression of proatherogenic proteins and relaxation. *In vitro* endothelium-dependent (bradykinin [BK] and acetylcholine [Ach]) and -independent (sodium nitroprusside [SNP]) relaxation were evaluated in harvested brachial and femoral arteries of 96 Yucatan miniature swine. In a subset of pigs ($n = 32$), expression of 18 pro-/antiatherogenic proteins was measured from brachial and femoral artery endothelial cell scrapes using immunoblot analysis. Vascular sensitivity (half-maximal effective dose) to BK, Ach and SNP was highly correlated between brachial and femoral arteries ($P < 0.01$). A significant correlation was found between brachial and femoral arteries for content of six of the 18 measured proteins ($P < 0.01$). Furthermore, expression of two proteins (eNOS and COX-1) was correlated with vasorelaxation function in the brachial artery ($P < 0.01$). We provide the first evidence of a relationship between brachial and femoral artery endothelium-dependent relaxation. Our data also suggest that, in general terms, endothelial expression of several established pro-/antiatherogenic proteins is not robustly associated between brachial and femoral arteries, and does not link strongly to vasorelaxation function.

Keywords: peripheral conduit arteries, endothelium-dependent relaxation, heterogeneity, atherosclerosis

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Introduction

Impairment of the endothelium has been proposed as the primary etiology of atherosclerosis.¹ Given that endothelial dysfunction is considered a final common pathway of the impact of traditional and novel cardiovascular risk factors, the assessment of endothelial dysfunction/function presents an attractive strategy to cumulatively evaluate atherosclerotic risk in humans.² In this regard, Celermajer *et al.*³ proposed a non-invasive technique to measure brachial artery flow-mediated (endothelium-dependent) dilation as an *in vivo* bioassay of endothelial function. Since this landmark paper, the measurement of brachial artery endothelial function has rapidly gained popularity, due in part to the widely held belief that brachial artery vasomotor function

represents a 'barometer' of systemic endothelial health.⁴ However, given the substantial evidence demonstrating phenotypic and functional heterogeneity of the endothelium throughout the arterial tree,^{5–7} the applicability of brachial artery findings to other vasculatures needs to be rigorously tested. In this report, we reasoned that if endothelium-dependent dilation in one vascular bed can in fact predict function in another, a correlation would exist between measures of endothelial function in vessels from the respective vascular beds (e.g. two peripheral conduit arteries). Furthermore, in this context, we hypothesized that the endothelial cell phenotype would be correlated between given vessels. Accordingly, the purpose of this study was to test the following hypotheses: (1) brachial and femoral

artery endothelium-dependent/independent relaxation is correlated; (2) endothelial expression of pro-/antiatherogenic proteins is correlated between brachial and femoral arteries; and (3) within vessel, there is a positive correlation between expression of antiatherogenic proteins and endothelium-dependent/independent relaxation, and an inverse correlation between expression of proatherogenic proteins and relaxation.

Material and methods

Experimental animals

We conducted a retrospective analysis of data from our swine database. It should be noted that data from a number of pigs used herein have been included in previous studies examining unrelated research questions.^{8–11} In the present investigation, we selected data from Yucatan miniature pigs in which *in vitro* endothelium-dependent and -independent relaxation of brachial and femoral arteries were assessed. The resulting sample size was 96 animals (64 male, 32 female). All pigs were purchased from a commercial breeder (Sinclair Research Farm, Columbia, MO, USA) and used with protocols approved by the Animal Care and Use Committee at the University of Missouri. All animals were housed in rooms maintained at 20–23°C with a 12:12-h light–dark cycle and provided a standard diet (Purina Laboratory Mini-pig Chow; 8% of daily caloric intake derived from fat). All pigs were 12–14 months of age and 25–40 kg body weight at time of sacrifice.

In vitro assessment of endothelium-dependent and -independent relaxation of brachial and femoral arteries

Procedures used to assess vasomotor responses of arterial rings have been published previously in detail.¹² In short, immediately following sacrifice, brachial and femoral arteries from each pig were harvested, trimmed of fat and connective tissue, and sectioned into 2–3 mm rings in cold Krebs bicarbonate buffer solution. Vasomotor reactivity was examined with the rings stretched to the length that produced maximal active tension ($L_{\max}$). Before dose–response curves were initiated, arterial rings were precontracted with prostaglandin F 2α (PGF $_{2\alpha}$) (30 μ mol/L). Endothelium-dependent relaxation was assessed by using bradykinin (BK; 10^{-11} – 10^{-6} mol/L). In a subgroup of pigs ($n = 27$), relaxation responses to acetylcholine (ACh; 10^{-10} – 10^{-4} mol/L) were additionally examined. Endothelium-independent relaxation was assessed with sodium nitroprusside (SNP; 10^{-10} – 10^{-4} mol/L). The bicarbonate buffer solution was replaced to wash out the drug and arterial segments were allowed to stabilize for one hour before initiation and between protocols. For each dose–response curve, half-maximal effective dose (ED $_{50}$) was calculated as an index of endothelial (BK, ACh) and smooth muscle (SNP) sensitivity using the GraphPad Prism v5.0a software.

