Original Article

SHIP-AHOY (Study of High Blood Pressure in Pediatrics: Adult Hypertension Onset in Youth)
Rationale, Design, and Methods

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Abstract—Although hypertension is identifiable in children and adolescents, there are many knowledge gaps on how to best define and manage high blood pressure in the young. SHIP-AHOY (Study of High Blood Pressure in Pediatrics: Adult Hypertension Onset in Youth) is being conducted to address these knowledge gaps. Five hundred adolescents will be recruited and will undergo ambulatory blood pressure monitoring, echocardiographic, vascular, and cognitive assessments, as well as epigenetic studies to identify mechanisms that underlie the development of hypertensive target organ damage. Details of the design and methods that will be utilized in SHIP-AHOY are presented here, as well as baseline characteristics of the first 264 study participants. The primary aim of the study is to develop a risk-based definition of hypertension in the young that will result in better understanding of the transition from blood pressure in youth to adult cardiovascular disease. (*Hypertension*. 2018;72:00-00. DOI: 10.1161/HYPERTENSIONAHA.118.11434.)
● **Online Data Supplement**

Key Words: adolescents ■ blood pressure ■ cardiovascular disease ■ hypertension ■ vascular stiffness

Target organ damage (TOD) related to high blood pressure (BP) levels measured in adulthood is strongly associated with significantly increased risk for cardiovascular events, such as stroke and myocardial infarction.[1,2] Ample data from large-scale clinical trials have demonstrated the cardiovascular benefits of BP reduction.[3] Because hypertension affects at least one-third of adults, the potential public health burden and associated expenditures related to hypertension are enormous.[4] Given this, substantial effort and resources have been expended to improve awareness and control of hypertension among adults.

Less attention has been paid to the earliest phases of hypertension, which likely have their origins in childhood. Although it is well known that hypertension and hypertensive TOD are detectable in the young, there are limited data available linking childhood BP levels with future cardiovascular disease (CVD). This point was underscored by an evidence review conducted for the US Preventative Services Task Force, which concluded that evidence linking childhood BP levels to the prediction of development of adult CVD is lacking.[5] A shortcoming of that analysis, however, is that it did not examine the adverse impact that elevated BP might have in a more immediate time frame for children and adolescents and also did not evaluate the level of BP at which this might occur.

In contrast to adult hypertension, childhood hypertension is statistically defined based on the distribution of BP in healthy children,[6] and treatment recommendations represent consensus opinion based on evidence from cross-sectional and longitudinal cohort studies.[6,7] Clarifying the relationship between BP levels and development of hypertensive TOD in youth is essential to establish evidence-based treatment guidelines. In addition, data on whether BP phenotype, as defined by the combination of casual and ambulatory BP measurements, can help predict the risk of hypertensive TOD in youth are scant. Finally, a better understanding of epigenetic pathways involved in the development of hypertensive TOD could help identify new targets for early therapy of hypertension in the young.

These knowledge gaps stimulated the development of SHIP-AHOY (Study of High Blood Pressure in Pediatrics: Adult Hypertension Onset in Youth), one of the American Heart Association's Strategically Focused Research Networks

in Hypertension. SHIP-AHOY consists of 3 projects (population, clinical, and basic) conducted on a single cohort of adolescents. The goals are to (1) redefine BP thresholds for the diagnosis of childhood hypertension, based on direct evidence; (2) better define the clinical phenotype of BP-associated TOD; and (3) focus on high BP in adolescence as an actual disease-causing, treatable condition in the young, rather than a risk factor.

Methods

At the completion of the study, data can be requested from the SHIP-AHOY Data Coordinating Center (DCC).

Study Population

SHIP-AHOY is recruiting a multiethnic cohort of 500 otherwise healthy adolescents across the BP spectrum, divided into 3 BP strata (see below). The BP groups are balanced for body mass index (BMI) category so that similar proportions of subjects in each BP group will be lean (BMI <85th percentile) or overweight/obese (BMI ≥85th percentile). Inclusion and exclusion criteria are presented in Table 1.

The study protocol has undergone institutional review board review and approval, and all study participants and their parents have provided written informed consent or assent according to local institutional review board requirements.

Table 1.   Inclusion and Exclusion Criteria

Inclusion
1. Age 11–<19 y
2. Written informed consent/assent per local IRB requirements
Exclusion
1. On current antihypertensive drug treatment or treated within the past 6 mo
2. Diabetes mellitus (type 1 or type 2) requiring pharmacological treatment
3. Drugs altering metabolic status including metformin and lipid-lowering agents
4. Significant proteinuria (verified by first-morning urine protein/creatinine ratio of ≥1.0)
5. Known history of chronic kidney disease or estimated GFR ≤90 mL/min per 1.73 m²
6. Congestive heart failure, obstructive valvular disease, or cardiomyopathy
7. Diagnosis of obstructive sleep apnea
8. Pregnant or breast-feeding female
9. Secondary hypertension (not including obesity)
10. BMI *Z* score ≥5 for age and sex
11. Uncorrected coarctation of the aorta or renal artery stenosis
12. Concurrent medications known to affect BP, including stimulant medications for ADHD, corticosteroids, calcineurin inhibitors, and oral decongestants
13. Any clinically significant unstable medical condition or chronic disease
14. Known major neurological condition known to affect cognitive function. Participants with milder neurological involvement, such as learning disabilities, will be marked, but not excluded from enrollment.
15. Symptomatic stage 2 hypertension

ADHD indicates attention-deficit hyperactivity disorder; BMI, body mass index; BP, blood pressure; GFR, glomerular filtration rate; and IRB, institutional review board.

