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I, Amanda J. Thopy, hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled:
Effects of the DASH diet on brachial artery flow mediated dilation in adolescents with pre-hypertension and hypertension

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Effects of the DASH diet on brachial artery flow mediated dilation in adolescents with pre-hypertension and hypertension

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Amanda Thopy
B.S., Indiana University, 2008

Committee Chair:
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Abstract

Effects of the DASH diet on brachial artery flow mediated dilation in adolescents with pre-hypertension and hypertension

By Amanda Thopy

Background: The presence of obesity in both adults and children has increased rapidly. Obesity in children is associated with arterial endothelial dysfunction and elevated blood pressure. Dietary interventions have empirically been shown to improve endothelial function in adults. The association between a Dietary Approaches to Stop Hypertension (DASH) dietary intervention and endothelial function in hypertensive or pre-hypertensive children has not been previously explored.

Objective: Endothelial function, as measured by brachial artery flow mediated dilation (FMD), and blood pressure were compared among adolescents with elevated blood pressure (BP) who participated in either a 6-month clinic-initiated behavioral nutrition intervention emphasizing the DASH diet or who received usual hospital-based nutrition care for BP management.

Methods: Sixty-four adolescents with pre-hypertension or stage one hypertension, newly enrolled in a hospital-based hypertension clinic, were randomized to the DASH intervention (DASH, n=33) or usual nutrition care (UC, n=31). Exclusion criteria included use of BP altering medications, receiving prior formalized diet therapy to manage BP, presence of target organ damage, having diagnosed diabetes or an eating disorder. The DASH intervention included 2 counseling sessions with a dietitian on the DASH diet, 6 mailings, and 15 telephone calls on behavioral strategies to promote dietary change. UC included 2 sessions with a dietitian on dietary guidelines consistent with those from the Fourth Pediatric Report of the National High
Blood Pressure Education Program. Adolescents in both groups were prescribed calorie levels for weight maintenance.

**Results:** Weight, height, blood pressure and FMD were assessed at pre-treatment (baseline) and after the 6 month intervention. FMD was adjusted for pre-treatment arterial diameter at rest. Among completers (DASH, n=27; UC, n=24), post-treatment mean values for FMD were significantly greater among DASH participants relative to UC after adjustment for age, gender, race, BMI z score and pre-treatment FMD level (adjusted means $\pm$ SD; 8.16 % $\pm$ 1.13 vs. 5.29 % $\pm$ 0.76, p=0.04). Post-treatment systolic and diastolic blood pressure was not significantly different between groups.

**Conclusion:** These findings suggest that a clinic-initiated behavioral nutrition intervention emphasizing the DASH diet with telephone and mail follow-up can lead to improved endothelial function in adolescents with above normal blood pressure over usual hospital-based nutrition care.

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Introduction/Literature Review

Obesity Epidemic and Hypertension

Over the past three decades, the prevalence of obesity has increased steadily and dramatically in the United States and become a major public health crisis (Wang & Beydoun, 2007; Woo, 2004). The epidemic has impacted both adults and youth. This rise in childhood obesity has occurred concurrent with a rise in pediatric chronic diseases including type 2 diabetes and hypertension (Woo, 2004; Din-Dzietham, 2007). There is much evidence showing a positive association between body weight and blood pressure in children, and it is generally believed that blood pressure in children has increased in parallel to the overweight epidemic (Chiolero, 2007). Data from school health screening programs, collected by Sorof and colleagues as part of a study on isolated systolic hypertension, obesity and hyperkinetic hemodynamic states in children, demonstrated that the prevalence of hypertension increased progressively with an increase in body mass index (BMI) (Sorof, 2002). Children are considered overweight if their BMI is greater than the 95th percentile, and in approximately 30% of overweight children from the study, hypertension was detectable (Sorof, 2002).

The ability of health professionals to identify children with abnormally elevated blood pressure has improved due to the development of a large database on normative blood pressure levels throughout childhood (National High Blood Pressure Education Program, 2004). An analysis of National Health and Nutrition Examination Survey (NHANES) data showed an increase in blood pressure from the 1988/1994 surveys to the 1999/2000 surveys in children and adolescents ages 8-17 years after adjustment for age, race/ethnicity, and gender (Muntner, 2004). The English Health Surveys found an increase in blood pressure in children between 1998 and 2001 and then again in 2004 (Chiolero, 2007). Longitudinal health data collected on children
who were followed into adulthood showed that hypertensive children and adolescents experience significant health consequences as adults, including stroke, myocardial infarction and cardiovascular mortality. To reduce risk of cardiovascular and related diseases, current treatment guidelines recommend that children and adolescents with pre-hypertension and hypertension have a comprehensive assessment for additional cardiovascular risk factors including obesity, diabetes, dyslipidemia, sedentary lifestyle and poor diet (NHBPEP, 2004).

**Chronic Hypertension and Target Organ Damage**

Chronic hypertension can contribute to damage to the heart and blood vessels, which is known as target organ damage (TOD). Specifically, left ventricular hypertrophy (LVH) may result from the heart attempting to accommodate the increase in pressure, and enlarging to exert an increase in force to pump the blood to distal locations in the body (NHBPEP, 2004). Structural and functional changes to blood vessels, also known as endothelial dysfunction, may occur from prolonged elevated blood pressure.

