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High Frequency Ultrasound for Evaluation of Intimal Thickness

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Abstract

Background: The measurement of carotid intima-medial thickness is a well validated measure of cardiovascular risk. Although atherosclerosis occurs in the intima, this arterial layer is not measured alone due to limitation in ultrasound resolution with standard frequency probes.

Methods: We evaluated the feasibility of using a 55-MHz ultrasound system with high resolution to measure intima thickness in several vascular territories compared to a “standard” frequency probe.

Results: The intima and medial thickness was measured in the brachial, radial and tibial arteries in 10 healthy subjects and 5 subjects with peripheral arterial disease. The high frequency ultrasound probe showed superior resolution compared to the standard frequency probe allowing for measurement of intima separately from media.

Conclusions: The intima can be measured independently of media with a high degree of reproducibility using a high frequency probe. This technology may allow for early detection of cardiovascular risk and extend knowledge about the physiological changes in the early atherosclerotic development.

Introduction

The early identification of individuals at of high cardiovascular risk and those with atherosclerosis is paramount to successful prevention treatment. One validated and well studied method for early evaluation of cardiovascular risk is ultrasound measurement of carotid intima-media thickness (IMT)⁽¹⁻⁴⁾. In clinical studies involving lipid reduction, carotid IMT measurement has proven a useful method for assessment of atherosclerotic disease ^(5, 6). Furthermore, it can be taught to “non-sonographers” and be administered in the office setting ⁽⁷⁾.

Atherosclerosis occurs in the intimal layer of the arterial wall and thickens with progressive disease. On the contrary, the medial layer does not thicken significantly from atherosclerosis. Therefore, an imaging modality that is capable of measuring the intimal thickness may be more useful for prediction of cardiovascular risk. We evaluated the feasibility of using a 55-MHz ultrasound system with high resolution in humans which was originally developed for imaging

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small animals. This high frequency system did not have enough penetration to visualize the carotid artery wall so we concentrated on more superficial arteries; brachial, radial and tibial. We report the results of high frequency ultrasound compared to standard frequency ultrasound for evaluation of intimal and medial thickness of these more peripheral, superficial vessels.

METHODS

Study Population

Ten healthy volunteers and 5 patients with peripheral artery disease (PAD) participated in the study and the demographic composition of the sample is given in Table 1. All subjects with PAD had a reduced ankle-brachial index. The subjects were recruited by word of mouth from volunteers and from clinic practices within the University of Pennsylvania Health System. The study was approved by the Institutional Review Board of the University of Pennsylvania. Written informed consent was obtained before study entry.

Ultrasound Measurements

High-Frequency Images—Sagittal-plane images from brachial (n=15), radial (n=15), and anterior tibial (n=10) arteries were collected with a high frequency mechanical scanner, Vevo 770, using a 55MHz transducer (Visualsonics, Toronto, Canada) with a fixed focal point. The power was 100% and the frame rate varied between 29Hz to 46 Hz depending on field of view. The smaller field of view had a higher frame rate. The anterior tibial artery images for patients with PAD were severely technically limited and measurement not feasible. The same transducer was used for all measurements. Measurements were taken at the distal portions of the brachial (just above antecubital space), radial, and anterior tibial arteries. Analyses were then made offline. Intima thickness (IT) was defined as the distance from the leading edge of the lumen-intima margin to the leading edge of the intima-media margin of the far wall. IMT was defined as the distance from the leading edge of the lumen-intima margin to the leading edge of the media-adventitia margin of the far wall. Media thickness (MT) was calculated as the difference between IMT and IT. In each subjects, the IT and IMT measurements were repeated on three different images. These measurements were averaged together to give the IMT and IT values each blood vessel in each subject. Coefficient of variation was calculated for each artery by taking the ratio of standard deviation to the mean. The interobserver variability was also calculated for images obtained from healthy subjects.

Standard-Frequency Images—B-mode real-time ultrasound with a Philips 5000 CL 15/7 transducer (peripheral vascular tissue preset; arterial sub-preset) (Philips Medical Systems, Bothell, WA) was used to obtain sagittal-plane images of the brachial, radial, and anterior tibial arteries for all 10 healthy study participants. This instrument has a frame rate of 30 Hz, dynamic range of 170dB and a single focal zone at or below the area of interest. Measurements were taken at the distal portions of the three arteries. Analyses were then made offline. IMT was defined as the distance from the leading edge of the lumen-intima margin to the leading edge of the media-adventitia margin of the far wall. Three measurements of IMT were made per artery for each subject and were averaged together. Coefficient of variation was calculated for each artery.

Image Analysis

Analyses of the images were completed using automated, edge-detection software (Carotid Analyzer for Research v 5.0.5, MIA Vascular Research Tools 5, Coralville, Iowa). Images were analyzed by an investigator proficient with the Carotid Analyzer software.