Solutions and drugs for vasomotor experiments

Krebs bicarbonate buffer solution contained (in mmol/L) 131.5 NaCl, 5.0 KCl, 1.2 NaH $_2$ PO $_4$, 1.2 MgCl $_2$, 2.5 CaCl $_2$, 11.2 glucose, 20.8 NaHCO $_3$, 0.003 propranolol and 0.025 EDTA. Solutions were aerated with 95% O $_2$ –5% CO $_2$ (pH 7.4) and maintained at 37°C. All drugs and chemicals were purchased from Sigma Chemical (St Louis, MO, USA).

Endothelial cell phenotype: immunoblot analysis

In a subset of male pigs ($n = 32$), expression levels of 18 proteins that are frequently altered in early atherosclerotic disease (Table 1) were measured from brachial and femoral artery endothelial cell scrapes as previously described.^{11,13} Protein samples from brachial and femoral endothelial scrapes were loaded on the same gel in an alternating pattern allowing within gel comparisons between vessels. Analysis of protein was performed with chemiluminescence and quantified by densitometry using Kodak 4000R Imager and Molecular Imagery Software.

Statistical analysis

Pearson correlations were used to determine the relationship between brachial and femoral artery sensitivity (ED $_{50}$) to vasodilators (BK, ACh and SNP). Pearson correlations were also used to examine whether protein expression (net intensity) was related between brachial and femoral arteries as well as with vascular sensitivity within a given vessel. Furthermore, paired *t*-tests were used to compare the expression of proteins between the brachial and femoral arteries. For all statistical tests, the alpha level was set at

Table 1 Relationship and comparison between brachial and femoral artery endothelial expression of pro-/antiatherogenic proteins in swine

	Pearson correlation		Paired <i>t</i> -test	
	<i>r</i> (<i>r</i> ²)	<i>P</i> value	Fold difference from brachial artery	<i>P</i> value
Proatherogenic proteins				
Arginase 1	0.15 (0.02)	0.408	0.91 $\pm$ 0.16	0.66
Caveolin-1	0.52 (0.27)	0.006*	1.25 $\pm$ 0.33	0.409
AT-1	0.83 (0.69)	0.0001*	0.97 $\pm$ 0.13	0.781
AT-2	0.11 (0.01)	0.57	0.86 $\pm$ 0.09	0.333
p47phox	0.17 (0.03)	0.359	1.67 $\pm$ 0.09	0.0001*
p67phox	0.56 (0.32)	0.003*	1.18 $\pm$ 0.23	0.359
Rac-1	0.16 (0.03)	0.478	2.43 $\pm$ 0.34	0.001*
COX-1	0.17 (0.03)	0.365	0.96 $\pm$ 0.09	0.744
VCAM-1	0.13 (0.02)	0.577	0.94 $\pm$ 0.10	0.666
MDA	0.62 (0.39)	0.0001*	1.08 $\pm$ 0.10	0.382
Antiatherogenic proteins				
eNOS	0.18 (0.03)	0.332	1.30 $\pm$ 0.08	0.007*
p-eNOS/ eNOS	0.58 (0.33)	0.001*	1.41 $\pm$ 0.22	0.037
p-Akt/Akt	0.68 (0.47)	0.0001*	0.83 $\pm$ 0.13	0.416
HSP90	0.42 (0.18)	0.018	1.31 $\pm$ 0.12	0.019
SOD-1	0.36 (0.13)	0.046	1.08 $\pm$ 0.02	0.002*
SOD-2	0.10 (0.01)	0.569	1.45 $\pm$ 0.07	0.0001*
SOD-3	–0.02 (0.00)	0.925	0.95 $\pm$ 0.09	0.732
Catalase	–0.17 (0.03)	0.343	1.45 $\pm$ 0.09	0.001*

**P* < 0.01

0.01 to account for the multiple correlations and/or comparisons. All analyses were performed with SPSS v.17.0. (SPSS, Inc, Chicago, IL, USA).