Two autopsy studies that evaluated tissue from adolescents and young adults who experienced sudden deaths from traumatic injury demonstrated a significant relationship between hypertension and the presence of atherosclerotic lesions in the aorta and coronary arteries (Berenson, 1998; McGill 2001). However, neither study examined whether presence of hypertension was related to arterial dysfunction (Berenson, 1998; McGill, 2001). The question was addressed in two recent clinical studies, using ultrasound methods to assess vasculature function. Davis and colleagues longitudinally assessed carotid intimal-medial thickness in 346 men and 379 women followed from childhood to 33-42 years of age (2001). Arnett also longitudinally assessed carotid intimal-medial thickness once to twice yearly in 1,207 children.
ages 10-14 until age 23 years (2001). Both trials found increased carotid intimal-medial thickness and larger artery compliance in young adults (Davis, 2001; Arnett 2001).

Until recently procedures to assess vasculature changes have been very invasive. Older techniques used skin biopsies, cannulation of the brachial artery to infuse agonists and antagonists followed by strain gauge venous plethysmography to evaluate vessel changes, and sublingual administration of nitroglycerin to induce artery dilation for doppler imaging (Deanfield, 2005). Researchers using these methods had difficulty getting parents to agree to have their children participate in clinical trials leading to insufficient power to look at associations between vascular measures and health risks such as blood pressure. Now newer ultrasound techniques have been adapted for use in children and adolescents and are able to look at diverse changes in blood vessel structure and function including carotid large artery compliance, pulse wave velocity, subcutaneous adipose tissue blood flow, and brachial artery flow mediated vasodilatation (FMD) (Deanfield, 2005). Through use of these new technologies, it is becoming clear that even mild blood pressure elevations in youth may have an adverse effect on vascular structure and function, although not all results are in agreement with this observation (Groner, 2006; Aggoun, 2008; Quyyumi; 2010). The technique used to assess arterial damage/dysfunction may be a contributing factor to equivocal findings. For example, FMD, which is a measure of blood vessel stiffness, has been found to worsen in adolescents with blood pressures on the higher end of normal (Groner, 2006). These results were confirmed by Aggoun et al. (2008) in prepubescent obese children, as compared to prepubescent lean children using FMD. In the same study, no association was found between elevated blood pressure and arterial dysfunction as was measured by Carotid Intima Media Thickness (cIMT). Ayer et al. (2009) found that systolic but non diastolic blood pressure was significantly and independently
associated with cIMT in a sample of 405 healthy eight year old children. Meyer et al. (2006) found that compared to non-obese children, obese children had significantly impaired FMD and increased cIMT. In this study these increased endothelial measures were significantly associated with several risk factors including hypertension. Farpour-Lambert et al. (2009) conducted a three month randomized control trial of an exercise intervention on 44 pre-pubertal obese children with measures of blood pressure and endothelial function as outcomes. At three months, significant changes were found in blood pressure, where blood pressure was lower at post-intervention than baseline. However, there was no associated change in cIMT or FMD (Farpour-Lambert et al, 2009). The conflicting evidence on the relationship between blood pressure and endothelial function in youth may be due to several factors include the need for larger studies of longer duration to show change in outcome and the lack of standardized measurement techniques for vascular function across clinics.

Challenges to Assessing TOD in youth

Echocardiography is recommended as the initial tool for evaluating patients with TOD (NHBPEP, 2004). Echocardiography uses standard ultrasound techniques to image a two dimensional views of the heart. From the images practitioners can perform comprehensive evaluations of the cardiovascular structure, function, and hemodynamics that characterize disease processes (Gottdiener, 2004). Echocardiography is routinely used to measure left ventricular mass (LVM), which is then used to determine the presence of left ventricular hypertrophy (LVH). LVH is the most prominent clinical evidence of TOD caused by hypertension in children and adolescents (NHBPEP, 2004). LVH is an independent risk factor for cardiovascular disease and can lead to significant health consequences including congestive heart failure, cardiovascular mortality and morbidity (Levy 1990, de Simone 2007). A conservative cut point that determines
the presence of LVH is a LV mass of 51 g/m^2.7. This cut point is >99th percentile for LV mass in children and adolescents and is associated with increased morbidity in adults with hypertension (NHBPEP, 2004). Echocardiographic recording has become increasingly sophisticated as techniques including tissue Doppler and three-dimensional reconstruction have become commonplace in the clinic (Jennings, 2007). Despite the improved Echocardiogram technologies, the quantitative assessment is still challenging. Newer techniques provide estimates of LV mass that are systematically lower than those obtained with the standard echocardiographic measurements and have required a reassessment of the normal values for LV structural variables in human populations. However, determining normal values is difficult because LV mass can vary several-fold amongst individual members of a healthy population. The methods for adjusting for body size, gender, and ethnic differences are not standardized. Researchers have also found athletes to have a larger LV mass, which needs to be taken into account (Jennings, 2007).

