Effects of Acute Stress on Brachial Artery Blood flow Measured via Ultrasound

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Abstract: We conducted a randomized, controlled trial to examine the effects of acute stress on vascular endothelial function in 29 men and women with elevated or normal cholesterol. In previous studies, a significant reduction in endothelial function in the immediate post stress period was observed. It is possible that differences in blood flow after stress may explain the variability observed by past studies. The goal of this research was to examine post-stress changes in brachial artery blood flow and blood velocity and to test whether cholesterol status alters this hemodynamic response to stress. We found significant reductions in baseline blood flow volume and blood flow velocity at 45 and 90 min post-stress. In addition, the percent increase in volume and velocity caused by cuff deflation was higher at 90 min post-stress vs. the resting baseline. There were no significant differences in blood flow responses between subjects with high vs. low cholesterol.

Introduction

Cardiovascular disease

Cardiovascular disease is the leading cause of death in industrialized countries. According to the American Heart Association, in 2002 cardiovascular disease impacted 70.1 million adults (34.2% of the total population) and the mortality rate was 927.4 thousand. This disease is prevalent in Western cultures. Some of the risk factors that are seen in individuals who have this disease are high blood pressure, tobacco use, high blood cholesterol, physical inactivity, overweight, obesity, and diabetes mellitus (AHA 2005).

There have been many studies to help inform researchers and the general population about this disease, but one important, landmark study has documented the progression of the disease over a period of time, within the same individuals. The Framingham Heart Study was funded by the federal government in Framingham, Massachusetts to learn the circumstances under which this disease effects the general population. In 1948, researchers recruited 5,029 men and women between the ages of 30 and 62 and began a project, which would impact society for many years to come. This study recruited a second generation in 1971 that consisted of 5,124 of the original participants' adult children and their spouses. The third generation (the children of the offspring cohort) is currently being recruited and examined to seek further understanding of how genetic factors relate to cardiovascular disease (NHLBI, 2002). The participants of this study have been monitored very closely through cardiovascular examinations, hospital admissions information, death information, information from physicians and other sources outside the clinic (AHA, 2005). The objective of this study was to identify that common factors or characteristics that contribute to cardiovascular disease by following the development of the disease over a period of time. This study has better informed researchers and the general public about the etiology of cardiovascular disease (NHLBI, 2002).

Although there are many factors, which contribute to cardiovascular disease atherosclerosis, is one of the major causes. Atherosclerosis is a build up of plaque (deposits of fatty substances, cholesterol, cellular waste products, calcium, and other substances) within the inner lining of an artery wall (AHA, 2005). This disease begins in childhood and progresses into adulthood. Studies have shown that this disease is genetic but it also is prevalent in individuals with elevated levels of cholesterol and triglycerides in the blood, those with high blood pressure, and individuals exposed to tobacco smoke (AHA, 2005).

Cardiovascular disease also has put a strain on the U.S. economy. According to American Heart Association, the U.S. spent an estimated $393.5 billion in 2005 on cardiovascular disease. These costs included direct costs (physicians, other professionals hospital, and nursing home services, medication, home health care and other medical supplies) and indirect costs (lost productivity resulting from morbidity and mortality). In contrast, the estimated cost of all cancers was half this amount (estimated at $190 billion-direct and indirect costs) in the same time period (AHA 2005).

Stress and Cardiovascular Disease

Cardiovascular disease and stress have been researched to show if there is a relationship between the two factors. Some of the factors that initiate stress are job strain, personal problems, and social interaction but more research needs to be done to better understand the mechanisms through which stress promotes vascular disease. Researchers are unsure if stress is an independent risk factor for cardiovascular disease; but stress may have a direct correlation with other risk factors such as high blood pressure, high cholesterol levels, smoking, physical inactivity, and unhealthy diet (AHA 2002). Strike and Steptoe (2004) summarized several studies on the role of psychological factors in CVD and reported a correlation between chronic stress, low socioeconomic status, depression, and social isolation and development of coronary artery disease and suggested that these characteristics can be seen as CVD risk factors (Strike and Steptoe 2004). In addition, in the Kuopio Ischemic Heart Disease Study, 901 Eastern Finnish men from four age cohorts (age 42 to 60 years) were studied to understand the correlation of cardiovascular reactivity to mental stress and atherosclerotic risk. Researchers found that men with the largest cardiovascular responses during exposure to mental stress had more severe atherosclerosis. (Kamarck et al.,1997). Although studies have shown the correlation between the two, there are many other factors, which contribute to this disease, and researchers acknowledge that psychosocial factors may not directly cause CAD (Strike and Steptoe, 2004).