Study Organization

Each of the 3 projects of SHIP-AHOY has individual principal investigators at 5 clinical sites (Figure 1) who regularly interact with the Strategically Focused Research Networks center director, who also oversees the DCC. Additional network oversight is provided by the consultants and Executive Steering Committee. The Cardiovascular Research Center (CVRC) in Cincinnati oversees echocardiography and pulse wave velocity (PWV) studies, the ambulatory BP monitoring (ABPM) Center in Seattle oversees performance and initial analysis of ABPM studies, and cognitive questionnaires and tests are scored at the Psychology Core in Chapel Hill. Blood and urine samples, including genetic samples used in the Basic Science project, are processed at each site, stored at −80°C and shipped in batches quarterly to Cincinnati Children's Hospital Medical Center (CCHMC) laboratories for analysis. Processing of samples for epigenetic studies is performed at the University of Cincinnati, with analyses of these data performed in conjunction with genetic and bioinformatics experts at CCHMC.

Study Assessments

Casual BP Measurement

SHIP-AHOY participants have casual BP measurements obtained in the right arm by auscultation at study entry, then again at the main study visit. All participating sites use the same aneroid sphygmomanometer (Mabis MedicKit 5; Mabis Healthcare, Waukegan, IL). Standardized training and certification in the auscultatory BP measurement protocol were provided to all study personnel responsible for casual BP measurement.

At each study visit, before BP determination, arm circumference is measured with a plastic measuring tape at the midpoint of the upper arm between the acromion and olecranon, and a cuff is then selected so that the length of the cuff bladder is equal to 80% to 100% of the arm circumference. After cuff selection, the peak inflation pressure is determined by inflating the cuff to 60 mm Hg and then gradually continuing to inflate in increments of 10 mm Hg until the radial pulse is no longer felt—thereby determining the pulse obliteration pressure. An additional 30 mm Hg is added to this value and recorded as the peak inflation pressure. The cuff is then inflated to this value for all BP measurements in each participant at that study visit.

After 5 minutes of rest, BP measurement begins. First, pulse is measured by palpation of the radial artery. Then, 4 BPs at 30-s intervals are obtained by auscultation of the brachial artery, using the first Korotkoff sound for systolic BP (SBP) and the fifth Korotkoff sound for diastolic BP. The average of the last 3 BPs is recorded as the participant's BP for the study visit. According to the average SBP, BP percentile rank is determined, and the participant is classified into 1 of 3 BP groups, which are based on published data demonstrating that hypertensive TOD may occur at BP levels as low as the 80th percentile[8]:
1. High-risk BP: Average SBP ≥90th percentile.
2. Mid-risk BP: Average SBP ≥80th and <90th percentiles.
3. Normal BP: Average SBP <75th percentile.

Ambulatory BP Monitoring

Ambulatory BP is measured with an oscillometric device (SpaceLabs OnTrak, Spacelabs Healthcare, Issaquah, WA). Using the arm circumference obtained during the auscultatory BP measurement, a properly sized cuff is selected, and the monitor is placed on the participant. Three resting BP's are obtained immediately after monitor placement and recorded for confirmation of correct placement and function of the device. BP monitoring is performed for 26 hours, with measurements obtained every 20 minutes. The monitor is returned to the site, and the data are downloaded to a laptop computer. Studies are divided into sleep-wake periods according to diaries provided by the participants. No hours of monitoring are discarded, consistent with current American Heart Association recommendations for pediatric ABPM.[8] Data files are transmitted electronically to the ABPM center in Seattle.