As mentioned previously, several tests are used to measure both structural and functional changes to the blood vessels. Some are more sensitive than others and data accuracy varies. Pulse wave velocity (PWV) is a measure of central arterial stiffness (Blumenthal, 2010). Typically doppler recordings measure aortic wave form in meters per second and a higher velocity indicates presence of atherosclerosis and decreased vascular function. Proven consequences of arterial stiffening are increased pulsatile blood pressure which contributes to higher systolic blood pressure thereby causing increased left ventricular after load and reduced coronary perfusion (Blacher, 1999). Although PWV is strongly related to the presence of atherosclerotic lesions, it does not measure structural changes to the endothelium. Another limitation of the measure is that arteries become longer and more tortuous with age, which may
lead to an underestimation of the arterial path length and an underestimation of PWV (Blacher, 1999). Shorter arterial dimensions are found in men with abdominal obesity and women with large bust size (Asmar, 2001). Therefore, PWV may be overestimated in these cases (Blacher, 1999).

CIMT is a fairly noninvasive measurement that enables visualization of the lumen and walls of the carotid artery using B-mode ultrasound (Greenland, 2000 & Lorenz, 2010). Several pathological studies have demonstrated that increased cIMT thickness in selected arteries is associated with aging and that medial thickness is related to hypertension (Greenland, 2000 & Lorenz, 2010). CIMT has been shown to be predictive of future adverse CVD events (e.g. stroke, myocardial infarction, and heart attack), and the technique is now recommended by the American Heart Association as an initial risk assessment tool for CVD and as a secondary prevention to monitor the affect of prescribed treatment over time (Veronique, 2011).

Unfortunately, cIMT takes at least 12 months to show response to treatment requiring long study lengths for its use in clinical trials (Veronique, 2011).

More recently vascular research has focused on brachial artery flow mediated dilation (FMD) as a primary measure of vascular function. FMD refers to the capacity of blood vessels to respond to physical and chemical stimuli in the lumen, and the ability of the vessel to self-regulate tone and to adjust blood flow and distribution in response to changes in the local environment (Correti, 2002). FMD is recorded using ultrasonagraphic assessment of the brachial artery during reactive hyperaemia induced by stress from cuff inflation (Hodson, 2010). FMD is calculated as the percentage increase in diameter from baseline (before cuff inflation) to the maximum value which is obtained after the cuff is deflated. A greater percent FMD indicates better endothelial function (Corretti, 2002). There is more research on pediatric populations
using IMT and FMD measures than PWV (Corretti, 2002). Empirical evidence shows that both FMD and IMT measures responded adversely to hypertension and favorably to pharmacotherapy and lifestyle intervention in children (Groner, 2006). Response time to intervention is reported to be shorter for FMD than cIMT, e.g., 6 months compared to 12 months (Woo, 2004). Evidence shows that FMD of the brachial artery is a reliable measure of vascular function and responds quickly to lifestyle modification (Correti, 2002).

**Therapy for Target Organ Damage**

Several randomized clinical trials have demonstrated improved vascular function in response to pharmaceutical and nutrient supplementation interventions. Bai et al. (2009) conducted a meta-analysis of twelve short term (3-30 day length), randomized, placebo controlled supplementation trials with L-arginine to determine if supplementation with this amino acid improved FMD. Ages of study participants spanned the lifecycle and those enrolled ranged from healthy to those with presence of or with risk factors for CVD. Results from this meta-analysis supported a beneficial relationship between L-arginine supplementation and brachial artery FMD, particularly when baseline FMD was low. Kovacs el al (2006) conducted a study of 50 subjects with low FMD testing the effects of two angiotensin converting enzyme inhibitors (ACEI) medications, Quinapril and Enalapril, on FMD. These researchers found, in post myocardial infarction patients, that endothelial dysfunction as assessed by FMD was improved by low dose Quinapril (Kovacs, 2006). Ghianadoni et al (2003) tested the affects of six antihypertensive drugs on FMD in 168 hypertensive patients for over six months (Ghiadoni, 2003). The drugs ranged from the ACE inhibitors, to calcium antagonists, AT-1 receptor antagonists, and β-blockers. All drug therapies similarly reduced blood pressure, but only the ACE inhibitor, perindopril, increased flow mediated dilation (Ghiadoni 2003). In general, these
trials support a beneficial role of drug therapy in improving endothelial function as measured by FMD.

The impact of dietary and lifestyle changes on endothelial function has been less well-studied. Several randomized clinical trials have demonstrated improved vascular function in adults and adolescents, as measured by flow-mediated dilation (FMD) in response to dietary interventions. Kelishadi et al. (2008) performed a 6-week diet and exercise intervention study in 35 obese children, ages 12 to 18 years. These researchers found improved FMD post-intervention compared to baseline and concluded that a short term trial of diet and exercise can reverse vascular damage. Woo et al. (2004) evaluated the reversibility of obesity-related arterial dysfunction in 82 overweight children, 9 to 12 years of age, participating in either a dietary or dietary/exercise intervention. Based on the results of the trial, these investigators concluded that vascular dysfunction is partially reversible with diet intervention. Additionally they also found that diet and exercise were more effective in improving FMD than diet alone. In a study by Pena et al. (2004), thirty-six adolescents with type 1 diabetes completed a randomized, double-blind crossover trial. The participants received eight weeks of oral folic acid (5 mg/day) and eight weeks of placebo, with an eight week wash out period between treatments. Pena and colleagues found that short-term folic acid supplementation, at a level of 5 mg/day, increased FMD, where placebo did not (Pena 2004). In summary, these few studies support a positive effect of dietary and exercise interventions on FMD. More studies are needed on the relationship between diet, blood pressure and vascular function as well as long term intervention effects on these outcomes.

