Importance of Measuring the Time Course of Flow-Mediated Dilatation in Humans

Mark A. Black, N. Timothy Cable, Dick H.J. Thijssen, Daniel J. Green

Abstract—Flow-mediated dilation (FMD) is widely used to describe conduit artery endothelial function. The traditional approaches to FMD calculation assess diameter change at arbitrary time points after occluding cuff deflation. The aim of this study was to examine the time course of brachial artery FMD after a 5-minute period of forearm ischemia in 12 young, 12 fitness matched older and 12 older untrained subjects. Edge-detection and wall tracking of high resolution B-mode arterial ultrasound images, combined with synchronized Doppler waveform envelope analysis, were used to calculate brachial artery diameter, blood flow, and shear rate continuously across the cardiac cycle after forearm ischemia. FMD was significantly higher in young healthy subjects (7.8 ± 3.2%) compared with sedentary older subjects (5.2 ± 2.8%, P < 0.05) but not trained older subjects (6.4 ± 2.3%). Time to peak diameter differed between young (50 ± 11 seconds) and both older groups (trained; 80 ± 21, P < 0.001; sedentary; 83 ± 36 seconds, P < 0.001). A large proportion (>42%) of true peak diameters fell outside the time frames typically used to assess FMD in the literature. When calculated according to the commonly used approach, ie, 60 secs after cuff deflation, FMD was significantly lower compared with true peak FMD in all groups (P < 0.001), and no differences were evident between the groups. The time course of FMD differs significantly between young and older subjects. Studies assuming that peak dilation occurs at an arbitrary time point, or within limited time windows, may draw misleading conclusions regarding differences between groups. More sophisticated approaches to measurement of FMD are required if it is to be considered a valid biomarker of vascular disease. (Hypertension. 2008;51:203-210.)

Key Words: blood flow • arterial diameter • high-resolution ultrasound • doppler • shear rate

Endothelium-dependent flow-mediated dilatation (FMD) describes the vasodilator response of a conduit artery to elevations in blood flow–associated shear stress after a 5-minute period of ischemia.1 In the past 15 years, this method has been widely used in research settings to describe differences in conduit artery endothelial function between healthy subjects and those with cardiovascular diseases2 or risk factors,3 or to assess the impact of various lifestyle4,5 or pharmacological interventions.6,7 The traditional approach to FMD calculation expresses the diameter at 60 seconds after cuff deflation relative to the preceding baseline diameter.1 This approach is currently used by many research groups, with recent publications in highly ranked peer reviewed journals.8–12

Nonetheless, little is known about the time course of change in arterial diameter and shear rate after a 5-minute period of forearm ischemia. Furthermore, there is a large variation in the literature regarding the time frame for postdeflation measurement of diameter changes. For example, recent studies examining brachial artery endothelial function in older subjects examined the FMD response at either 1 minute,8 between 70 to 90 seconds,13,14 or up to 215 or 316 minutes after cuff deflation. Indeed, even those studies which have assessed diameter at multiple time points after cuff deflation may fail to identify the true peak diameter if this falls between, or outside, the selected discrete measurement periods. Although reviews have been published regarding the methodology used to assess FMD,17,18 these do not describe the optimal method for assessing time-to-peak diameter or deal specifically with the correction of FMD for the eliciting shear rate stimulus.19

Because of our previous anecdotal observations that time-to-peak diameter often occurs outside the typical ranges used for assessment, the aim of the present study was to continuously assess changes in brachial artery diameter after a 5-minute ischemic stimulus applied to the forearm in healthy young subjects, fitness-matched older subjects, and older sedentary subjects. We used a continuous edge-detection and wall tracking system, which calculates arterial diameter, blood flow, and shear rate at 30 Hz. We hypothesized that differences would exist in the time course of diameter change between young and older subjects and between fit and unfit older individuals.