The ABPM center then performs preliminary quality review of the ABPM data before transmittal to the DCC. Summary variables are generated, including mean 24-hour, wake and sleep BP, BP load
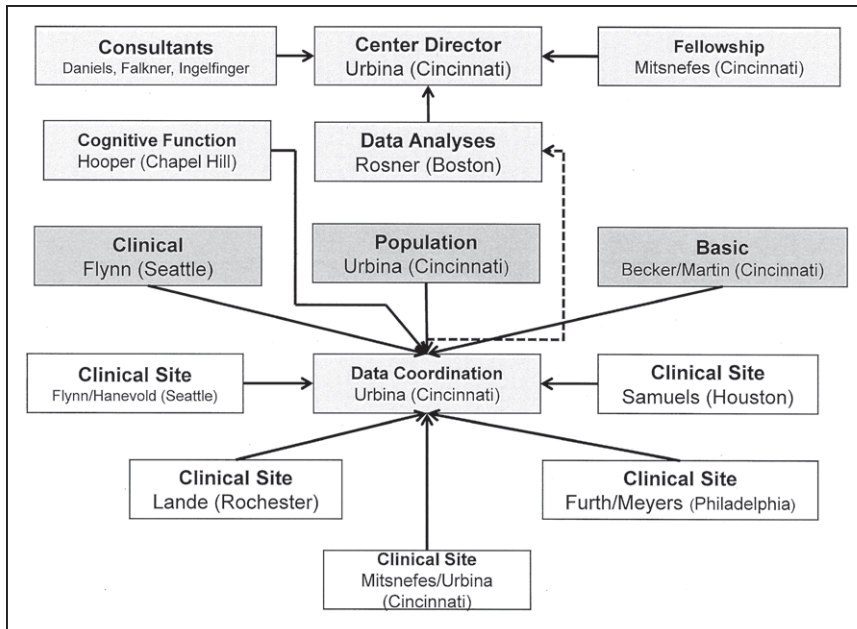
Figure. Illustration of the SHIP-AHOY organization (Study of High Blood Pressure in Pediatrics: Adult Hypertension Onset in Youth).

(percentage of readings >95th percentile), heart rate, pulse pressure, and BP variability. The casual BP and ABPM results are then used to derive the categorical assignment of BP phenotype (Table 2) according to current guidelines.[9]

Cardiac Measurements

Echocardiography is performed using standard cardiac ultrasound systems at each site. Cardiac sonographers participated in a web-based training in the echocardiographic protocol. Fourteen images are obtained with the participant supine. The images planes include parasternal long-axis view, parasternal short-axis view, apical 4-chamber view, apical 2-chamber view, high parasternal short-axis view, and suprasternal notch view. Loops of at least 3 cardiac cycles are obtained. All images are uploaded to a cloud-based image repository for later analysis by the CVRC. The absence of structural heart disease is confirmed, and any abnormalities found in the study are flagged and sent to the clinical site PI.

Left ventricular (LV) end-diastolic dimension, LV end-systolic dimension, end-diastolic interventricular septal thickness, and LV end-diastolic and end-systolic posterior wall thickness measurements are obtained offline by a trained sonographer using a Cardiology Analysis System (Digisonics, Houston, TX). LV mass (LVM) is calculated from 2-dimensional–guided M-Mode images of the left ventricle at end diastole[10,11] using the Devereux equation.[12] To adjust for body size without overcompensating for the adverse effect of obesity, LV mass index is calculated as $LVM/ht^{2.7}$, as described by DeSimone.[13]

Relative wall thickness at end-diastole is also calculated as the ratio of the sum of the interventricular septum and posterior wall divided by the end-diastolic dimension ([LV end-diastolic posterior wall thickness+end-diastolic interventricular septal thickness]/LV end-diastolic dimension). LV geometry is categorized as normal, concentric remodeling, concentric hypertrophy, or eccentric hypertrophy based on whether a participant has normal or elevated LVMI or relative wall thickness.[14]

Systolic function is evaluated by calculation of midwall fractional shortening, a measure that better reflects myocardial performance in hypertrophied hearts.[15] LV strain and strain-rate imaging is performed using TOMTEC software (TOMTEC Corporation, Chicago, IL) to quantify intraventricular dyssynchrony and evaluate components of myocardial function.[16]

For diastolic function, mitral inflow velocities are obtained with pulsed wave Doppler in the apical 4-chamber view. The Doppler cursor is placed parallel to mitral inflow, and maximal velocity is measured with the sample volume at the mitral valve leaflet tips. The mitral peak E (early filling) and A (inflow with atrial contraction) waves are measured offline, and E/A ratio is calculated. Tissue Doppler imaging myocardial flow velocities are acquired in the apical 4-chamber view. The peak (Ea) and late velocities (Aa) of mitral annular flow were recorded at the septal and lateral annulus; both lateral and septal Ea/Aa ratios and their averages are calculated. Two-dimensional measures of left atrium by body surface area are also obtained.[17] Each of the above parameters has been shown to correlate with invasive measures of diastolic function and LV end-diastolic pressure.[18]

Vascular Measurements

PWV is measured using the SphygmoCor CPV System (AtCor Medical, Sydney, Australia) as an assessment of vascular stiffness.[19] All research coordinators from the clinical sites were trained by the CVRC to perform the PWV measures. The patient is asked to lie supine in a comfortable position with their head flat on the bed. Using calipers, the average of 3 measures of sternal notch to femoral artery distance is obtained and entered into the software as the distal femoral distance. The distance from the carotid artery to the suprasternal notch is then obtained and entered as the proximal carotid measurement. ECG leads are then placed on the chest in standard positions. A tonometer is then used to obtain arterial waveforms gated to the R wave on the ECG tracing from the carotid and femoral artery. PWV is the difference in the carotid-to-femoral path length divided by the difference in R-wave-to-foot of the pressure wave times. The software requires a minimum of 3 data pairs to calculate a result and includes specific quality control indicators. Data are then downloaded from the device and sent to the CVRC.