Stress and Endothelial Function

The endothelium layer of the vascular walls regulates the blood flow and helps prevent atherosclerosis. This layer also responds to changes in blood flow such as shear stress; where it secretes vasodilators (nitric oxide (NO), prostacyclin) and vasoconstrictors (endothelin-1, angiotensin II), which regulate the dilation and constriction of vessels (West, 2001). Some recent studies have suggested that endothelial function is altered by stress (Ghiadoni, et al, 2000). The following coronary risk factors are also associated with the impairment of endothelial function: smoking, elevated cholesterol, saturated fat, obesity, and diabetes. (AHA 2005). Endothelial function is also related to flow mediated dilation (FMD), which is a non-invasive measure for endothelial health. Brachial artery flow mediated dilation is the most commonly used measure of endothelial function.   FMD is based on the understanding of how vascular smooth muscle responds to increases in blood flow (Sinoway et al, 1989). Blood vessels respond to their environment by dilating or constricting depending on the conditions they are under. The increase in blood flow causes shear stress on the endothelium, triggering NO release in healthy arteries, and brachial artery FMD measures how well the artery dilates in response to the physical stress of increased flow.

We recently completed a study that examined whether acute stress impacted the magnitude of arterial blood flow. FMD was measured during a rest and a stress period in participants over time increments (baseline, 10 min, 45 min, and 90 min). The results of this study showed that FMD was lower during the stress testing session than during the rest testing session, but significant reductions in FMD at 90 min post-stress were not present (Krick 2004). However, in our preliminary analyses, we did not measure the magnitude of the flow stimulus across the 4 time points. This is important because the size of the flow stimulus is a direct determinant of the magnitude of flow-dependent dilation.

Purpose of the present study

It appears that stress may be involved in the development of atherosclerosis (Strike and Steptoe, 2004). However, the mechanism(s) for this effect are not known. Previous studies have examined changes in forearm blood flow during stress. In general, they find that baseline blood flow significantly increases and that this increased blood flow returns to baseline within 10 min (Lindqvist 2004). However, in studies of FMD it is also interesting to know whether acute stress changes the peak hyperemic flow (the rate of blood flow after the release of a blood pressure cuff). Unfortunately, few papers have examined hyperemic flow after stress. This is due, in part, to use of older technologies for measuring forearm blood flow. In a recent study, Ghiadoni et al (2000) examined changes in forearm blood flow and FMD under resting conditions and at 30, 90, and 240 min post stress. Although they did not show the data on hyperemic flow in the manuscript, they noted that hyperemic flow was not changed in the 240 min after stress (Ghiadoni et al., 2000). Therefore, the present study investigated the effects of brief stressor on brachial arterial blood flow to examine one potential mechanism through which stress affects CVD risk.

Methods

Participants

Subjects for this study were carefully screened to ensure that they were healthy and they met the criteria for inclusion. Subjects were recruited through fliers and by contacting eligible subjects involved in previous studies in Dr. West's lab. After an initial telephone interview, potential subjects were scheduled for a screening visit at the General Clinical Research Center at the Pennsylvania State University campus to assess height, weight, blood pressure, and fasting serum lipids. In total, 29 individuals were recruited 14 healthy subjects (2 women) and 15 hypercholesterolemic subjects (1 woman). Inclusion criteria were as follows: LDL-C and total cholesterol between the 50-90th percentile as defined by NHANES III, triglycerides between 100-350 mg/dl, and age 35-65 years. We enrolled all eligible men and eligible post-menopausal women who were not on hormone replacement therapy (HRT). The following criteria were used to exclude potential participants from participation: current use of lipid-lowering medications (statins, niacin, bile acid binding resins) or anti-hypertensive medications (ACE-

inhibitors, beta-blockers, calcium-channel blockers, diuretics), use of oral or injected corticosteroids (steroid medication), inhaled beta-agonists, atherosclerotic disease, history of MI or angina; vascular disease, including peripheral vascular ischemia, intermittent claudication, retinopathy or significant neuropathy; inflammatory disease; tobacco use (cigarettes, cigars, chewing tobacco); allergy to latex or adhesive tape. It was important to ensure that all participants were healthy, expressing no clinically evident cardiovascular disease or diabetes, so that underlying disorders, such as metabolic syndrome, could be excluded as a confound.