Gaps in the Literature

Evidence that supports the efficacy of nonpharmacologic interventions for improving endothelial function in children is limited. Since the literature supports a positive relationship
between blood pressure and vascular function, it would seem logical to examine dietary approaches to blood pressure management to determine whether these therapeutic dietary strategies could effectively modify vascular function as well as blood pressure. The Dietary Approaches to Stop Hypertension (DASH) diet has been shown to lower blood pressure in adults (Appel et al, 1997) and adolescents (Couch et al., 2008). In adults, this diet has been shown to remediate vascular damage, including FMD, but this same relationship has not been examined in youth (Hodson, 2010). It would be beneficial to know if restoration of vascular function is observed in response to a DASH diet intervention on adolescents as it would have major therapeutic value in early remediation and prevention of CVD.

**Purpose Statement**

Endothelial function, as measured by FMD and blood pressure was compared among adolescents with elevated blood pressure (BP) before and after participating in either a 6-month clinic-initiated behavioral nutrition intervention emphasizing the DASH diet or usual hospital-based nutrition care for BP management. The purpose of this study was to determine whether change in FMD from baseline to post-intervention differed in those who participated in the DASH intervention compared to usual care.

**Major Hypothesis**

As compared to adolescents receiving usual care, DASH-4-Teens participants will have a greater change in FMD and blood pressure post-intervention compared to baseline.

**Methods**

This thesis will report the results from a sub-study of participants who completed a randomized clinical trial (RCT) that began in February 2008 and is currently ongoing. One hundred and thirty six subjects are currently enrolled in the RCT that is designed to study the
effects of behavioral nutrition intervention emphasizing the DASH diet on blood pressure and vascular function in adolescents with hypertension and pre-hypertension. This study will report on a sample size of 51 subjects from the RCT who had completed their baseline and 6-month study assessment visits as of December 2010.

Subjects

Participants were recruited from the Cincinnati Children’s Hypertension Clinic (CCHC), a referral program for the diagnosis, evaluation and treatment of children with hypertension at the Cincinnati Children’s Hospital Medical Center (CCHMC). The center is staffed by the division of Cardiology and is directed by Dr. Elaine Urbina M.D. This study was approved by the IRB at CCHMC and the University of Cincinnati.

Subjects were included in the study if they were between the ages of 11 and 18 years and had diagnosed hypertension or pre-hypertension. Diagnosis of hypertension or pre-hypertension was made by the patient’s attending physician in the CCHC and was based on three blood pressures made on different days. A mercury sphygmomanometer with appropriate size blood pressure cuff was used to make all blood pressure diagnoses.

Subjects were excluded if they had stage 2 hypertension or secondary hypertension, were being treated with anti-hypertensive medications or any other medication that could alter blood pressure, had received prior formalized diet therapy to manage their blood pressure within the last 6 months, had presence of Target Organ Damage (TOD) as defined by LVH, had diagnosed type 1 or 2 diabetes, were unwilling to stop the use of vitamins, minerals, or antacids, did not speak English, had a diagnosed eating disorder, had a psychological or medical condition that may preclude them from full participation or did not have full medical clearance from a physician to participate. All children under the age of 18 years signed informed assents and
parents signed parental permission forms. Subjects over the age of 18 years signed informed consents.

Intervention

Participants who entered the study were randomly assigned to either a 24-week behavioral nutrition intervention that emphasized the DASH dietary pattern (DASH-4-Teens intervention) or a 24-week routine nutrition care intervention (Usual Care). Randomization occurred using a table of random numbers. Treatment assignments were stratified based on age (11-14 years and 15-18 years), gender, and hypertension status (pre-hypertension or stage 1 hypertension). All study physicians were blinded to group assignment.

The DASH-4-Teens intervention emphasized gradual dietary changes using a DASH-type dietary pattern of 3 servings a day of low fat dairy foods, 8 servings a day of fruits and vegetables, and 2 servings or less a day of DASH unfriendly foods. DASH unfriendly were defined as foods that contain more than 3 grams of fat or 480mg of sodium per serving (Couch, 2008). Participants randomized to the DASH treatment group received face-to-face DASH diet specific counseling with a dietitian at baseline and three months. Participants received a 10 module illustrated manual that detailed the DASH-4-Teen curriculum, tips for incorporating DASH food groups into the diet, foods lists, self assessment activities and behavioral strategies for changing intake. After the initial face-to-face counseling session, weekly goals for servings of fruits and vegetables, low-fat dairy foods and DASH unfriendly foods, action plans and behavioral strategies to achieve goals were delivered by way of 15 phone calls and six mailings from trained research interventionists. Subjects received monetary compensation for meeting study-related food goals, which were verified by study interventionist upon review of a completed diet self-monitoring booklets.
The Usual Care (UC) intervention for adolescents followed the guidelines in use and recommended by the Fourth Pediatric Report of the National High Blood Pressure Education Program (NHBPEP). The UC intervention did not deviate from the nutrition care that is routinely provided to all newly admitted patients to the CCHC. Participants randomly assigned to UC received the booklet *Your Guide to Lowering Blood Pressure* and had two face-to-face counseling sessions with a dietician at baseline and three-months. The booklet contains current dietary recommendations including strategies to reduce weight, eat more fruits and vegetables and control sodium and fat in the diet. The booklet also contained information on the DASH diet. It provided DASH serving recommendations and included strategies for adopting the DASH dietary pattern. Consistent with current routine care, no behavioral skills were discussed.

