Noninvasive Low-Frequency Ultrasound Energy Causes Vasodilation in Humans

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OBJECTIVES
We evaluated the potential vasodilator effects of transcutaneous low-frequency ultrasound (US) in human brachial arteries.

BACKGROUND
Recent data show that transthoracic low-frequency US energy results in canine coronary artery vasodilation.

METHODS
Brachial artery diameters were measured before and after low-frequency US (29 kHz, 1.4 W/cm²) exposure using US imaging with a linear-array transducer. We assessed the time course of diameter changes after US in 20 subjects. In 10 of 20 subjects, brachial artery flow-mediated vasodilation (FMD) was measured to compare the effect of US to a standard method of evaluating endothelial function.

RESULTS
Significant vasodilation was seen after 2 min of US compared with baseline values. At 5 min of US, the brachial artery diameter increased by 4.1%. In addition, the arteries continued to dilate after US exposure. At 3 min after US there was a 5.4%, and at 5 min after US a 6.0% increase in vessel diameter (p < 0.001). These diameters returned to baseline dimensions about 20 min after stopping US. Ultrasound-mediated vasodilation and percentage FMD showed good correlation (r = 0.87; p < 0.001).

CONCLUSIONS
This is the first study to demonstrate that noninvasive transcutaneous low-frequency US energy dilates human brachial arteries. This arterial vasodilator effect has a rapid onset (within 2 min), lasts about 20 min, and is similar in magnitude to that of FMD. The vasodilator effect of US may have diagnostic and therapeutic potential in patients with or at risk for vascular disease. (J Am Coll Cardiol 2006;48:532–7) © 2006 by the American College of Cardiology Foundation

Ultrasound (US) is generally used for diagnostic imaging using MHz frequencies. It is also being developed as a therapeutic tool related to the direct mechanical energy effects of low-frequency (kHz) US. Low-frequency US energy accelerates thrombolysis by itself or when used in conjunction with a thrombolytic agent in vitro and in vivo (1–7). Low-frequency US energy also improves tissue perfusion and reverses acidosis in ischemic tissues in animal models (7–9). Thus, low-frequency US may have a potential vasodilator effect in humans. Invasive, catheter-delivered, low-frequency (20 kHz) high-intensity US has previously been shown to cause vasodilation in peripheral and coronary arteries in animals and humans (10–12). Recently our group showed that noninvasive, transcutaneous low-frequency US energy results in canine coronary vasodilation. Within 5 min of US exposure, the cross-sectional area (CSA) in canine coronary arteries increased by 21%. This vasodilator effect was reversible and similar in magnitude to that induced by nitroglycerin (13). In this study, we evaluate if transcutaneous low-frequency US has a vasodilator effect on human brachial arteries in vivo. In addition, to investigate the mechanism of the vasodilator effect, we compare the effect of low-frequency US to brachial artery flow-mediated vasodilation (FMD), which is a standard method for the evaluation of endothelial-dependent vasomotor function.

METHODS
Twenty participants were enrolled in this study (mean age ± SEM: 36 ± 2 years; 19 men). One had hypertension on treatment, 1 had borderline dyslipidemia, 2 had a family history of coronary artery disease, and 1 was a current smoker. The Cedars-Sinai Medical Center Institutional Review Board approved the study, and all participants provided written informed consent.

Study protocol. Low-frequency transcutaneous US (29 kHz, 1.4 W/cm²) was applied for 1 to 5 min to identify the onset as well as magnitude of the US vasodilator effect in human subjects. Because endothelial function is affected by food, caffeine, and time of day (circadian rhythm), the study was performed in the morning in a fasting state. After baseline US imaging of the brachial artery, low-frequency transcutaneous US was applied to the arm for 1 min, 2 min, or 3 min in 10 subjects. Images were obtained in this group after 1, 2, or 3 min of US. In 20 subjects, US was applied continuously for 5 min. In the group receiving 5 min of continuous US, subsequent vascular imaging and measurements of the brachial artery were repeated immediately after the 5 min of US exposure as well as 3 min and 5 min after