After the screening visit, participants engaged in 1 or 2 additional visits. All participants took part in the stressor testing sessions, and a subset also returned for a rest only testing session. However, this report is restricted to the results of that acute stress session only. Participants were asked to fast for 12 hours prior to testing. Upon arriving at the General Clinical Research Center, participants under went a blood vessel function ultrasound test (described below). During the speech task, participants were presented with a problematic scenario, and then given 2 min to prepare a 3-min speech. Participants believed they were being videotaped as they presented; however, no actual recording occurred. Upon completion of the stressor portion of the session, participants were asked to rest for 10-min, at which time the second FMD and blood flow measurement was collected. A third FMD and blood flow measurement was collected 45-min following completion of the speech task (15-min after the previous FMD measurement). The final FMD and blood flow measurement was collected 90-min following completion of the speech task. Testing sessions lasted approximately 3-hours, and participants remained in a supine position for the duration of the testing sessions. Studies have shown that posture does not inhibit hemodynamic reactivity to stress, and responses to the speech stressor should agree with previous literature, regardless of posture (Sherwood and Turner 1993; Radaelli et al. 1994).

FMD of the brachial artery and brachial artery blood flow were assessed in the right arm four times during each visit once at baseline, and again at 10 min, 45 min, and 90 min post stress. This assessment was completed under controlled conditions by trained and blinded sonographer. The brachial artery above the elbow of the dominant arm was scanned in longitudinal sections. Changes in diameter were assessed by external B mode ultrasound imaging (using an Acuson Aspen equipped with 10 MHz linear array transducer; Acuson, Mountain View, California). This technique allowed for reliable detection of changes in vascular diameter as small as 0.1 mm. Participants wore a standard blood pressure cuff on the opposite arm and measurements were taken once every other minute during each rest and stress period. All blood flow velocity measurements were collected using pulsed Doppler flow with the probe at a 70 degree angle to the vessel.

Baseline measurements of blood flow, blood pressure, and heart rate were obtained after 15 min of supine rest. A pneumatic tourniquet was placed around the forearm distal to the target artery and inflated to a pressure of 200 mmHg for 5 min. Increased flow was induced by sudden cuff deflation. Images were recorded continuously during cuff deflation and they continued for 120 seconds afterwards.

Ultrasound images were stored on high quality sVHS tapes and analyzed by a single experienced observer. The rate of blood flow (ml/min) was calculated using customized software for measuring velocity time integrals of Doppler flow wave forms as follows [flow (ml/min)= velocity time integral x cross-sectional area of the vessel x heart rate].

Statistical Analyses

All variables were analyzed using a mixed models approach using SAS PROC MIXED (SAS v.8, Cary, NC). Models included condition, time, visit number, and condition order as fixed effects, and subject as a random effect. There were no significant effects of condition order. Tukey-Kramer adjusted *P*-values were used to examine the source of significant effects. Text and tables report least squares means ± SE, and probability values ≤ 0.05 were considered statistically significant.

Results

Effects of subject characteristics on flow

Examination of the cholesterol status of the participants in this study did not show any differences in flow volume or velocity between the groups. Due to this observation we were able to combine the two groups together and analyze their results as a whole.

Effects of acute stress on flow

Results are presented for six variables: base flow (ml/min), peak flow (ml/min), percent change in flow (%), base velocity (M/s), peak velocity (M/s), and percent change in velocity (%). Each of these variables were examined separately across the 4 time intervals (0, 10, 45, and 90 min post stress). We observed significant main effects of time for baseline blood flow volume; baseline flow velocity, percent change in blood flow volume, and the percent change in blood flow velocity (all P's ≤0.003). Figure 1 and Table 1 show that basal blood flow volume (the amount of blood flowing through the vessels before the cuff was inflated) showed significant changes over time. Baseline flow (ml/min) at 10 min post stress was unchanged compared to the resting value. However, at 45 min and 90 min post-stress there was a significant decrease in basal blood flow volume. Peak blood flow (ml/min, measured after the cuff was released) was unchanged at 10 min, 45 min, and 90 min vs. the pre-stress baseline. (Table 1, Figure 2). The percent change in blood flow after cuff release was not changed 10 min post-stress vs. the pre-stress baseline. However, this variable was significantly increased at 45 and 90 min post stress (Table 1, Figure 3).