Results

BK, Ach and SNP elicited a concentration-dependent relaxation of the brachial and femoral arteries (data not shown). As illustrated in Figure 1, vascular sensitivity to all three vasodilators was highly correlated ($P < 0.0001$) between brachial and femoral arteries ($r = 0.83$, 0.78 and 0.68 , respectively). Similar relationships were observed in male and female pigs; therefore, data were pooled across gender. Within vessel, vascular sensitivity to BK and Ach was not correlated ($P > 0.01$), nor were either correlated with SNP ($P > 0.01$). Table 1 summarizes the correlation and comparison between brachial and femoral artery endothelial expression of pro-/antiatherogenic proteins. For six of the 18 measured proteins, significant correlations ($P < 0.01$) were found between brachial and femoral arteries. Expression levels of these six proteins appeared to be similar ($P > 0.01$) between vessels. Furthermore, endothelial nitric oxide synthase (eNOS) content of the brachial artery endothelium was correlated with increased sensitivity to Ach ($r = 0.559$; $P = 0.001$) and SNP ($r = 0.567$; $P = 0.001$). In addition, brachial artery cyclooxygenase 1 (COX-1) content was related with decreased sensitivity to Ach ($r = 0.465$; $P = 0.007$). None of the remaining correlations between protein expression and vasorelaxation function reached statistical significance ($P > 0.01$).

Discussion

The present study tested the hypothesis that vasomotor reactivity is related among conduit arteries of the peripheral circulation. We provide the first evidence of a relationship between brachial and femoral artery endothelium-dependent relaxation. Conversely, our data also indicate that endothelial expression of most of the measured pro-/antiatherogenic proteins does not correlate between brachial and femoral arteries and are not correlated with endothelium-dependent/independent relaxation responses in either arteries. Brachial and femoral arteries of pigs resemble those of humans with respect to size and endothelium/smooth muscle content.¹⁴ Furthermore, similar to humans, porcine femoral arteries are markedly susceptible to atherosclerotic disease.¹⁵ In short, the use of swine allowed for a controlled experimental environment and the supply of ample vascular tissue for endothelial phenotypic characterization.¹⁶

An explosion of human studies utilizing brachial artery endothelium-dependent dilation as an index of endothelial function has become apparent in recent years.^{2,17,18} The widespread belief that brachial artery vasomotor function provides an *in vivo* bioassay of systemic endothelial function is primarily founded on two highly cited studies by Anderson *et al.*¹⁹ and Takase *et al.*²⁰ reporting an association between brachial and coronary artery endothelial function in humans. Interestingly, in the Anderson *et al.* study, brachial artery flow-mediated dilation (FMD) was only

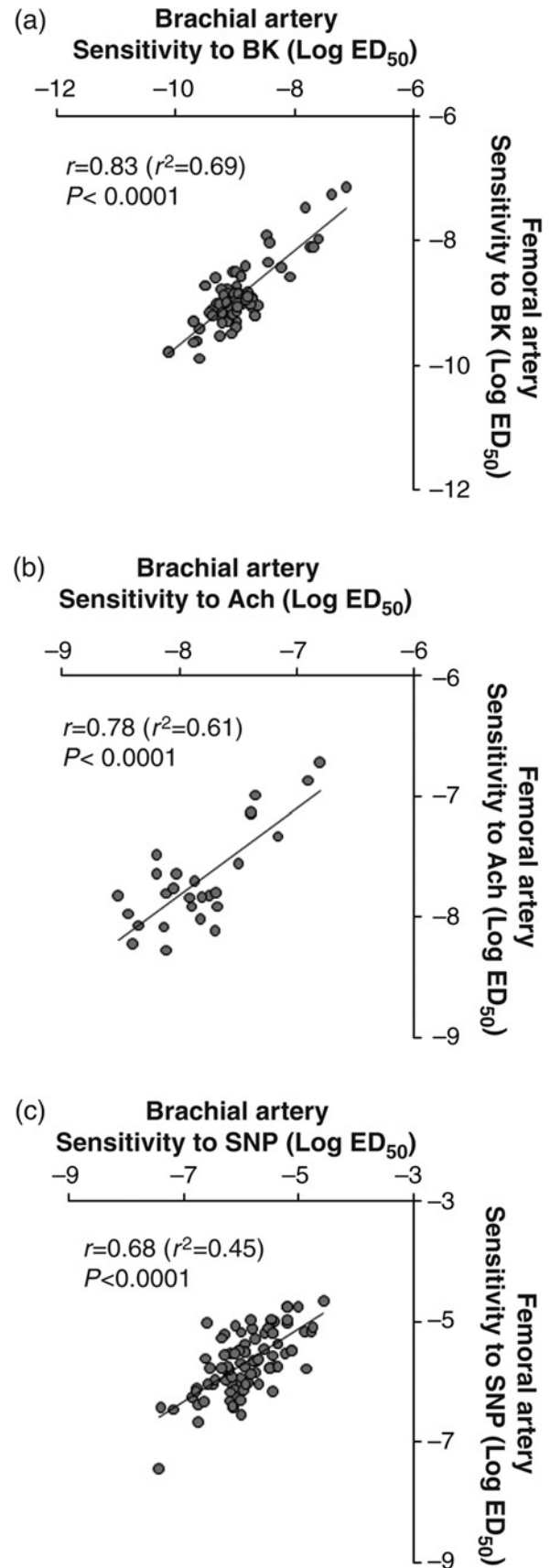


Figure 1 Relationship between brachial and femoral artery endothelium-dependent (a,b) and -independent (c) relaxation in swine. BK, bradykinin; Ach, acetylcholine; SNP, sodium nitroprusside; ED₅₀, half-maximal effective dose