Cognitive Measurements

Cognitive testing in SHIP-AHOY focuses on the assessment of executive functions as an estimate of hypertensive TOD. Executive

Table 2. BP Phenotypes

Phenotype*	Casual BP	Ambulatory BP
Normotensive	Normal	Normal
White coat hypertension	Elevated	Normal
Ambulatory hypertension	Elevated	Elevated
Masked hypertension	Normal	Elevated

ABPM indicates ambulatory BP monitoring; and BP, blood pressure.

*Categories defined as per 2014 American Heart Association Scientific Statement on pediatric ABPM.[9]

functions provide critical support for goal-directed behaviors that include inhibition, working memory, and attention regulation, abilities that are critical for the regulation of learning and behavior. Each of the tasks chosen for this study (Table S1 in the online-only Data Supplement) were selected because (1) they represent key cognitive functions that have previously been identified as important in the hypertension literature[20,21]; (2) they are age-appropriate and have well established age-based normative data; (3) they maintain strong psychometric properties; (4) they are well standardized and relatively straightforward with respect to their administration, assuring that nonpsychologists can be trained to administer them; and (5) they have few language barriers in their administration. All components of the test battery have been validated in children.[22–25]

The executive function tasks for this study were selected to measure attention regulation and inhibitory control, working memory, and nonverbal problem solving. Ratings of executive functions also will be obtained from the parent and participant. As with other assessments in SHIP-AHOY, standardized training and certification in the cognitive protocol have been provided to all study personnel responsible for conducting these assessments. Sites send the cognitive test results to the Cognitive Reading Center at the University of North Carolina for scoring and quality assurance checks.

Laboratory Analyses

Analyses of blood and urine samples are performed by the CCHMC clinical laboratory. Laboratory measures include fasting lipids, glucose, insulin, hsCRP (high-sensitivity C-reactive protein), and uric acid level. Urine albumin and creatinine, obtained from a properly collected first-morning urine sample, are used for calculation of urinary albumin excretion and urine sodium (Na) and potassium (K) for calculation of the urinary Na/K ratio. Girls have a urine pregnancy test performed at the local site because early pregnancy may affect BP. Microalbuminuria is defined as 30 to 300 mg albumin/g creatinine. Values ≥300 mg/g are considered proteinuria (macroalbuminuria). Glucose and insulin are used to estimate insulin resistance using the Homeostasis Model Assessment equation.[26] This equation correlates significantly ($P<0.001$) with insulin sensitivity measured directly with the insulin clamp.[27] Homeostasis Model Assessment will be analyzed as a continuous variable, and we will use Homeostasis Model Assessment >3.25 as a categorical criterion for insulin resistance. Total cholesterol, total triglycerides, and HDL-C (high-density lipoprotein-cholesterol) are measured, and LDL-C (low-density lipoprotein-cholesterol) is calculated.

Evaluation of Epigenetic Changes

One of the major challenges in studying hypertension is the marked heterogeneity in outcomes. Potential sources for phenotypic variation are acquired genetic, also known as epigenetic, modifications and alterations in gene regulation. Epigenetic processes alter gene activity without fundamentally changing the DNA[28] and have been shown to play a major role in the phenotypic expression of diseases, such as hypertension. Multiple studies have supported a role for epigenetics in BP control and downstream or tissue-level sequela from poor control. Gene methylation alterations have been implicated in BP control[29,30] and carotid intima-media thickness.[31] Micro-RNA (miRNA), as major regulators of gene activity, are associated with cardiac injury, angiogenesis, and cellular changes.[32–36]

Although genome-wide evaluation of epigenetic changes is possible, this approach would require a prohibitively large cohort. Thus, we opted to leverage existing biological data to select candidate genes for our epigenetic study. First, from studies performed in hypertensive adults, we compiled a list of genes which had DNA hypertension-associated alterations.[37–40] From this list, we identified 106 genes that had also been reported to have either altered methylation or to be targets for miRNAs with resulting altered gene expression. We subsequently used ToppFun (http://toppgene.cchmc.org/),[41] a gene list enrichment software to narrow the number of candidate genes for additional testing. Lastly, we used the GRAIL system (Gene Relationships Across Implicated Loci) to identify functional interactions, epigenetic modifications, and altered regulatory miRNAs between genes associated with hypertension and LV hypertrophy. The final list of 14 candidate genes (Table S2) cluster with the following

biologically plausible pathway for the development of hypertension and BP-related TOD:

Sympathetic stimulation→release of renin-angiotensin-aldosterone→altered sodium reabsorption in the kidney→vasoconstriction→LVH.