Received September 4, 2007; first decision September 21, 2007; revision accepted November 26, 2007.
From the Research Institute for Sport and Exercise Science (M.A.B., N.T.C., D.H.J.T., D.J.G.), Liverpool John Moore’s University, UK; the Department of Physiology (D.H.J.T.), Radboud University Nijmegen Medical Centre, The Netherlands; and the School of Human Movement and Exercise Science (D.J.G.), The University of Western Australia, Crawley.
Correspondence to Dr Danny Green, Research Institute for Sport and Exercise Science, Henry Cotton Campus, Liverpool John Moores University, 15-21 Webster Street, Liverpool, L3 2ET. E-mail d.j.green@ljmu.ac.uk
© 2008 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.107.101014
Materials and Methods

Subjects
Twelve healthy young recreationally active volunteers (6 m, 6 f; 26.2±3.3 years), 12 older healthy sedentary (6 m, 6 f; 58.9±5.1 years), and 12 age-matched trained older subjects (6 m, 6 f; 57.3±3.9 years; Table 1), were recruited from the community. Premenopausal females were tested during the early follicular phase of their cycle. All subjects were screened for cardiac abnormalities and cardiovascular disease before entering the study and performed a 12-lead ECG-graded treadmill exercise test. No subject reported having been diagnosed with cardiovascular disease or risk factors such as hypercholesterolemia or hypertension. Subjects who smoked or were on medications of any type were excluded. The study procedures were approved by the Ethics Committee of Liverpool John Moores University, adhered to the Declaration of Helsinki, and all subjects gave prior written consent.

Experimental Design
Under standardized conditions and at the same time of day, the brachial arterial diameter and shear rate changes to a 5-minute ischemic period were continuously recorded, using high-resolution B-mode ultrasound imaging. All measures were performed after a 6-hour fast, 8-hour abstinence from caffeine or alcohol, and at least 24 hours after strenuous physical activity.

Experimental Procedures

Assessment of Physical Fitness
Exercise testing was undertaken on a treadmill ergometer (H/P/Cosmos, Phasor 4.0, Nussdorf-Traunstein, Germany), with initial workload set at 4 km/h at 5% gradient and step-wise increments in speed and grade every 3 minutes until volitional exhaustion. Heart rate and rhythm were continuously recorded by 12-lead ECG, and blood pressure was measured during the last 30 sec of each 3 minutes stage. The volume of oxygen consumption (VO₂) during exercise was calculated from minute ventilation, measured using a pneumotach and simultaneous breath-by-breath analysis of expired gas fractions (Medgraphics CPX/D). Minute ventilation, measured using a pneumotach and simultaneous breath-by-breath analysis of expired gas fractions (Medgraphics CPX/D).

Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (Dinamap; GE Pro 300V2) on the contralateral arm. A rapid inflation/deflation pneumatic cuff was positioned on the upper arm immediately distal to the olecranon process to provide a stimulus to forearm ischemia. A 7.5-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Aspen, Acuson) was used to image the brachial artery in the distal third of the upper arm. Ultrasound parameters were set to optimize longitudinal B-mode images of the lumen/arterial wall interface. Continuous pulsed wave Doppler velocities were obtained using the Aspen, and data were collected using the lowest possible insonation angle (always <60°), which did not vary during each study.

Flow-Mediated Dilation
To examine the brachial artery FMD response, a rapid inflation/deflation pneumatic cuff was used to provide the ischemic stimulus. After an initial 15-minute resting period, baseline scans assessing resting vessel diameter and flow were recorded over 1 minute. The cuff was then inflated to >200 mm Hg for 5 minutes. Diameter and flow recordings resumed 30 seconds before cuff deflation and continued for 3 minutes thereafter. Peak artery diameter and flow, and the time taken to reach these peaks after the release of the occlusion, were recorded.