mildly correlated with Ach-induced vasodilation of the coronary artery ($R^2 = 0.13$), whereas in the Takase *et al.* study, when the same stimulus (shear stress) was utilized in both vasculatures, a much higher correlation ($R^2 = 0.61$) was found. Taken together, these data suggest that the relationship in vasomotor activity between two vessels may be dampened when different stimuli are utilized to signal endothelium-dependent relaxation (dilation). We tested this hypothesis by examining whether the response to different vasodilators was correlated in each artery. Interestingly, in brachial and femoral arteries, we observed a lack of correlation between vascular sensitivity to BK and Ach, both endothelium-dependent dilators. This disassociation between responses in the same arteries suggests that heterogeneity in the signal transduction mechanism by which a given vessel dilates may exist for these two agents.

To our knowledge, the present study is the first to evaluate the relationship in vasomotor function between two peripheral conduit arteries. The observation that brachial and femoral artery endothelium-dependent relaxation is correlated (Figure 1) is important in view of the plethora of human research targeting the assessment of vasomotor function exclusively at the brachial artery. While we advocate that caution should be used when inferring information from one conduit artery to another, the present data indicate that brachial artery endothelial function may reflect function of the femoral artery and, therefore, perhaps other peripheral conduit arteries as well. Further research is warranted to determine whether the correlation between brachial and femoral artery endothelium-dependent dilation is also observed in humans with the use of the FMD technique; a question that could be addressed using currently existing large data-sets from established human laboratories. In this regard, Thijssen *et al.*⁷ eloquently described the differences in FMD among five conduit vessels including brachial and femoral arteries in a group of young healthy subjects. The authors reported an inverse correlation between artery size and FMD across the five vessels. Importantly, this relation remained significant despite normalization of shear rate; thus suggesting that artery structure *per se* may play an important role in the dilatory response and explain the heterogeneity in FMD observed throughout the vasculature.⁷ Similar to humans, the femoral arteries of swine have a greater diameter than the brachial arteries.¹³ However, this difference in diameters does not appear to be associated with altered endothelial responsiveness in pig arteries in the same fashion as observed for FMD in humans.⁷ It is possible that the impact of artery size on endothelium-dependent relaxation is specific to the signal (e.g. shear versus Ach) used to evoke the dilation; however, further research is needed to explore this phenomenon.

Regional heterogeneity within the endothelium has been recognized for over 50 years. Endothelial cells from different vascular beds (e.g. arteries versus veins) or different regions within a vessel (curvature versus straight segment) display a remarkable variation in endothelial phenotype.⁵ In fact, Aird has stated that 'no two endothelial cells are identical'.⁶ Such heterogeneity may result from the influence of distinct microenvironments on intrinsically identical cells and/or from epigenetic modifications that lead to discrete phenotypes

despite similar microenvironments.⁶ Our finding that only six of the 18 pro-/antiatherogenic proteins assessed were significantly correlated between brachial and femoral arteries and that of those only three exhibited an R^2 greater than 0.35 (Table 1) supports the notion of spatial heterogeneity of endothelial phenotype between arteries of similar architecture and physiological function. Given that porcine brachial and femoral arteries, unlike humans, are exposed to similar hydrostatic forces,¹³ future studies are needed to elucidate whether the mechanisms contributing to the apparent phenotypic heterogeneity between limb vasculatures are attributed to shear stress profile disparities and/or intrinsic factors.

Another novel finding of the study was the overall lack of association between vasomotor function and endothelial expression of the pro-/antiatherogenic proteins examined. Only two proteins out of 18 exhibited statistically significant correlations with vasorelaxation responses. Of note, eNOS content of the brachial artery was correlated with increased sensitivity to Ach and SNP. In addition, brachial artery COX-1 content was related with decreased sensitivity to Ach. The apparent absence of correlation between protein expression and vasorelaxation function is important when considering that the majority of vascular research, at least in humans, relies on functional outcomes (e.g. FMD). In this context, the current data suggest that prudence should be taken when solely using vasomotor function as a surrogate marker of endothelial health. These results also indicate that more work is needed to establish the relationships between endothelial cell phenotype and endothelium-dependent dilator function.

In summary, the present investigation provides support, for the first time, that brachial artery endothelium-dependent dilation can reflect vasomotor function in the femoral artery of pigs. Our data also suggest that, in general terms, endothelial expression of several established pro-/antiatherogenic proteins is not robustly associated between brachial and femoral arteries, and does not link strongly to vasorelaxation function.

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