Both interventions focused on weight maintenance in discussion on daily food serving goals.

**Outcomes**

Vascular function was measured by percentage brachial artery flow-mediated dilation (%FMD). This measure was evaluated in participants at (pre-treatment) baseline, at 6-months (immediately post-treatment) and at 18-months (1 year post-treatment). For this thesis sub-study only the baseline and 6-month measurements were considered. The FMD assessments were done at the CCHMC Cardiovascular Imaging Core Research Laboratory by experienced pediatric vascular ultrasonographers using B-mode ultrasound imaging with a GE Vivid 7-V7916 Ultrasound System (Horton Norway). Subjects underwent assessment of FMD in a quiet and stable temperature environment after refraining from ingesting caffeine, using tobacco or exercising for 8 hours. After 10 minutes of rest in the supine position, subjects extended their left arm 80-90 degrees from the body. A pneumatic blood pressure tourniquet was applied to the
widest part of the forearm below the antecubital fossa. The diameter of the left brachial artery was measured with a high resolution linear array M12L, 5.0-11.0 MHz vascular ultrasound transducer. Resting images were obtained at baseline (pre-inflation) 2-15 cm above the elbow. The depth and gain settings of the transducer were used to obtain an optimal image which shows a segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall and two parallel lines representing echoes that arise from the blood intimal and media adventitia boundaries on the near and far walls. Anatomic landmarks (veins and facial planes) were noted for reproducible transducer placement. Reactive hyperaemia was then induced by inflation of the blood pressure cuff to 75mmHG above SBP (max 240-300). After 4 minutes, the tourniquet deflated automatically. Brachial artery diameters were obtained at 60, 90, and 120 seconds after deflation. Three end diastolic measures of diameter were made from the digital images stored on the Camtronic Medical digital image storage and reading system and used the “trailing edge” of adventitia-media interface of near wall to the “leading edge” of the media-adventitia interface of the far wall. FMD was adjusted for pre-treatment arterial diameter at rest and expressed as the proportion of change from rest. Percent FMD, at 60, 90 and 120 seconds were calculated using the formula: %FMD= (LDp-LDb)/LDb * 100 where LDp is the lumen diameter post-inflation at 60, 90 and 120 seconds, respectively and LDb is the lumen diameter at rest. The % FMD maximum (% FMD max) dilation was considered the peak change in arterial diameter from baseline to within 120 seconds of hyperemia. Any values for %FMD maximum in the negative range were considered indicative of endothelial spasm and were reset to zero.

Gender and age were self-reported by participants at baseline and parents self-reported the race of their child. Weight was measured at baseline and 6 month assessment with participants wearing light indoor clothing and no shoes using a calibrated triple-beam balance.
scale. Standing height was measured using a wall-mounted stadiometer. Each measure was taken twice and the average of the two readings was calculated. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. BMI z scores were determined from recent Centers for Disease Control growth charts containing safe-specific median, standard deviation, and distribution scenes correction information using the LMS method. Blood pressure measurements are performed with a mercury sphygmomanometer according to standardized procedures as outlined in the NHBPEP (NHBPEP, 2004). After the adolescent sits quietly for 5 minutes, three separate blood pressures were taken in the seated position on the right arm using the appropriate size cuff. Blood pressure measurements were taken 30 seconds apart. Systolic BP (SBP) was determined as the appearance of the first Korotkoff sound (k1), and diastolic BP (DBP) as the disappearance of the Korotkoff sounds (K5). At each patient assessment in the study, blood pressure was the mean of all available measurements (baseline: 9 blood pressure measurements across 3 visits; 6-month assessment: 3 blood pressure measurements at 1 visit; and 18-month assessment: 3 blood pressure measurements at 1 visit.) Hypertension status was defined according to the fourth Pediatric Report of the NHBPEP (NHBPEP, 2004). Blood pressure was considered a secondary outcome in these analyses.