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Ultrasound image acquisition and determination of FMD. The brachial artery was imaged with an HDI-5000 US system and broadband L12-5 linear-array transducer (Philips Medical, Bothell, Washington). Studies were performed in a quiet, temperature-controlled (23°C) room. The subjects were positioned supine with the arm in a comfortable position for imaging of the brachial artery, which was imaged above the antecubital fossa. A transverse plane with the use of color flow Doppler facilitated location of the brachial artery, which was then imaged in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall was selected for continuous two-dimensional (2D) grayscale imaging. The 2D image was optimized using the depth function in the US system. Imaging depth and gain settings were kept constant throughout the study. The baseline image was then acquired. Brachial artery diameter was measured at the same time in the cardiac cycle using electrocardiogram gating during image acquisition. The onset of the R-wave was used to identify end-diastole, which corresponds to the minimum diameter of the artery. A pulse-wave (PW) Doppler was obtained from the mid-artery. Flow velocity and velocity-time integral (VTI) were calculated on an average of consecutive 3 heart cycles at baseline and after US exposure. Absolute flow (ml) during 1 heart cycle was calculated by multiplying the cross-sectional area and VTI. Brachial artery diameter measurements were performed off line by 2 cardiologists using a semiautomatic quantitative analysis system after the procedure. The second cardiologist performing the measurements was blinded to the data obtained by the first cardiologist. All diagnostic US data were digitized, and off-line measurements were performed by the the cessation of US exposure. Brachial artery percentage ultrasound-mediated vasodilation (%UMD) was defined as (highest diameter in these 3 different time points after US exposure – baseline diameter)/(baseline diameter). Brachial artery Doppler was also performed 30 s, 3 min, and 5 min after stopping the application of US to assess changes in flow.

In 10 of the 20 subjects, the low-frequency US study was repeated to assess the reproducibility of the vasodilator effect. In this study group, the brachial artery diameter was measured every 5 min until the arterial diameter decreased to its baseline value. Brachial artery flow-mediated vasodilation (FMD) was also measured in these subjects to compare the effect of low-frequency US to the standard method for evaluation of endothelial-dependent vasomotor function.

**Vericis Echo Review application** (Camtronics Medical Systems, Hartland, Wisconsin). All the data were measured twice, and final values were averaged. The inter- and intra-observer coefficients of variation (CV) for brachial artery diameters were 0.9% and 0.5%, respectively. The mean error of brachial artery diameters between measurements was 0.028 mm, and the absolute error ranged from 0.002 to 0.086 mm.

The method for determining brachial artery FMD followed the guidelines of the International Brachial Artery Reactivity Task Force (14). In brief, after measurement of baseline brachial artery diameter, arterial flow was interrupted for 5 min by a blood pressure cuff placed on the proximal forearm at least 50 mm Hg higher than arterial pressure. Using electrocardiographic triggering, end-diastolic images were captured at baseline and for 2 min after cuff deflation. Brachial artery %FMD induced by reactive hyperemia was expressed as relative change from baseline (%FMD = [(60-s maximum diameter – baseline pre-cuff diameter)/(baseline pre-cuff diameter)] × 100).

**Transcutaneous US device.** The US system consists of a generator and a US transducer delivering 29-kHz pulsed-wave US (Timi3 Systems, Santa Clara, California). The transducer, which transmits US energies through a water-filled bladder, was placed over the upper arm covering the brachial artery as shown in Figure 1. The transducer-radiating area is about 60 cm², and the surface was coupled to the skin with US transmission gel (Graham-Field, Bay Shore, New York). US maximum intensity is approximately 1.4 W/cm² (corresponding to spatial-average, temporal-average intensity of about 0.12 W/cm²). The US signal is pulsed wave, and the US is on 30% of the time and off 70% of the time (30% duty cycle). The pulse repetition frequency of the ultrasound system is 25 Hz, and its temporal maximum US power is about 23 W. The shape of the US field diverges and covers the brachial artery. Because of the diverging beam shape the maximum US intensity is at the
entrance point of US, i.e., on the skin surface. The output of the transducer was measured with a calibrated hydrophone (model 8103, Brüel & Kjær, Nærum, Denmark) in accordance with the existing guidelines and standards (15–17).

Skin temperature measurements. Skin temperature measurements were made during 5 min of US exposure in 10 subjects. The skin temperature directly beneath the US probe was measured with a thermometer (HH202A, Omega Engineering, Stamford, Connecticut).