Similar patterns were seen for measurements of blood flow velocity (thrust and speed of blood flow). The baseline velocity of blood flow (M/s) showed no significant changes in blood flow from 0 min to 10 min post-stress. At 45 min and 90 min post-stress, there was a significant decrease in basal blood flow velocity (Table 1, Figure 4). However, the peak velocity of blood flow (M/s) showed no significant change at 10 min, 45 min, and 90 min post-stress (Table 1, Figure 5).

The percent change of velocity showed no change at 10 min or 45 min, but this velocity measurement did increase at 90 min post-stress (Table 1, Figure 6).

Discussion

Our results suggest that a brief stressor will alter blood flow volume and blood flow velocity, and that these effects are only evident 45 – 90 min post-stress. This is in contrast to previous studies showing that baseline blood flow significantly increases during stress and that this increased blood flow returns to baseline within 10 min (Lindqvist, 2004). Our study showed significant reductions in basal blood flow volume and blood flow velocity at 45 min and 90 min post stress. Peak (post-deflation) blood flow and blood velocity remained unchanged. Therefore, the percent increase in blood flow and blood velocity at 90 min was higher in the post-stress period than under resting conditions.

In addition, our study also showed that participants with high or low cholesterol produce the same magnitude of change in blood flow and velocity after being stressed. Ultimately, our work suggests that acute stressors do have an impact brachial artery blood flow volume and blood flow velocity, that that these parameters need to be monitored for more than 10 min post stress to see this result. One important limitation of this analysis is that we did not include data from the stress-free testing session. Therefore, the changes in blood flow and velocity that we observed at 45 and 90 min post-stress may actually result from subjects continuing to fast during the post-stress period. Future studies must include time-matched control sessions to rule out this possibility.

This study has several other limitations. First, we examined the response to a controlled stressor in the laboratory. It is possible that subject exhibit a different pattern of responses to real stressors encountered in their daily lives. In addition, we may have seen a difference between high and low cholesterol participants if we had studied patients with a wider range of cholesterol levels. Women were under-represented in the sample due to concerns about menstrual cycle fluctuations impacting vascular responses. Therefore, it is not clear whether gender differences exist in blood flow responses to stress. Future studies could address by recruiting more women. Finally, our study was only done when the participant was in a supine position and most stressors are encountered when people are standing or seated; a different position could have different results.

Further studies

We suggest that future studies include a control session and that they assess changes in blood flow for longer periods of time in order to understand how stressor exposure affects vascular function. Researchers may want to recruit representative samples of women and men to see if they show different results. Also, future studies should enroll participants with a wider range of cholesterol levels to test whether patients with more advanced metabolic problems show larger post-stress changes in blood flow

Table 1. Effects of acute stress on blood flow during the 90 minute recovery period. Data are presented as mean ± SE.

	Pre-stress	10 min Post-stress	45 min Post-stress	90 min Post-stress
Base flow (ml/min) [a]	167.6 ± 11.5	137.4 ± 11.5	125.1 ± 11.5*	122.8 ± 11.5*
Peak flow (ml/min)	1074.3 ± 60.7	1081.6 ± 60.7	1097.2 ± 60.7*	1115.4 ± 60.7*
% change flow (%) [a]	681.7 ± 71.5	787.8 ± 71.5	876.4 ± 71.5	944.5 ± 71.5
Base velocity (M/s) [a]	0.90 ± .02	0.89 ± .02	0.84 ± .02*	0.83 ± .02*
Peak velocity (M/s)	1.71 ± .05	1.72 ± .05	1.72 ± .05	1.74 ± .05
% change velocity (%) [a]	94.1 ± 6.4	95.5 ± 6.4	106.6 ± 6.4	112.1 ± 6.4*