Statistical Analysis

Subjects included in these analyses were study completers (those with baseline and post-treatment, e.g., 6-month data). Means and standard deviations were derived at baseline and post-treatment for continuous variables and frequencies for categorical variables. Distributions of the residuals were checked for normality assumptions and based on these findings no outcome variables needed transformation. Unpaired t-tests were used to compare group differences at
baseline and post-treatment for continues variables, e.g., FMD, and chi-squared for categorical data, e.g., gender. Mixed effects models were used to assess the effect of the intervention-type (DASH versus Usual care) on change in FMD from baseline to post-treatment. Pearson correlations were used to examine the relationships between selected subject characteristics and outcomes. Significant correlations were used to determine covariates for final prediction models. Final models for FMD were adjusted for age, BMI z-score and baseline peak % FMD. Statistical analyses were performed with SAS software (version 9.2, SAS Institute, Cary, North Carolina) P values <0.05 were considered to be statistically significant.

Results

Fifty one adolescents who completed a baseline and 6-month post-treatment study assessment were included in this thesis subsample, and their demographics and baseline hypertension status are described in Table 1. Participants in the DASH and Usual Care groups were comparable for age, gender, and race with no significant difference between them. Hypertension status at baseline was also not different between groups.

Table 1: Baseline Characteristics of the DASH-4-Teens versus Usual Care Completers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DASH-4-Teens</th>
<th>Usual Care</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Age, years (SD)</td>
<td>14.5 ± 1.8</td>
<td>14.5 ± 2.0</td>
</tr>
<tr>
<td>Gender, n (males/females)</td>
<td>17/10</td>
<td>14/10</td>
</tr>
<tr>
<td>Race, n (white/black/other)</td>
<td>14/9/4</td>
<td>17/7/2</td>
</tr>
<tr>
<td>Hypertension Status (Pre-hypertension/Stage 1 hypertension)</td>
<td>15/12</td>
<td>12/12</td>
</tr>
</tbody>
</table>

Completer= participants who completed baseline and 6-month study assessments

In comparing intervention groups BMI and BMI z score changes from baseline to post-treatment, there were no significant differences between groups for change in BMI or BMI z-score (p= 0.27 for both) from pre to post intervention. This finding indicates that weight status remained relatively stable for both groups over the 6-month intervention period (Table 2). The
DASH group experienced a mean reduction in SBP of ~4 mm Hg from baseline to post treatment versus a mean SBP reduction of ~1 mm Hg in the UC group; the SBP change from pre to post intervention was not significantly different between groups. Mean DBP decreased by ~ the same amount in each group (~1 mm Hg) pre to post intervention; again the difference between groups for DBP was not significant. The mean z-score changes in BP from pre to post intervention showed similar results and were not significantly different between groups.

Comparison of changes in vascular function from pre to post-intervention in Table 2 showed that there was a trend for DASH participants to have greater dilation of vessels (an increase in FMD) post-treatment at 120 seconds post cuff-deflation compared to UC (p=.08). Notably, the dilation of blood vessels in the UC group appeared to decrease or worsen across all FMD measurement times. This decrease was particularly noteworthy at 60 seconds and 120 seconds post-cuff deflation. Also, the %FMD max, which represents peak dilation of blood vessels across FMD measurement times, showed a trend for greater improvement in the DASH group relative to the UC group at post treatment (p=.10).