This targeted approach allows us to evaluate how environmental influences, such as obesity, metabolic derangement, and lifestyle, might influence gene expression and regulation leading to BP-related TOD.

DNA Methylation Measurement by Pyrosequencing

Individual bisulfite-treated DNA samples are subjected to polymerase chain reaction amplification.[42–44] Pyrosequencing generates continuous measurements of DNA methylation ranging from 0% to 100% (methylation%=peak height methylated/[peak height methylated+peak height unmethylated]). Methylation% is also logit transformed (log2[methylation%/1−methylation%]). After standard normalization procedures, we will use ANOVAs to test for differences between groups and t tests for pairwise comparisons between groups.

Micro-RNA Profiling

In addition to the targeted approach previously summarized, we also performed unbiased genome-wide miRNA-sequencing. The Illumina TruSeq small RNA kit (Illumina, Inc, San Diego, CA) was used as per the manufacturer's recommendations, except for the library size selection (18–25 bases), resulting in a minimum of 2 mol/L reads per sample. To produce miRNA profiles, we used the miRExpress software package (http://mirexpress.mbc.nctu.edu.tw)[45] to successively group identical reads, trimming the library adaptor sequence off the end of the reads and aligning the reads to the pre-miR reference sequences in the latest release of the mirBase database. Finally, the alignments to each pre-miR are tallied into individual miRNA counts to obtain a digital readout of how many molecules of each miRNA was sampled in the experiment. That number is proportional to the miRNA's expression blood level. After appropriate normalization, we search for miRNAs that are upregulated or downregulated between groups. As we expect many genes to be differentially expressed, we will use pathway analyses to identify the key biological processes underpinning the differential expression patterns.

Multicenter Study Coordination

Uniform methods of collecting data were developed across projects. Questionnaire (medical, family, diet, exercise) and other phenotypic data (age, race/ethnicity, sex, anthropometrics, casual/office BP) are entered into a REDCap web-based database (www.projectredcap.org) hosted at CCHMC. Laboratory results processed in Cincinnati are also transmitted to the DCC in electronic format, where abnormal clinical values are flagged and transmitted to the site principal investigators to communicate to the participants. Echocardiograms and PWV data are downloaded and transmitted electronically to the DCC for reading at the CVRC. Similarly, ABPM data and cognitive testing scores are transmitted electronically to the DCC from the ABPM center and cognitive reading center, respectively.

Data quality is monitored and controlled at many points in the data collection process. The DCC at CCHMC is staffed by a biostatistician, programmer, and coordinator. The DCC evaluates all data sources for outliers and biologically implausible results every 6 months and then adjudicates them. Reproducibility of repeat measures of LV structure and PWV are calculated and monitored.

Statistical Analyses

The cross-sectional nature of the study will allow us to test the hypotheses that adolescents with high-risk BP will have significantly greater LVMI and PWV compared with adolescents with normal BP. Sample size calculations (see online-only Data Supplement) were performed to ensure that we would have sufficient power to test this hypothesis.

χ^2 analyses will be used to relate BP category (low risk/midrisk/high risk) to TOD at baseline. In addition, logistic regression will be used to relate TOD to BP phenotype after adjusting for (1) age,

sex, BMI at baseline; and (2) all variables in (1) plus additional CVD risk factors (eg, lipids, insulin resistance, glucose). The primary analyses will be linear regression models. Similar analyses will be applied for TOD measures of executive function (individually and then combined executive composite) and urine albumin excretion.

Primary Outcomes

Outcome variables of TOD include LVMI, PWV, urine albumin excretion, and cognitive function scores with a focus on executive functions.

Results

To date, 264 adolescents have been recruited into SHIP-AHOY. Participant characteristics and cardiovascular variables of interest are summarized in Table 3. Mean age is 15.7±7.7 (range 11.2–18.9), 51% are male, and 61% are white. BMI and other measures of obesity are generally highest in the high-risk BP group.

There has been good separation of casual BP between the low, medium, and high-risk BP groups (Table 3). Similarly, there has been good separation of mean ambulatory BP among the 3 BP groups. Nocturnal dipping has not significantly varied between groups thus far. Success in obtaining research-quality ambulatory BP studies has been good, with average recording times of 25.5 hours and 82% of planned readings obtained.

Discussion

Although the association between primary hypertension and cardiovascular death in adults has been well described, the causality and natural history, including genetic, epigenetic, and environmental influences that impact the clinical expression of primary hypertension in youth remain unclear. A better understanding of primary hypertension over time, from childhood forward, will be facilitated by long-term population studies that include a mechanistic backbone. At some point in the continuum of primary hypertension, LVH, increased arterial stiffness, and renal damage can develop. Similarly, measures of TOD, such as LVH, increased PWV, decline in renal function, and impaired cognitive function themselves become preclinical diseases and powerful, independent risk factors for ischemic heart disease, congestive heart failure, arrhythmias, and sudden cardiac death. Understanding the determinants of these 2 transition points (from risk factor to TOD to clinical disease) may greatly impact the investigation and examination, management, and health of patients with primary hypertension. Understanding the epigenetic modifications of the existing genetic background may also explain the heterogeneity in the hypertensive phenotype.