Image Quality

Three independent readers rated the quality of the high-frequency and standard-frequency images to determine if there was a difference in the general quality of the images. Readers looked at raw images from both frequencies for the brachial, radial, and anterior tibial arteries of the study participants. If an artery was not able to be located in high-frequency, the corresponding image in standard-frequency was not rated for that subject. Readers gave each image a score of 1-5 using a five-point scale based on the clarity of the posterior intima-lumen and media-adventitia margins of the entire image. This scale is reproduced in Table 2.

Statistics

Results were expressed as mean $\pm$ SD. For high-frequency measurements, radial and anterior tibial artery IT and IMT were normally distributed; brachial IT and IMT were not normally distributed. For standard-frequency measurements, the anterior tibial artery IMT was normally distributed, while the brachial and radial artery IMTs were not. Coefficient of variation = mean SD of differences between measurements divided by the mean of these measurements multiplied by 100. Student *t* tests and Wilcoxon Signed Rank tests were used to examine the differences in IMT and image quality using high-frequency and standard-frequency ultrasound imaging for each arterial location. A Kruskal-Wallis test was conducted to examine differences in IT for the three arteries. All tests used were 2-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Reproducibility

The coefficient of variation for the high frequency probe was 13% and 6% for brachial arterial IT and IMT, 10% and 5% for radial arterial IT and IMT, and 23% and 5% for anterior tibial arterial IT and IMT, respectively. The Coefficient of variation for the standard frequency probe was calculated for each artery and was 7% for brachial arterial IMT, 5% for radial arterial IMT, and 7% for anterior tibial arterial IMT. The interobserver variability for the high frequency imaging was calculated for the brachial and radial arteries for the healthy subjects. The interobserver variability for the brachial artery was 15% for the IT and 12% for the IMT and for the radial artery was 9% for the IT and 4% for the IMT. The subject variability for the brachial artery was 32% and 35% for IT and IMT, respectively and for the radial artery was 11% and 28% for IT and IMT, respectively.

IMT Measurement

Brachial—A Wilcoxon Signed Ranks test was conducted to evaluate the difference between high-frequency and standard-frequency ultrasound in the measurement of brachial artery IMT thickness. High-frequency ultrasound measurements ($M=0.19$, $SD=0.05$) were significantly smaller than standard-frequency ultrasound measurements [$M=0.29$, $SD=0.07$, $Z=-2.67$, $p<0.01$, $N=10$].

Radial—A Wilcoxon Signed Ranks test was conducted to evaluate the difference between high-frequency and standard-frequency ultrasound in the measurement of radial artery IMT thickness. High-frequency ultrasound measurements ($M=0.19$, $SD=0.06$) were significantly smaller than standard-frequency ultrasound measurements [$M=0.22$, $SD=0.06$, $Z=-2.32$, $p<0.05$, $N=10$].

Anterior Tibial—A paired-sample Student's *t*-test was conducted to evaluate the difference between high-frequency and standard-frequency ultrasound in the measurement of anterior tibial artery IMT thickness. High-frequency ultrasound measurements ($M=0.21$, $SD=0.07$)

were significantly smaller than standard-frequency ultrasound measurements [$M=0.33$, $SD=0.07$, $t(6)=-2.60$, $p<0.05$, $N=7$].

IT Measurement

The results of IT analysis of high-frequency brachial, radial, and anterior tibial arterial images are presented in Table 3. A Kruskal-Wallis test was conducted to examine any differences among the IT measurements of three arteries. Brachial, radial, and anterior tibial IT measurements were not found to significantly differ ($p=0.14$) among the healthy subjects. The IT and IMT was significantly thicker in the PAD patients compared to healthy subjects (Table 4). The IT was not able to be assessed in any subjects using standard frequency ultrasound due to low resolution.

Image Quality

Figure 1 displays a comparison between high-frequency and standard-frequency images of the same patient's artery in each of the three arterial locations. As anticipated, the high frequency ultrasound probe showed superior resolution compared to the standard frequency probe. Figure 2 shows a representative ultrasound image for three arteries from one of the patients with PAD.

Brachial—A Wilcoxon Signed Ranks test was conducted to evaluate the difference in image quality between the best high-frequency and standard-frequency brachial image for each patient ($N=10$). High-frequency ultrasound image quality ($M=2.27$, $SD=0.91$) was significantly better than standard-frequency ultrasound image quality [$M=2.67$, $SD=0.88$, $Z=-2.06$, $p<0.05$] for the best image of the brachial artery.

Radial—A Wilcoxon Signed Ranks test was conducted to evaluate the difference in image quality between the best high-frequency and standard-frequency radial image for each patient ($N=10$). High-frequency ultrasound image quality ($M=1.63$, $SD=0.72$) was significantly better than standard-frequency ultrasound image quality [$M=3.57$, $SD=1.07$, $Z=-4.52$, $p<0.001$] for the best image of the radial artery.