Simple correlations were run to determine the association between weight status and blood pressure change and %FMD max. There were no significant associations between change in BMI z-score and %FMD max (r=.20, p=.88), SBP z score (r=.22, p=.13), or DBP z-score (r=.15, p=.30).
Table 2: Weight status, blood pressures and vascular function outcomes for intervention completers at baseline and post-treatment (Mean +/- SD)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Time Point</th>
<th>DASH Intervention</th>
<th>UC Intervention</th>
<th>Mean Difference</th>
<th>P-Values</th>
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<tbody>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BMI, kg/m^2</td>
<td>Baseline</td>
<td>30.62 ± 8.75</td>
<td>34.37 ± 13.02</td>
<td>-3.74</td>
<td>.23</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>30.97 ± 9.33</td>
<td>35.22 ± 13.49</td>
<td>-4.25</td>
<td>.19</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>0.36 ± 1.47</td>
<td>0.86 ± 1.71</td>
<td>-0.50</td>
<td>0.27</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>Baseline</td>
<td>1.75 ± 0.80</td>
<td>2.04 ± 0.80</td>
<td>-0.28</td>
<td>.21</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>1.72 ± 0.84</td>
<td>2.06 ± 0.80</td>
<td>-0.34</td>
<td>.15</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-0.03 ± 0.19</td>
<td>0.02 ± 0.14</td>
<td>-0.05</td>
<td>0.27</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm HG</td>
<td>Baseline</td>
<td>126.77 ± 6.69</td>
<td>126.28 ± 6.22</td>
<td>0.50</td>
<td>.78</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>122.55 ± 9.20</td>
<td>125.17 ± 8.88</td>
<td>-2.61</td>
<td>.31</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-4.22 ± 6.89</td>
<td>-1.11 ± 7.51</td>
<td>-3.11</td>
<td>0.13</td>
</tr>
<tr>
<td>SBP z-score</td>
<td>Baseline</td>
<td>1.31 ± 0.66</td>
<td>1.18 ± 0.60</td>
<td>0.14</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>0.84 ± 0.88</td>
<td>0.98 ± 0.89</td>
<td>-0.14</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-0.47 ± 0.64</td>
<td>-0.20 ± 0.74</td>
<td>-0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>DBP, mm HG</td>
<td>Baseline</td>
<td>78.09 ± 6.16</td>
<td>77.85 ± 7.34</td>
<td>0.25</td>
<td>.89</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>76.85 ± 6.95</td>
<td>76.66 ± 7.99</td>
<td>0.19</td>
<td>.49</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-1.25 ± 6.88</td>
<td>-1.18 ± 7.05</td>
<td>-0.07</td>
<td>0.97</td>
</tr>
<tr>
<td>DBP z-score</td>
<td>Baseline</td>
<td>1.18 ± 0.57</td>
<td>1.08 ± 0.68</td>
<td>0.09</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>1.04 ± 0.60</td>
<td>0.88 ± 0.82</td>
<td>0.16</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-0.14 ± 0.58</td>
<td>-0.20 ± 0.68</td>
<td>0.07</td>
<td>0.70</td>
</tr>
<tr>
<td>Flow Mediated Dilation</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD Max 60, %</td>
<td>Baseline</td>
<td>6.60 ± 7.19</td>
<td>6.02 ± 5.29</td>
<td>0.57</td>
<td>.75</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>5.92 ± 7.96</td>
<td>3.86 ± 4.48</td>
<td>2.05</td>
<td>.27</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-0.68 ± 7.45</td>
<td>-2.15 ± 6.59</td>
<td>1.48</td>
<td>0.46</td>
</tr>
<tr>
<td>FMD Max 90, %</td>
<td>Baseline</td>
<td>5.29 ± 7.42</td>
<td>3.65 ± 4.08</td>
<td>1.64</td>
<td>.34</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>4.46 ± 7.99</td>
<td>2.85 ± 3.90</td>
<td>1.61</td>
<td>.38</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>-0.83 ± 8.31</td>
<td>-0.80 ± 5.20</td>
<td>-0.03</td>
<td>0.99</td>
</tr>
<tr>
<td>FMD Max 120, %</td>
<td>Baseline</td>
<td>2.82 ± 6.68</td>
<td>2.96 ± 4.74</td>
<td>-0.14</td>
<td>.93</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>3.75 ± 6.76</td>
<td>0.96 ± 3.90</td>
<td>2.79</td>
<td>.08†</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>0.93 ± 7.49</td>
<td>-2.00 ± 6.65</td>
<td>2.93</td>
<td>0.15</td>
</tr>
<tr>
<td>FMD Max, %</td>
<td>Baseline</td>
<td>7.82 ± 6.89</td>
<td>6.97 ± 4.76</td>
<td>0.85</td>
<td>.62</td>
</tr>
<tr>
<td></td>
<td>Post Treatment</td>
<td>7.99 ± 7.71</td>
<td>5.13 ± 3.39</td>
<td>2.86</td>
<td>.10†</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>0.18 ± 6.60</td>
<td>-1.84 ± 5.20</td>
<td>2.01</td>
<td>0.24</td>
</tr>
</tbody>
</table>

† Indicates a trend, p ≤ 0.10.
Table 3: Mixed model effects of intervention-type (DASH versus UC) on change in %FMD.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Effect of Intervention on FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
</tr>
<tr>
<td>DASH</td>
<td>0.97 (1.24)</td>
</tr>
<tr>
<td>UC</td>
<td>-1.91 (0.76)</td>
</tr>
<tr>
<td>Difference between DASH and UC</td>
<td>2.88 (1.38)</td>
</tr>
</tbody>
</table>

*models adjusted for age, gender, race, BMI z-score, and pre-treatment FMD levels.

In the mixed effects model (Table 3), the variable %FMD Max was used as the dependent variable rather than dilation at 60, 90 or 120 minutes, since it represents the peak dilation of blood vessels during the %FMD procedure. After adjustment for age, gender, race, BMI z-score and pre-treatment %FMD levels, the DASH intervention was related to a greater positive change in %FMD Max compared to the UC group (P=.04). In examining mixed effects models by group, the UC intervention showed a significant decrease in blood vessel dilation of 1.91% from pre to post-intervention after adjustment for potential confounders (p=.02). In the DASH group, the FMD max increased, but the change was not significant from pre to post-intervention.

**Discussion**

Findings from this study support our original hypothesis that the DASH-4-Teens participants would have a greater change in %FMD from baseline to post treatment compared to adolescents who participated in the UC intervention. Notably, the DASH-4-teens intervention participants were able to maintain their % FMD max at pre-treatment levels or improve slightly from baseline, while the UC participants experienced a worsening in vascular function over the course of the trial. These findings are in line with the results of other DASH intervention trials in adults. For example, in a four week intervention on 144 patients with high blood pressure, Blumenthal et al. (2010) found that the DASH diet significantly improved %FMD, compared to controls receiving usual nutrition care to manage their blood pressure. In a 30 day dietary intervention of 27 participants assigned to either the DASH diet or to their usual diet (control
Hodson et al. (2010) found improved %FMD measures from baseline to post treatment in both groups; differences between groups were not significant. Hodson and colleagues suggest that their trial may not have been long enough nor had enough power to detect group differences in FMD (Hodson, 2010). Notably, in our study we found that participants in the DASH intervention experienced some improvement or at least maintenance of pre-treatment blood vessel dilation from pre to post-intervention while the UC participants tended to experience a worsening in this measure. This finding was not observed in other studies. Reasons for these unexpected results are unclear. Factors that may contribute to a worsening in vascular function include weight gain, dyslipidemia, and ongoing blood pressure elevation (Quyyumi, 2010). In this study, the UC participants had a slight increase in BMI and BMI z-score; however, BMI change was not significant from pre to post-intervention. In the UC group, mean SBP and DBP remained stable from pre to post-intervention, suggesting persistent pre-hypertension and hypertension, over the 6 month trial. This outcome may have contributed to the adverse FMD change in this group. Serum lipid changes were not measured.