Statistical analysis. Continuous variables were expressed as mean ± SEM or percentage change. Paired t test was used to assess changes in brachial arterial diameter, Doppler measurements, and skin temperature before and after US exposure. Repeated measures analysis of variance (RMANOVA) was used to assess changes in brachial artery diameter across the 4 time points. Contrasts were used to compare each post-US mean with the baseline mean; the significance level for each contrast was adjusted to 0.0167 by the Bonferroni method. Pearson’s correlation coefficient was calculated to show the relation of the US effect to brachial artery %FMD and the reproducibility of the US effect. Unpaired t test was used to compare brachial artery %UMD to %FMD and responders to minimal responders divided by US effect. A p value of <0.05 was considered statistically significant.

RESULTS

Ultrasound measurements. Percentage brachial artery diameter changes before and immediately after US exposure according to different durations of US exposure are shown in Figure 2. There is significant vasodilation after 2, 3, and 5 min of US exposure compared to the baseline values. The skin temperature did not change before and after 5 min of US exposure (30.5 ± 0.44°C to 30.7 ± 0.41°C; p = NS; n = 10).

A representative case of a B-mode vascular US image before and after 5 min of US exposure is shown in Figure 3. Brachial artery dilates from 3.938 mm at baseline to 4.567 mm after US exposure. Figure 4 shows the time course of changes in mean brachial artery diameter in response to US exposure. The RMANOVA model was significant and each time comparison to baseline had a p value of <0.001. Mean brachial artery diameter increased from 4.06 ± 0.08 mm at baseline to 4.30 ± 0.09 mm at 5 min after US exposure. After 5 min of US exposure, mean

![Figure 2](image)

**Figure 2.** Percentage brachial artery diameter changes before and immediately after ultrasound (US) exposure in different time durations. There is significant vasodilation after 2, 3, and 5 min of ultrasound exposure compared with the baseline values. Images of the brachial artery were digitized and measured using the Vericis Echo Review application (Camtronics Medical Systems, Hartland, Wisconsin). The R-wave of the electrocardiogram was used to time the measurements of vascular diameter. *p < 0.01; †p < 0.001; mean ± SEM (%), n = 10.

![Figure 3](image)

**Figure 3.** A representative case of B-mode ultrasound image on brachial artery at baseline (left) and after 5 min ultrasound exposure (right). Brachial artery diameter was measured off-line after the procedure by the Vericis Echo Review application (Camtronics Medical Systems, Hartland, Wisconsin) using a semiautomatic quantitative analysis system (3.938 mm at baseline and 4.567 mm after ultrasound exposure). *p < 0.001, mean ± SEM (mm), n = 20.
brachial artery diameter increased by 4.1% (p < 0.001 compared with baseline). The arteries further dilated to 5.4% at 3 min after stopping US (p < 0.001) and continued to dilate to 6.0% at 5 min after stopping US exposure (p < 0.001). In 10 subjects, the brachial artery diameter was measured every 5 min until it returned to baseline. The brachial artery decreased to baseline measurements at 21 ± 3 min after US exposure.

Individual variations were observed in response to US exposure. Some of the subjects responded by augmenting well to US and some of them did not respond. We defined subjects who increased the brachial artery diameter by ≥0.1 mm as nonresponders (0.02 ± 0.02 mm; n = 5) and those with >0.1 mm increase in diameter as responders (0.28 ± 0.03 mm; n = 15). Nonresponders were different in age compared with responders (45 ± 3 years vs. 33 ± 2 years, respectively; p < 0.05). In addition, 3 of 5 nonresponders had other cardiovascular risk factors. Namely, 1 nonresponder had hypertension, 1 had dyslipidemia, and 1 was a smoker.

**Doppler study.** Overall, VTI did not change significantly but tended to slightly decrease after 5 min of US exposure compared with baseline (18.3 ± 1.6 cm to 17.6 ± 1.8 cm; p = NS; n = 20). Conversely, pulsatility index (PI), which is an index of peripheral vascular resistance calculated as (peak systolic velocity − minimum diastolic velocity)/mean velocity, had a small nonsignificant increase (6.6 ± 1.4 to 7.5 ± 1.3; p = NS; n = 20). Absolute flow did not change before and after 5 min of US exposure (2.2 ± 0.2 ml/beat to 2.3 ± 0.2 ml/beat; p = NS; n = 20). However, individual variations were observed in response to US exposure in VTI. Five of 20 subjects increased more than 9% (range 9.4% to 57.5%) in VTI compared with baseline. All of these 5 cases dilated their brachial artery (responders) after US exposure. Another 5 of 20 subjects had essentially no or minimal change in their VTI (<5%, range −4.9% to 3.6%); 1 was a nonresponder and 4 were responders. The other 10 of 20 subjects had a decrease in VTI of more than 9% (−38.5% to −9.3%); 4 were nonresponders and 6 were responders.