Brachial Artery Diameter and Blood Flow Analysis
Post-test analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias. Briefly, the video signal was taken directly from the ultrasound machine and, using an IMAQ-PCI-1407 card, was encoded and stored as a digital DICOM file on the PC. Subsequent software analysis of this data were performed at 30 Hz using an icon-based graphical programming language and toolkit (LabVIEW 6.02, National Instruments). The initial phase of image analysis involved the identification of regions of interest (ROI) on the first frame of every individual study (Figure 1). These ROIs allowed automated calibration for diameters on the B-mode image and velocities on the Doppler strip. An ROI was then drawn around the optimal area of the B-mode image within this ROI a pixel-density algorithm automatically identified the angle-corrected near and far-wall e-lines for every pixel column within the ROI. The algorithm begins by dividing the ROI into an upper half, containing the near wall lumen-intima interface, and a lower half containing the far wall interfaces. The near-wall intimal edge is identified by a Rake routine that scans from the bottom to the top of the upper half of the ROI. The position of the edge is established by determining the point where the pixel intensity changes most rapidly. Typical B-mode ROIs therefore contained approximately 200 to 300 diameter measurements per frame, the average of which was calculated and stored. This process occurred at 30 frames per second. We did not use the R-wave
gating function in our software because of previous observations that at 30 Hz the continuous assessments of diameter yields similar results.

A final ROI was drawn around the Doppler waveform and automatically detected the peak of the waveform (Figure 1). The mean diameter measure derived from within the B-mode ROI (above) was synchronized with the velocity measure derived from the Doppler ROI at 30 Hz. Ultimately, from this synchronized diameter and velocity data, blood flow (the product of cross-sectional area and Doppler velocity) and shear rate (4 times velocity divided by diameter) were calculated at 30 Hz. All data were written to file and retrieved for analysis in a custom designed analysis package (Figure 2). We have shown that reproducibility of diameter measurements using this semiautomated software is significantly better than manual methods, reduces observer error significantly, and possesses an intraobserver CV of 6.7%.

Furthermore, our method of blood flow assessment is closely correlated with actual flow through a “phantom” arterial flow system.

**Data Analysis**

Baseline diameter, flow, and shear rate were calculated as the mean of data acquired across the 1 minute preceding the cuff inflation period (Figure 2B). After cuff deflation, a custom designed software program was used to determine peak diameter from the 30 Hz of mean diameter data derived according to the methods described above. Peak diameter after cuff deflation was automatically detected according to an algorithm which identified the maximum bracket of data subsequent to performance of a moving window smoothing function. This smoothing routine calculates the median value from a bracket of data which shares 20% overlap with the preceding bracket. The maximum value of all the calculated median values is then automatically detected and chosen to represent the peak of the diameter curve (Figure 2A). FMD was calculated as the percentage rise of this peak diameter from the preceding baseline diameter. The time to peak diameter (in seconds) was calculated from the point of cuff deflation to the maximum post-deflation diameter. Calculation of FMD and time to peak were therefore observer independent and based on standardized algorithms applied to data, which had undergone automated edge-detection and wall tracking. For the purpose of comparison, we also calculated the FMD response according to the traditional guideline, which suggests that peak dilation occurs at 60 seconds after deflation.

**Statistics**

In accordance with recent findings, we expressed FMD data normalized to the shear rate stimulus responsible for endothelium-dependent FMD. The postdeflation shear rate data, derived from simultaneously acquired velocity and diameter measures at 30 Hz (Figure 2B), was exported to a spreadsheet and the area under the shear rate curve (AUC) calculated for data up to the point of maximal postdeflation diameter (FMD) for each individual. In this way an individual’s FMD was normalized to the area under their own shear rate curve between the point of deflation and maximal dilation for that individual.

Statistical analyses were performed using SPSS 14.0 (SPSS) software. All data are reported as mean (SD) and statistical significance was assumed at P<0.05. ANOVA and post-hoc unpaired t tests were used to assess significance of difference between groups and the impact of age and fitness status on the changes in brachial artery diameter, blood flow, and shear rate.

**Results**

Peak oxygen consumption and body mass index were similar between young subjects and older fit subjects, whereas sedentary older subjects demonstrated a significantly lower peak oxygen consumption and higher body mass index compared with the other 2 groups (Table 1). There were no significant differences in resting diastolic blood pressure between the 3 distinct groups, whereas the older groups demonstrated a significantly higher systolic blood pressure.