Anterior Tibial—The available images that could be evaluated for the anterior tibial artery, compared to brachial and radial arteries, were limited ($N=90$ (radial and brachial); $N=63$ (anterior tibial)) due technical problems mainly from limited penetration of ultrasound beam. A Wilcoxon Signed Ranks test was conducted to evaluate the difference in image quality between the best high-frequency and standard-frequency anterior tibial image for each patient. High-frequency ultrasound image quality ($M=2.33$, $SD=1.28$) was not significantly better than standard-frequency ultrasound image quality [$M=2.95$, $SD=1.16$, $Z=-1.70$, $p=0.09$] for the best image of the anterior tibial artery. This indicates that ultrasound quality is similar for both high frequency and standard frequency in the same individual in this vascular territory. However, only high frequency was able to discern the intima alone whereas standard frequency was only able to measure the intima and media combined.

DISCUSSION

The results show that high frequency ultrasound allows for imaging of the intimal arterial layer which is not seen with standard ultrasound frequency. Even in this relatively small study, the IT for patients with PAD was found to be significantly higher than healthy subjects. Ultrasound pulses with shorter spatial pulse length yield higher axial resolution. Although the precise characteristics of the ultrasound pulses of the commercial scanners used in this study are not known, higher frequency transducers in general provide shorter pulses and higher resolution, which is consistent with our observations. The intima could be clearly seen, and IT measured independently of IMT, using high-frequency ultrasound (for radial, brachial, and anterior tibial

arteries), but not when using standard-frequency ultrasound. Radial, brachial, and anterior tibial arterial IT measurements did not differ significantly among the study participants. Anatomical and technological constraints more severely affected the high-frequency measurement of the anterior tibial artery compared with the radial and brachial arteries. Although the anterior tibial artery did not reach significance, it is the belief of the investigators that high-frequency images are of better quality than standard-frequency images in those patients whose anterior tibial arteries can be optimally scanned in high-frequency. High-frequency IMT measurements were consistently significantly smaller than standard-frequency IMT measurements for brachial, radial, and anterior tibial arteries. The reason for this difference is not known but could be related to higher spatial and contrast resolution associated with higher frequency imaging.

High-frequency ultrasound using a 55-MHz probe is technique commonly used in small animal research, as it allows for the detection of physiological structures down to $\sim 30\mu\text{m}$ in size. In contrast to small animal research, only one other study to date has explored the use of high-frequency ultrasound in a human sample.⁽⁸⁾ In this study, researchers examined the feasibility of high-frequency ultrasound imaging in human vascular research. Investigators found it possible to separately visualize the IT from the MT in radial and anterior tibial arteries. Radial and anterior tibial IT measurements positively correlated with standard-frequency carotid IMT measurements (a standard non-invasive marker of atherosclerosis). Additionally, patients with PAD had significantly larger radial artery IT and IMT measurements compared to healthy age- and sex-matched controls.

The current study extends the work of Osika et al as this is the first study to our knowledge comparing the high-frequency ultrasound imaging to standard-frequency ultrasound imaging in the brachial, radial, and anterior tibial arteries of humans with and without PAD. Additionally, ours is the first study to examine the quality of a large sample of high-frequency of brachial, radial, and anterior tibial images, and further, make comparisons between high-frequency and standard-frequency image quality for these three vessels. IMT, which combines measurements of the intima and the media, has been shown to be a reliable marker of cardiovascular health and atherosclerosis risk.⁽⁹⁾ While combined intimal and medial thickness is an important cardiovascular risk factor, the intimal and medial layers undergo different changes during the development and progression of atherosclerosis; the intimal layer thickens.⁽¹⁰⁾ Since different changes occur in these layers during atherosclerosis, and because these two layers play different physiological roles in vascular health, assessment of IT independently of IMT may be a useful additional variable for atherosclerosis. Also, independent IT measurement may make it possible to detect atherosclerotic physiological changes earlier than what is currently possible using combined IMT measurement.

In addition to allowing IT to be measured independently of IMT, the increased spatial resolution of high-frequency ultrasound produces imaging studies of very fine quality for the brachial and radial arteries for healthy subjects and patients with PAD. The high-frequency ultrasound images of the anterior tibial arteries were of good quality for the healthy subjects but poor quality for patients with PAD. As evidenced by Figure 1, under optimal scanning conditions, high-frequency images appear sharper and have more clearly defined vessel layer margins than standard-frequency ultrasound images. The highly defined lumen-intima and media-adventitia boundaries facilitate easier and more precise measurement of vessel IMT. This increased study readability could make high-frequency ultrasound a useful addition or alternative to standard-frequency ultrasound in IMT imaging studies of the superficial vessels.