Alternatively there may have been changes in the dietary intake of nutrients and food components among participants in the DASH group compared to those in UC that contributed to maintenance or some improvement in blood vessel function in DASH participants over UC. The dietary data was currently not available for this study. Since the DASH diet contains nutrients and food components that may improve vascular function as predicted, if those who participated in the DASH-4-Teens intervention versus UC had greater dietary compliance to a DASH diet, it may have contributed to improved blood vessel function. Greater dietary compliance to a DASH diet among participants in the DASH-4-Teens intervention relative to UC would be expected given the intensity of the DASH intervention. The DASH group received
weekly telephone counseling sessions with the dietitian, which addressed behavioral skills and constantly reinforced the dietary guidelines for blood pressure management. The UC group only received two sessions with a dietician with no behavioral skill use included to assist in DASH dietary compliance.

Along these lines, several studies have shown that high intakes of sodium significantly impair vascular function, while low sodium diets improve function (Dickinson, 2011; Dickinson, 2009, Todd, 2010 & Jablonski, 2009). The DASH-4-Teens program encouraged foods that were low sodium (less than 480 mg/serving) and low fat (<3 g/serving). If participants in the DASH intervention were able to lower sodium significantly more than UC participants or UC participants increased their sodium intake over the course of the trial, this may have contributed to the negative results observed. There is not data in adults to support a direct relationship between fruit and vegetable intake and FMD, although changes in intake of fruits and vegetables may modify blood pressure.

The DASH diet emphasizes fruits and vegetables high in potassium. Endothelial potassium channels are responsible for maintaining the resting potential of endothelial cells and modulating the release of vasoactive compounds (Félétou, 2009). Vitamins C and E, which are also found in fruits and vegetables, have shown to improve endothelial function through their antioxidant properties (Engler, 2003). Low fat dairy foods are also encouraged as part of the DASH diet. Ca^{2+} activates the endothelial potassium channels and decreased levels may cause smooth muscles to vasoconstrict (Félétou, 2009).

There did not appear to be a significant correlation between blood pressure and %FMD max in this small subsample. Researchers do not fully understand the relationship between FMD and blood pressure, and the evidence on the relationship between blood pressure and endothelial
function is conflicting. Several studies have shown associations between low sodium diets and both reduced blood pressure and improved endothelial function (Quyyumi, 2010). However, as was seen in our study and by several others, reduced BP was not consistently associated with improved FMD (NHBPEP, 2004; Quyyumi, 2010). Similar to our results, in a 2009 study on 29 overweight normotensive adults, Dickinson et al. found that a low salt diet significantly improved FMD scores compared to usual salt intake, e.g., 3300 mg/day; however, there was no significant relationship between reduced blood pressure and improved FMD. These results suggest that low sodium diets may improve endothelial function independent of blood pressure changes. In a study assessing the effects of high salt versus low salt meals on 16 healthy normotensive participants, Dickinson et al. (2011) found a significant correlation between high salt meals and decreased FMD scores, but no changes in blood pressure or a significant relationship between blood pressure and FMD. In a large scale longitudinal study of 3,500 patients, Shimbo and colleagues found no significant relationship between FMD and the incidence of hypertension over a 4.8 year period (2010). Hypertension is a complex multifactoral disease, where the initiating injury may be in the kidney, central nervous system, blood vessels, or a combination of factors (Quyyumi, 2010).

A limitation of the current study was a small sample size, which may explain the inability to detect significant differences in blood pressure and significant relationships between blood pressure and FMD. Also, as dietary information was not yet available for our analysis, but will be examined in the larger randomized clinical trial, it is not known how specific diet changes that occurred in response to the intervention may have contributed to our findings. We cannot claim that it was a reduction in sodium, an increase in fruits, an increase in vegetables or some other nutrient or food group change that contributed to the maintenance or slight improvement in
endothelial function in the DASH group relative to UC. Our findings, must be attributed to an overall DASH-4-Teens intervention effect relative to UC on maintenance and improvement in %FMD max.

In conclusion, findings from this study suggest that the DASH-4-teens dietary intervention can lead to maintenance or slight improvement in endothelial function, as measured by %FMD, compared to adolescents who receive usual hospital based nutrition care. To our knowledge, this study is the first to report the efficacy of the DASH diet on modifying blood vessel function in adolescents. Findings from this study add valuable insight to our understanding of the relationship between the DASH diet and FMD in youth.
References