[a] Significant main effect of time, $p < 0.05$

* Significantly different vs. the pre-stress measurement (Tukey test $P \leq 0.05$).
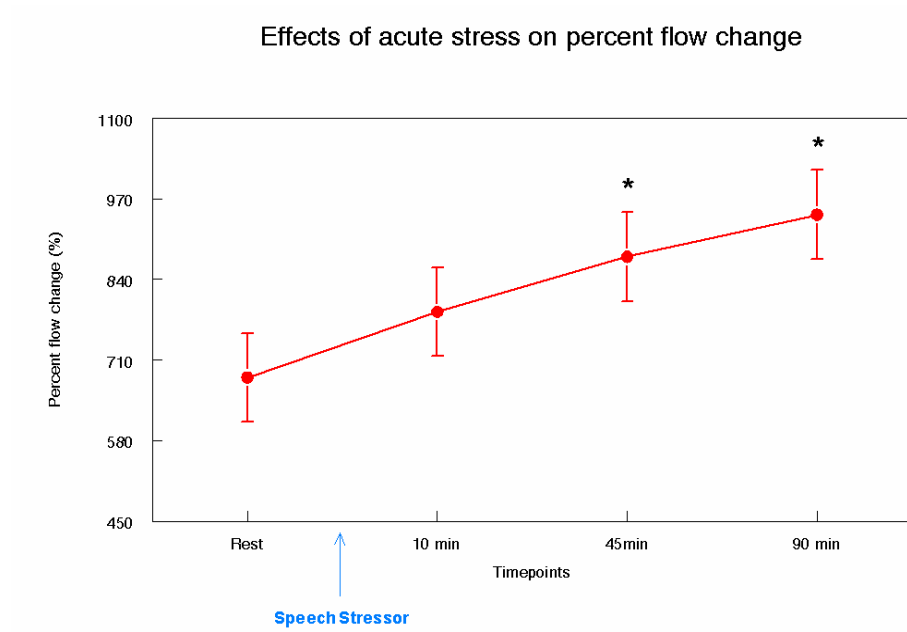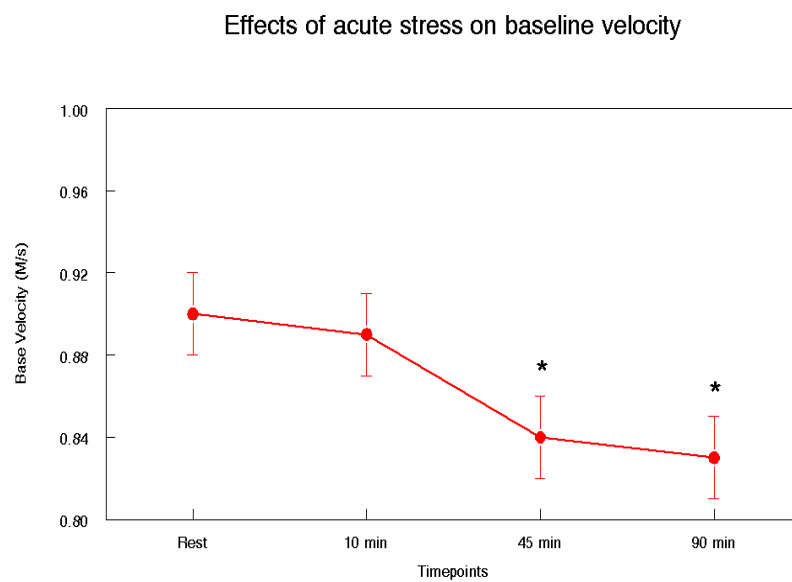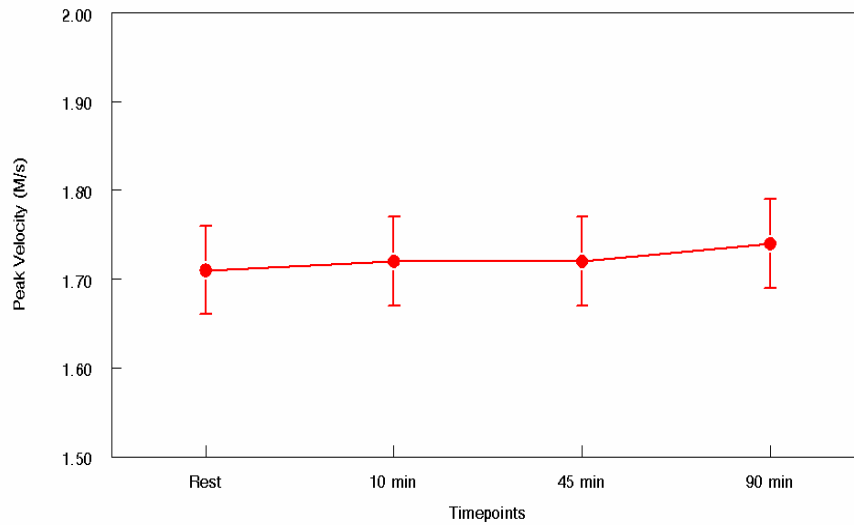
Figure 2