SHIP-AHOY has several unique design elements that should allow us to answer many of the questions surrounding the development of hypertension starting in the young. First, it is the largest multicenter, multiethnic study of BP and BP-associated TOD in adolescents. Second, it includes youth across the BP distribution to determine the risk for TOD, even in adolescents with BP <90th percentile. Third, it combines auscultatory casual BP measurements with ABPM data to determine the BP phenotype, which will better characterize BP and what BP parameter signifies greater risk. Finally, the

Table 3. SHIP-AHOY Participant Characteristics*

Variable	Low-Risk BP (N=130)	Mid-Risk BP (N=60)	High-Risk BP (N=74)
Demographics/anthropometrics			
Age, y	15.7±1.5	16.1±1.7	15.4±1.6
Sex (male), %	50.0	65.0	58.1
Race (white), %	63.9	60.0	56.8
Hispanic, %	12.3	15.0	16.2
Height, m	1.68±0.09	1.72±0.10	1.69±0.09
Weight, kg	74.2±22.5	85.7±26.4	85.6±26.7
Waist, cm	83.6±17.7	89.7±20.4	93.3±21.0
BMI, kg/m²	26.3±6.7	29.3±9.6	29.8±8.3
Waist/height ratio	49.7±9.8	52.1±11.4	55.1±11.7
Hemodynamics			
K1 SBP, mm Hg	110.8±10.0	126.0±5.6	133.6±7.4
K5 DBP, mm Hg	74.5±9.6	82.3±7.5	86.1±9.3
MAP, mm Hg	86.8±9.3	96.9±5.5	101.9±7.3
SBP percentile, %	43.6±26.3	83.0±9.4	95.0±4.3
DBP percentile, %	71.0±23.5	87.0±11.5	91.4±12.7
Heart rate, bpm	71.4±11.9	68.3±12.4	71.4±12.6
ABPM parameters†			
Mean wake SBP	116.7±9.2	125.5±9.1	130.4±9.9
Wake SBP index	0.87±0.07	0.93±0.07	0.97±0.08
Wake SBP load, %	9.6±13.5	23.7±20.9	35.7±28.0
Mean wake DBP	68.6±6.5	73.6±6.6	75.2±7.8
Wake DBP index	0.83±0.08	0.89±0.08	0.91±0.10
Wake DBP load, %	9.6±11.7	21.3±15.9	27.0±21.5
Mean sleep SBP	103.1±9.3	109.8±11.1	114.0±10.1
Sleep SBP index	0.88±0.07	0.93±0.10	0.96±0.09
Sleep SBP load, %	11.4±17.0	26.6±27.5	34.4±29.0
Mean sleep DBP	55.8±5.7	58.4±6.0	60.7±8.5
Sleep DBP index	0.84±0.09	0.88±0.09	0.91±0.13
Sleep DBP load, %	12.7±16.7	19.7±18.5	27.8±26.8
SBP dipping, %	11.6±6.1	12.6±6.0	12.5±5.7
DBP dipping, %	18.4±7.6	20.6±6.2	19.4±7.4

ABPM indicates ambulatory BP monitoring; BMI, body mass index; BP, blood pressure; DBP, diastolic BP; MAP, mean arterial pressure; SBP, systolic BP; and SHIP-AHOY, Study of High Blood Pressure in Pediatrics: Adult Hypertension Onset in Youth.

*Data expressed as mean and SD or as frequencies.

†Index is calculated as the subject's BP value divided by the 95th percentile BP; load is defined as the percentage of readings above the 95th percentile for that time period; dipping percentage is calculated as ([mean wake SBP/DBP−mean sleep SBP/DBP]/mean wake S/DBP)×100.

inclusion of advanced echocardiographic measures, such as tissue Doppler imaging and strain, arterial stiffness measurements (PWV), and cognitive functioning as secondary measures of TOD, is unique. This will provide significant additional evidence on the intermediate effects of high BP in the young.

The characteristics of the participants enrolled thus far demonstrate the feasibility of recruiting a well-characterized cohort of adolescents that will allow us to address fundamental questions about the effects of high BP in youth. The SHIP-AHOY is likely to change how BP is evaluated and diagnosed in youth, moving from a statistical definition of risk to one that is based on thresholds for intervention based on intermediate measures of TOD. These data may also inform development of more specific treatment guidelines and, by identifying those at highest risk, may be able to modify the CVD risk trajectory into adulthood.

Perspectives

Although much is known about childhood hypertension, uncertainty remains on how to best define BP thresholds for diagnosis and treatment. SHIP-AHOY will provide new data that should help to close these and other knowledge gaps, thereby advancing our understanding of the earliest phases of hypertension and hypertension-associated CVD.

Sources of Funding

Disclosures

None.