**Assessment of Brachial Artery Responses in Young, Old Fit, and Old Unfit Subjects**

Brachial FMD was similar between healthy young and fitness-matched older subjects, but significantly lower in sedentary older subjects compared with young healthy persons (Figure 3). Importantly, when brachial artery FMD was calculated according to the traditionally accepted 60-second postdeflation time point, no differences were found between the 3 groups. Moreover, the 60-second postdeflation FMD differed significantly from the true peak FMD in all groups (Figure 3). The time to peak diameter after cuff release was significantly briefer in the young healthy subjects compared
Figure 2. Upper panel, The flow-mediated dilation (FMD) edge detection and wall tracking software “output” screen. Each dot represents the mean of 200 to 300 diameter measures for a given frame, with a frame rate of 30 Hz. The vertical cursors are placed at times corresponding to baseline or FMD periods. The peak responses after FMD are calculated by applying a smoothing algorithm which (Continued)
with both older groups, whereas older fit and unfit subjects did not differ (Figure 4). If FMD was assessed within specified time windows, as is common in the literature (50 to 70 seconds or 70 to 90 seconds), 42% to 100% of the subjects fell outside these predetermined brackets (Table 2). Even continuous assessment of postdeflation diameter up to 90 seconds after cuff deflation results in the true peak dilation being missed in 42% of the trained and sedentary older subjects (Table 2).

The AUC for shear rate from cuff deflation to peak diameter was not different between young, trained older, and sedentary older subjects (18042±10934, 20590±9470, 19576±10038 U, respectively). Correcting the brachial artery FMD for the shear rate AUC until peak diameter, which was recently indicated as the most appropriate method to correct for the eliciting stimulus,22 revealed no differences between groups (Figure 5). When the AUC for shear rate was calculated from 0 to 30 seconds after cuff deflation, as is common practice in the literature, no difference was evident between groups (13564±7389, 13152±4393, 11088±5576 U, respectively), but this data were significantly lower compared with the AUC until the true peak diameter. Thus, use of a limited time frame to correct for the shear rate stimulus (eg, 1 to 30 sec after deflation), results in different corrected FMD values compared with the correction applied up to the true peak diameter in all groups (Figure 5).

**Figure 2 (Continued).** determines the median value of a moving window of consecutive data points. The “peak” of the smoothed median values detected in this way is then used to calculate %FMD via comparison to the preceding baseline. Lower panel, Still frame of B-mode ultrasound data “Display” software. Continuous traces of brachial artery diameter (white), velocity (red), and flow (yellow) against time. Vertical “begin” and “end” cursors are placed to zoom in on selected data and, ultimately, calculate mean (MBF) blood flows, area under the flow curve, and similar data for shear rate.

**Figure 3.** Brachial artery FMD values after a 5-minute period of forearm ischemia in healthy young (n=12), old fit (n=12), and old unfit (n=12) subjects. FMD was calculated as the dilation at 60 seconds after cuff deflation (“60 sec FMD,” black symbols) and at the true peak (“true FMD,” white symbols) after cuff deflation. Error bars represent the standard error of the mean for each group.

**Figure 4.** Mean and individual brachial artery diameter time to peak dilation after a period of 5 minutes of forearm ischemia in healthy young (n=12, black dots), old fit (n=12, black squares), and old unfit (n=12, open squares) subjects. Error bars represent standard error of the mean.

**Discussion**

The findings of this study have important implications for the assessment of FMD in humans. First, when using continuous diameter assessment, the time to peak diameter change in response to a 5-minute ischemic stimulus in the brachial artery differed significantly between young and older subjects. In addition, brachial FMD calculated according to the traditional approach adopted in the literature, that is from diameters assessed at 60 seconds after deflation, was significantly lower in all groups compared with the true peak FMD values we observed. Indeed, a large proportion of peak diameter measurements occurred outside the commonly used assessment windows for postdeflation diameter assessment.