**Comparison with FMD and reproducibility of US effect.** In 10 of 20 subjects, in addition to the response to US we measured brachial artery FMD to compare with the standard method for evaluating endothelial function. Mean brachial artery %FMD was 5.9 ± 1.0% (range 2.9% to 12.6%) and mean brachial artery %UMD was 5.6 ± 1.1% (range 0.3% to 11.6%) in these repeated subjects (n = 10). There was a similar change in %FMD and in %UMD (5.9 ± 1.0% vs. 5.6 ± 1.1%; p = NS). In addition, there was a good correlation between brachial artery %UMD and %FMD, as shown in Figure 5 (r = 0.87; p < 0.001). The absolute differences between UMD and FMD ranged from 0.003 to 0.181 mm (mean 0.102 mm) for brachial artery diameters and 0.05% to 2.76% (mean 1.38%) for percentage change. The reproducibility of low-frequency US vasodilator effect in the same patients at 2 different time intervals was also good (r = 0.80; p < 0.005). The absolute differences between 2 different measurements ranged from 0 to 0.60 mm (mean 0.10 mm) for brachial artery diameters and 0.19% to 5.72% (mean 1.64%) for %UMD. The coefficients of variation between two different time studies were 1.7% for brachial artery diameters and 16% for %UMD measurements.

**DISCUSSION**

This is the first study to demonstrate that noninvasive transcutaneous low-frequency US energy dilates human arteries. There are also no earlier data regarding the duration of vasodilator effect after low-frequency US exposure in vivo. We found that this vasodilator effect has a rapid onset (within 2 min) and that it lasts about 20 min. A few studies...
have demonstrated that invasive catheter–delivered low-frequency US dilates coronary and peripheral arteries in animals and humans (10–12). In a study of noninvasive transcutaneous low-frequency US in dogs, it was shown by intravascular coronary US that US energy dilates coronary arteries (13). In that study, the vasodilator effect was apparent after 30 s (4.6% increase in diameter) of US exposure, after 2 min (8.2% increase in diameter) of US, and further after 5 min (10% increase in diameter) of US in dogs’ coronary arteries. These findings in a canine model are consistent with our data regarding the onset of US vasodilation in humans.

Although the mechanism of vasodilation induced by low-frequency US is not entirely known, it is likely to be due to a local vasodilator effect related to stimulation of endothelial cells by local vibrations from low-frequency US. Vascular endothelial cells normally respond with several mechanical stresses, including shear tension and compression. Ultrasound is a propagating pressure wave that transfers mechanical energy to tissues. Krasovitski and Kimmel (18) have found that shear stress is induced at a tissue wall in an US field. Suchkova et al. (8) have suggested that US can be viewed as a method to expose endothelial cells to controlled mechanical stress. Shear stress is particularly important in regulating endothelial cell function, because it alters cell morphology, metabolism, and gene expression. Fluid shear stress induces a rapid, large, and sustained increase in nitric oxide (NO) activity. In the very acute setting (seconds) of shear stress, calcium-activated potassium channels open and increase NO production (19,20).

Nitric oxide contributes to vessel dilation by inhibiting vascular smooth muscle constriction (21). Several factors appear to be operant for the increase in NO in response to shear stress. Acute changes appear to be sensitive to the increase in intracellular calcium which happens as potassium ion channels open. Over minutes, phosphorylation of endothelial NO synthase owing to shear stress also occurs, and this has been hypothesized as being important for sustained generation of NO (22).