References

1. Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB, Levy D. Impact of high-normal blood pressure on the risk of cardiovascular disease. *N Engl J Med*. 2001;345:1291–1297. doi: 10.1056/NEJMoa003417.

2. Psaty BM, Furberg CD, Kuller LH, Cushman M, Savage PJ, Levine D, O'Leary DH, Bryan RN, Anderson M, Lumley T. Association between blood pressure level and the risk of myocardial infarction, stroke, and total mortality: the cardiovascular health study. *Arch Intern Med*. 2001;161:1183–1192.

3. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA*. 2003;289:2560–2572. doi: 10.1001/jama.289.19.2560.

4. Go AS, Mozaffarian D, Roger VL, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics–2014 update: a report from the American Heart Association. *Circulation*. 2014;129:e28–e292. doi: 10.1161/01.cir. 0000441139.02102.80.

5. Thompson M, Dana T, Bougatsos C, Blazina I, Norris SL. Screening for hypertension in children and adolescents to prevent cardiovascular disease. *Pediatrics*. 2013;131:490–525. doi: 10.1542/peds.2012-3523.

6. Flynn JT, Kaelber D, Baker-Smith CM, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics*. 2017; 140:e20171904.

7. Flynn JT. High blood pressure in the young: why should we care? *Acta Paediatr*. 2018;107:14–19. doi: 10.1111/apa.14110.

8. Falkner B, DeLoach S, Keith SW, Gidding SS. High risk blood pressure and obesity increase the risk for left ventricular hypertrophy in African-American adolescents. *J Pediatr*. 2013;162:94–100. doi: 10.1016/j.jpeds.2012.06.009.

9. Flynn JT, Daniels SR, Hayman LL, Maahs DM, McCrindle BW, Mitsnefes M, Zachariah JP, Urbina EM; American Heart Association Atherosclerosis, Hypertension and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young. Update: ambulatory blood pressure monitoring in children and adolescents: a scientific statement from the American Heart Association. *Hypertension*. 2014;63:1116–1135. doi: 10.1161/HYP.0000000000000007.

10. Gottdiener JS, Bednarz J, Devereux R, Gardin J, Klein A, Manning WJ, Morehead A, Kitzman D, Oh J, Quinones M, Schiller NB, Stein JH, Weissman NJ; American Society of Echocardiography. American Society of Echocardiography recommendations for use of echocardiography in clinical trials. *J Am Soc Echocardiogr*. 2004;17:1086–1119. doi: 10.1016/j.echo.2004.07.013.

11. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005;18:1440–1463. doi: 10.1016/j.echo.2005.10.005.

12. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol*. 1986;57:450–458.

13. de Simone G, Devereux RB, Daniels SR, Koren MJ, Meyer RA, Laragh JH. Effect of growth on variability of left ventricular mass: assessment of allometric signals in adults and children and their capacity to predict cardiovascular risk. *J Am Coll Cardiol*. 1995;25:1056–1062.

14. Ganau A, Devereux RB, Roman MJ, de Simone G, Pickering TG, Saba PS, Vargiu P, Simongini I, Laragh JH. Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. *J Am Coll Cardiol*. 1992;19:1550–1558.

15. Shimizu G, Hirota Y, Kita Y, Kawamura K, Saito T, Gaasch WH. Left ventricular midwall mechanics in systemic arterial hypertension. Myocardial function is depressed in pressure-overload hypertrophy. *Circulation*. 1991;83:1676–1684.

16. Dandel M, Lehmkuhl H, Knosalla C, Suramelashvili N, Hetzer R. Strain and strain rate imaging by echocardiography - basic concepts and clinical applicability. *Curr Cardiol Rev*. 2009;5:133–148. doi: 10.2174/157340309788166642.

17. Farias CA, Rodriguez L, Garcia MJ, Sun JP, Klein AL, Thomas JD. Assessment of diastolic function by tissue Doppler echocardiography: comparison with standard transmitral and pulmonary venous flow. *J Am Soc Echocardiogr*. 1999;12:609–617.

18. Kasner M, Westermann D, Steendijk P, Gaub R, Wilkenshoff U, Weitmann K, Hoffmann W, Poller W, Schultheiss HP, Pauschinger M, Tschöpe C. Utility of Doppler echocardiography and tissue Doppler imaging in the estimation of diastolic function in heart failure with normal ejection fraction: a comparative Doppler-conductance catheterization study. *Circulation*. 2007;116:637–647. doi: 10.1161/CIRCULATIONAHA.106.661983.

19. Savant JD, Furth SL, Meyers KE. Arterial stiffness in children: pediatric measurement and considerations. *Pulse (Basel)*. 2014;2:69–80. doi: 10.1159/000374095.

20. Lande MB, Kupferman JC, Adams HR. Neurocognitive alterations in hypertensive children and adolescents. *J Clin Hypertens (Greenwich)*. 2012;14:353–359. doi: 10.1111/j.1751-7176.2012.00661.x.