Of more importance is the observation that opposite conclusions would have been reached using our continuous diameter assessment methodology in comparison with the usual fixed window approach. For example, it would be concluded that no differences in brachial artery FMD between groups were evident if the traditional 60-second FMD approach was adopted, whereas the true peak FMD data revealed a significant difference between sedentary older subjects and healthy subjects. These data clearly demonstrate that the method used to assess peak diameter after cuff deflation significantly alters FMD values and also the implications drawn from the same data set. Studies which assume that peak dilation occurs at an arbitrary time point or within an arbitrary time window may draw misleading conclusions regarding differences in true peak dilation responses between groups. We contend that these results support continuous assessment of arterial diameter, flow, and shear rate at high temporal resolutions for a prolonged time period when examining conduit artery endothelial function, something which is not universally, or even commonly, performed at present in the clinical and physiological literature.

The FMD response to a 5-minute ischemic period is a frequently used method to assess endothelial function in humans. As of August 2007, several thousand articles have used FMD as a principal outcome measure. Many of these, including recent high-impact articles,8–12 use the arbitrary 60-second time point to determine postdeflation arterial
responses. This traditional approach, based on the seminal studies of Celermajer et al.\(^1,23\) is not based on any rigorous published scientific data regarding time to peak diameter change in different subject or patient groups. While no consensus exists regarding the most appropriate time frame for peak response measurement,\(^17,18\) many studies have assessed diameter within fixed postdeflation time windows, most commonly before 90 seconds after deflation.\(^13,24–26\) Had this approach been adopted in the present study, the true time to peak of at least 42% of older subjects would still have been missed. An analysis window of 20 seconds within the 0 to 90 second postdeflation period could have resulted in all true peak diameters being missed in the present study (Table 2). Furthermore, studies which have assessed diameter in fixed time windows postdeflation have typically adopted discrete predetermined analysis time points, for example diameter assessments at 30-second or 1-minute intervals.\(^27\) In the absence of continuous diameter measurement, the possibility therefore remains that true peak diameters may have fallen between these discrete measurement intervals.

The different time to peak diameter we observed between groups leads to an intriguing question regarding the potential reasons for this observation. Advanced age is associated with an increased arterial stiffness.\(^28\) This change in compliance of the arterial wall with age may explain the delayed time of peak diameter. In addition, the biological aging process is linked mechanistically to altered pharmacodynamic responses to vasoactive drugs.\(^29\) It is conceivable that changes in enzyme rate constants with age may delay endothelial hormone production or smooth muscle cell responsiveness. In addition, age-related changes in free radical production may influence the rate of diameter change, as agents such as superoxide anions are known to interact with endothelial-derived vasodilators.\(^30\) These explanations remain speculative, and future studies should further examine the potential reasons for the delayed time to peak during the FMD response between subjects or after interventions.

An associated issue which the present study raises is that related to the correction of FMD data to shear rate. Pyke and Tschakovsky have elegantly demonstrated\(^22\) that it is important to correct FMD responses for the shear rate stimulus that elicits the diameter change. Ideally, the hemodynamic change that occurs within the artery after cuff deflation should be represented as the area under the shear rate curve up to the point at which peak diameter is observed for every individual.\(^22,31\) However, most studies that undertake FMD correction do so by calculating the shear rate AUC for a set period of time, for example 30 seconds after cuff deflation. It follows that significant differences in time to peak between groups lead to different area under the curve calculations for shear rate between groups. This difference is ignored if an arbitrary period of assessment such as 30 seconds is used. In the present study shear rate area under the curve calculated to 30 seconds after deflation was significantly lower than that calculated to the point of true peak dilation in each group. As a consequence, using these different values to correct FMD will potentially result in spurious conclusions being drawn.