Gori et al recently published a paper proposing an alternative to traditional non-invasive endothelial function tests.⁽¹¹⁾ The investigators advocated the use of the radial artery to measure flow-mediated constriction as a measure of endothelial health. As high-frequency ultrasound produces higher quality radial images compared to standard-frequency ultrasound,

future studies on this technique and other research utilizing the radial artery may benefit from using high-frequency versus standard-frequency ultrasound.

Study Limitations

The 55 MHz frequency used in high-frequency ultrasound allows for very high spatial resolution ($\sim 30\mu\text{m}$), but due to higher attenuation, the penetration depth is limited. The current Visualsonics system has a fixed focal zone of 4.5 mm below the skin's surface and, while useful for evaluating superficial arteries, it cannot be used to examine deeper arteries like the carotid and femoral. As such, high-frequency ultrasound in the present form would not be a viable alternative to standard-frequency ultrasound for carotid IMT imaging studies. Also, there is no "gold standard" of intimal thickness against which to judge the accuracy of measures of intimal thickness using the high frequency probe.

High-frequency ultrasound generally produces high-quality images of superficial arteries; however, individual variations in a person's anatomy, such as vessel location and depth, can affect image quality. Readable anterior tibial artery images were unable to be obtained for three study participants due to anatomical constraints. Similar to the high-frequency images, standard-frequency images of the anterior tibial arteries for these three participants were also of mediocre to poor quality. Thus, individual patient variation plays a role in the quality of image acquired by high-frequency ultrasound.

The current study was conducted as a feasibility study to investigate the feasibility of using high-frequency ultrasound in IT and IMT studies of superficial arteries (brachial, radial, and anterior tibial); as well as to compare the relative qualities of high-frequency versus standard-frequency ultrasound images for these arteries. As such, the sample size of the project was relatively small. Future studies using larger sample sizes should be conducted to replicate and validate our reported results with greater statistical power and evaluate disease populations. Thus, it is possible that these results would not generalize to a population with more significant cardiovascular risk factors.

Conclusions

Radial and brachial ITs can be seen and measured independently of the media with a high degree of reproducibility using high-frequency ultrasound. The high frequency image quality was good for the anterior tibial arteries in healthy subjects but poor for those with PAD. Under optimal conditions, high-frequency ultrasound produces better quality images of superficial arteries than standard-frequency ultrasound, and high-frequency ultrasound may be a useful tool in superficial artery IMT studies. Future studies should aim to compare high-frequency and standard-frequency ultrasound in larger samples and in samples with more significant cardiovascular risk factors, such as peripheral arterial disease and diabetes mellitus. High-frequency ultrasound may allow for earlier detection of the vascular changes of atherosclerosis due to ability to measure the intima thickness directly compared to traditional IMT measurement which may not be as sensitive to presence of early intimal thickening.

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Figure 1.

Comparison of high-frequency (55 MHz) (1a-3a) and standard-frequency (15/7 MHz) (1b-3b) images of peripheral arteries in three subjects. In the high-frequency images, the intimal layer is white; immediately beneath is the black medial layer (arrows delineate thickness). IMT indicates intima-media thickness; IT, intima thickness.

Figure 2.
Representative ultrasound images from three arteries using high frequency probe from patient with peripheral artery disease. Arrows indicate intima-media thickness.

Table 1

Participant Demographics

	Healthy (N=10)	Peripheral Artery Disease (N=5)
Age	38.3 ± 11.0 [‡]	71.6 ± 13.6
Sex	7 Female	3 Female
Race	7 C; 3 A*	3C,2AA
Cardiovascular Risk Factors		
Congestive Heart Failure	0	1
Current Smoker	1	1
Diabetes Mellitus	0	4
High Cholesterol	0	3
Hypertension	1	4
Myocardial Infarction	0	1

A = Asian, AA African American, C=Caucasian

Table 2

Image Quality Rating Scale

Score	Clarity of Image
1	Margins clearly visible over entire image
2	Margins visible over 75% of image
3	Margins visible over 50% of image
4	Margins visible over 25% of image
5	Margins visible over less than 25% of image

Table 3

IT Thickness of Brachial, Radial, and Anterior Tibial Arteries

Artery	<u>High-frequency</u>		<u>Standard-frequency</u>	
	Mean	SD	Mean	SD
Radial	0.058	0.009	N/A	N/A
Brachial	0.051	0.007	N/A	N/A
Tibial	0.059	0.012	N/A	N/A

Measurements are in mm. N/A indicates that intimal thickness was not able to be measured.

Table 4

IT Thickness of Brachial and Radial Arteries in Patients and Normal Control

Artery	Normal Control (N=10)	Patient (N=5)	Student T test (p)
Radial	0.058±0.0003	0.12±0.009	<0.01
Brachial	0.051±0.007	0.104±0.015	<0.01

Measurements are in mm.