Recently, it has been demonstrated that low-frequency US causes vasodilation and improves tissue perfusion in ischemic limbs (7,8) and myocardium (9) in animals. It seems likely that the US-induced capillary dilation and enhanced tissue perfusion are due to an NO-dependent mechanism, because these effects were completely blocked by NO inhibition. Furthermore, Altland et al. (23) demonstrated that low-frequency (27 kHz continuous wave) US increases human umbilical vein endothelial cell NO synthase activity and NO synthesis in vitro. In that study, the cells showed a rapid response in NO synthesis with a maximum increase during 1 to 5 min after US exposure. The NO synthesis declined to baseline 30 min after US exposure. The duration of the vasodilator effect in the present study was about 20 min and thus parallels the increases in NO in that earlier study. This suggests that the vasodilator effect acts not only through the ion-channel mechanism but also through enzymatic phosphorylation.

The mechanism of vasodilation due to US is not the same as that due to FMD, which is recognized as a method for evaluating endothelial function by the release of NO (24). Flow-mediated dilation is caused by an increase in volume flow, and our data does not support this as a mechanism for US-induced vasodilation. In most of the cases, absolute flow did not change before and after US exposure. There was a heterogeneous response of brachial artery flow velocities to US. Because the US energy only affects the segment exposed to US (as seen in Fig. 1), flow velocities are determined by the resistance vessels in the hand and forearm. However, there is a good correlation between the magnitude of the US effect and FMD in the present study. It seems likely that both are mediated by an NO-dependent mechanism, because NO is an important regulator of vasomotor tone. Vasodilation secondary to smooth muscle relaxation from a vasoinhibitory effect of US can not be excluded in the present study.

Analysis of individual responses to low-frequency US showed that nonresponders (vasodilation ≤0.1 mm; n = 5) were different in age than responders (vasodilation >0.1 mm; n = 15) (45 ± 3 years vs. 33 ± 2 years; p < 0.05). The finding that age affects the response to low-frequency US is also consistent with the good correlation between UMD and FMD in the current study, because FMD has been shown to correlate with age (25). Mean %FMD in nonresponders was 3.8% in the present study. This was almost same value as for the group of subjects between 50 and 54 years old in the Framingham Study (25). In addition, 3 of the 5 nonresponders had cardiovascular risk factors. These factors could have affected the age differences between the Framingham study and our study.

A meta-analysis of 211 selected articles (11,984 patients) reporting on FMD and cardiovascular risk factors was recently published (26). In that meta-analysis, mean brachial artery %FMD was 6.8% in low-risk subjects divided into tertiles of Framingham risk score (27) (mean risk score 1.1% risk/10 years; mean age 30 years). Mean brachial artery %FMD in our study was 5.9 ± 1.0%. The %FMD of our study subjects is similar to that of the low-risk subjects in the meta-analysis. Because our study subjects are slightly older than the low-risk subjects in the meta-analysis (mean age 36 years vs. 30 years, respectively), there is a possibility that brachial artery %FMD is lower in our study subjects than in low-risk subjects in meta-analysis. These data suggest that our study group is representative of a low-risk patient population.

The safety of low-frequency US for animals using this intensity (1.4 W/cm²) and frequency (29 kHz) has already been shown in previous reports (1,2). High-intensity US can heat tissue (7,28), and tissue heating can cause vasodilation (29). However, previous studies using this same device have demonstrated that vasodilation as well as other effects occur during application of this device without an
increase in tissue temperature (1,9,13). In this study, we measured skin temperature at the site of US exposure using a thermometer. There was no evidence of any heating during US exposure to the subjects’ arm, and the skin temperature did not change during US exposure.

There are limitations of measurement of brachial artery diameter by the semiautomatic quantitative analysis system. However, the reproducibility of the low-frequency US vasodilator effect in the same patients at 2 different time intervals was good ($r = 0.80$; $p < 0.005$; mean absolute differences 1.64% for %UMD). This suggests that there is a consistent vasodilator effect of low-frequency US in human brachial arteries and that the methodology of this study using a semiautomatic quantitative analysis system is reasonable. Factors that significantly affect the reproducibility of UMD may be due to physiologic variables and secondarily due to technical errors.

In conclusion, the findings of the current study indicate that transcutaneous low-frequency US energy has a vasodilator effect with rapid onset (within 2 min) and a duration of about 20 min. The vasodilator effect of low-frequency US may have diagnostic as well as potential therapeutic applications in patients with vascular disease or dysfunction. Additional studies will be needed to address this issue.

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