**Limitations**

Hypertension and obesity are important cardiovascular risk factors and are strongly related to an impaired endothelial function.\(^4,12\) Differences in blood pressure and body mass index between the young and older groups in the present study may contribute to the delayed time to peak dilation in the older groups. Although both older groups demonstrated significantly higher systolic blood pressures, all subjects were within the normal range and hypertension was an entry exclusion criterion. Whereas obesity was present in 5 older sedentary subjects, no differences regarding the time to peak dilation were present between the obese (83±42 seconds) and

---

**Table 2. Proportion of Subjects (%) in Whom, Compared to Continuous Assessment to Find the True Peak Flow Mediated Dilation (FMD), FMD Was Correctly Identified Using the Most Common Assessment Methods in the Literature**

<table>
<thead>
<tr>
<th>Assessment Method</th>
<th>Young Subjects (n=12)</th>
<th>Trained Old Subjects (n=12)</th>
<th>Sedentary Old Subjects (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–120 seconds</td>
<td>100%</td>
<td>100%</td>
<td>83%</td>
</tr>
<tr>
<td>70–90 seconds</td>
<td>58%</td>
<td>25%</td>
<td>8%</td>
</tr>
<tr>
<td>0–90 seconds</td>
<td>100%</td>
<td>58%</td>
<td>58%</td>
</tr>
<tr>
<td>50–70 seconds</td>
<td>58%</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>0–60 seconds</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Traditional 60-second value</td>
<td>8%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Figure 5. Brachial artery corrected FMD values after a period of 5 minutes of ischemia in healthy young (n=12), old fit (n=12), and old unfit (n=12) subjects. FMD is corrected by dividing by the area under the curve for shear rate from 0 to 30 seconds (“AUC 0 to 30 sec,” black bars) or the area under the curve for shear rate up to the time point of true peak dilation (“AUC peak dilation,” white bars). Error bars represent the standard error of the mean.**
nonobese older sedentary subjects (82±35 seconds). Similarly, we cannot exclude the possibility that differences existed between young, older, and fit subjects in terms of blood lipid profiles or glycemic control. However, all of the older subjects were healthy, none had not been diagnosed with any risk factors or cardiovascular diseases and none was taking medications of any sort. These findings suggest that the delayed time to peak we observed between groups was indeed related to age, rather than the small differences in cardiovascular risk factors. Another limitation of the present study relates to sample size, and we cannot rule out the possibility that differences between groups may have become significant if more subjects were studied.

Clinical Relevance
In this study, we demonstrate that using an arbitrary time point or time window to determine the FMD response leads to significant underestimation of true FMD in the brachial artery within groups and to misleading conclusions regarding the presence of differences between groups. Accordingly, the bracket of time to identify the true peak FMD in healthy older subjects must be increased to at least 3 minutes from cuff deflation. Preferably, diameter assessments should be performed continuously across this period. Given the strong relationship between advanced age and increased cardiovascular risk, one may hypothesize that subjects with other cardiovascular risk factors or diseases may exhibit longer times to peak diameter relative to healthy controls and this should be considered in all future FMD studies which compare groups or the impact of interventions.

Perspectives
The present study was designed to assess changes in brachial artery diameters after a 5-minute ischemic stimulus in healthy young subjects, fitness-matched older subjects, and older sedentary subjects. The results demonstrated that the time to peak diameter was significantly different between young and older subjects. Consequently, FMD calculated by the traditionally adopted methods significantly underestimated the true peak FMD value, and furthermore, opposing conclusions are drawn according to the method of analysis used. Using arbitrary time points or time windows to determine FMD responses, or to calculate shear rate for FMD correction, potentially biases study outcomes. Our data suggest that more sophisticated approaches to measurement of FMD are required if it is to be considered a valid and standardized surrogate biomarker of vascular disease.

Acknowledgments
We thank Chris Reed for his assistance with development of the edge detection and wall tracking software.

Sources of Funding
D.H.J.T. is financially supported by The Netherlands Organization for Scientific Research (NWO-grant 82507010). M.A.B. is supported by the grant from the British Heart Foundation (FS/05/117/19771).

Disclosures
None.

References


Importance of Measuring the Time Course of Flow-Mediated Dilatation in Humans
Mark A. Black, N. Timothy Cable, Dick H.J. Thijssen and Daniel J. Green

Hypertension. 2008;51:203-210; originally published online December 17, 2007;
doi: 10.1161/HYPERTENSIONAHA.107.101014

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/51/2/203

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/