Effects of acute stress on peak flow

Figure 3

Effects of acute stress on percent flow change

Figure 4

Effects of acute stress on baseline velocity
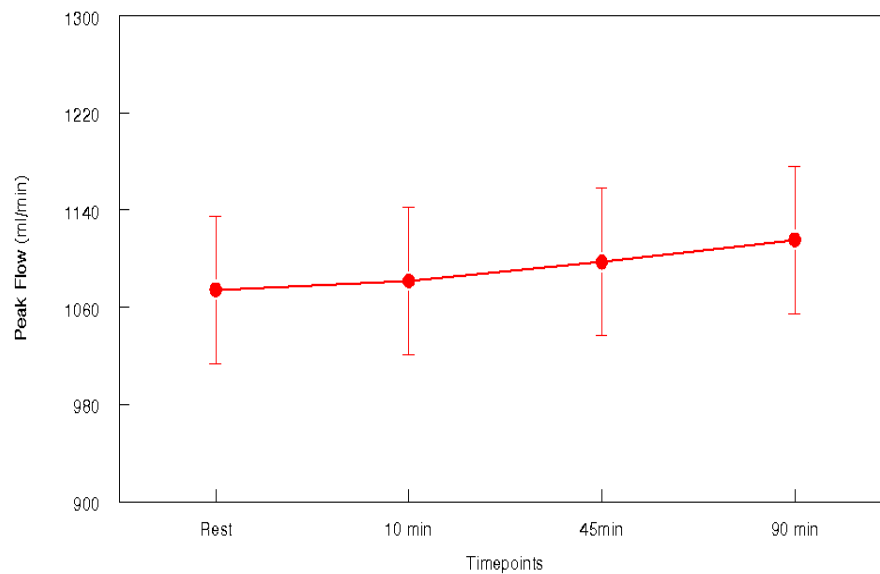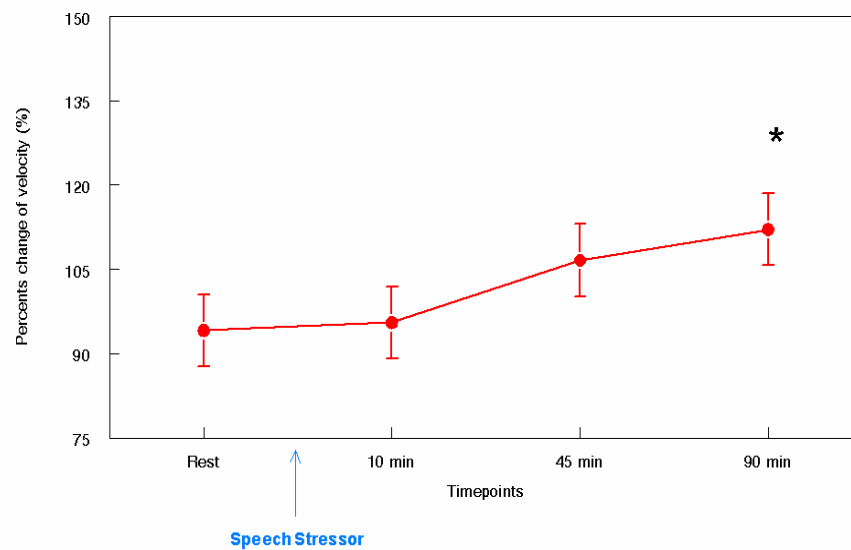
Figure 5

Effects of acute stress on peak velocity

Figure 6

Effects of acute stress on percent change of velocity

Acknowledgements

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