21. Lande MB, Adams H, Falkner B, Waldstein SR, Schwartz GJ, Szilagyi PG, Wang H, Palumbo D. Parental assessment of executive function and internalizing and externalizing behavior in primary hypertension after anti-hypertensive therapy. *J Pediatr*. 2010;157:114–119. doi: 10.1016/j.jpeds.2009.12.053.

22. Conners CK. *Conners Continuous Performance Test*. 3rd ed. North Tonawanda, NY: Multi-Health Systems Inc; 2014.

23. Shrank F, Mather N, & McGrew KS. *Woodcock-Johnson IV Tests of Cognitive Abilities*. Itasca, IL: Riverside Publishing; 2014.

24. Gioia GA, Isquith PK, Guy SC, Kenworthy L. *Behavior Rating Inventory of Executive Functions*. Lutz, FL: Psychological Assessment Resources, Inc; 2015.

25. Hammill DD, Pearson NA, Wiederholt JL. *Test of Nonverbal Intelligence-2*. Lutz, FL: Psychological Assessment Resources, Inc; 1990.

26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell

function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.

27. Cheng C, Campbell KL, Kushner H, Falkner BE. Correlation of oral glucose tolerance test-derived estimates of insulin sensitivity with insulin clamp measurements in an African-American cohort. *Metabolism*. 2004;53:1107–1112.

28. Weinhold B. Epigenetics: the science of change. *Environ Health Perspect*. 2006;114:A160–A167.

29. Udali S, Guarini P, Moruzzi S, Choi SW, Friso S. Cardiovascular epigenetics: from DNA methylation to microRNAs. *Mol Aspects Med*. 2013;34:883–901. doi: 10.1016/j.mam.2012.08.001.

30. Alexeeff SE, Baccarelli AA, Halonen J, Coull BA, Wright RO, Tarantini L, Bollati V, Sparrow D, Vokonas P, Schwartz J. Association between blood pressure and DNA methylation of retrotransposons and pro-inflammatory genes. *Int J Epidemiol*. 2013;42:270–280. doi: 10.1093/ije/dys220.

31. Breton CV, Park C, Siegmund K, Gauderman WJ, Whitfield-Maxwell L, Hodis HN, Avol E, Gilliland FD. NOS1 methylation and carotid artery intima-media thickness in children. *Circ Cardiovasc Genet*. 2014;7:116–122. doi: 10.1161/CIRCGENETICS.113.000320.

32. De Rosa S, Curcio A, Indolfi C. Emerging role of microRNAs in cardiovascular diseases. *Circ J*. 2014;78:567–575.

33. Li RC, Tao J, Guo YB, et al. *In vivo* suppression of microRNA-24 prevents the transition toward decompensated hypertrophy in aortic-constricted mice. *Circ Res*. 2013;112:601–605. doi: 10.1161/CIRCRESAHA.112.300806.

34. Pan W, Zhong Y, Cheng C, Liu B, Wang L, Li A, Xiong L, Liu S. MiR-30-regulated autophagy mediates angiotensin II-induced myocardial hypertrophy. *PLoS One*. 2013;8:e53950. doi: 10.1371/journal.pone.0053950.

35. Thum T, Lorenzen JM. Cardiac fibrosis revisited by microRNA therapeutics. *Circulation*. 2012;126:800–802. doi: 10.1161/CIRCULATIONAHA.112.125013.

36. Villar AV, Merino D, Wenner M, Llano M, Cobo M, Montalvo C, García R, Martín-Durán R, Hurlé JM, Hurlé MA, Nistal JF. Myocardial gene expression of microRNA-133a and myosin heavy and light chains, in conjunction with clinical parameters, predict regression of left ventricular hypertrophy after valve replacement in patients with aortic stenosis. *Heart*. 2011;97:1132–1137. doi: 10.1136/hrt.2010.220418.

37. Johnson AD, Newton-Cheh C, Chasman DI, et al; Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium; Global BPgen Consortium; Women's Genome Health Study. Association of hypertension drug target genes with blood pressure and hypertension in 86,588 individuals. *Hypertension*. 2011;57:903–910. doi: 10.1161/HYPERTENSIONAHA.110.158667.

38. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, Cupples LA, Myers RH. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study. *Hypertension*. 2000;36:477–483.

39. Mitchell GF, Guo CY, Kathiresan S, et al. Vascular stiffness and genetic variation at the endothelial nitric oxide synthase locus: the Framingham Heart study. *Hypertension*. 2007;49:1285–1290. doi: 10.1161/HYPERTENSIONAHA.106.085266.

40. Ehret GB, Munroe PB, Rice KM, et al; International Consortium for Blood Pressure Genome-Wide Association Studies; CARDIoGRAM consortium; CKDGen Consortium; KidneyGen Consortium; EchoGen consortium; CHARGE-HF consortium. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109. doi: 10.1038/nature10405.

41. Chen J, Xu H, Aronow BJ, Jegga AG. Improved human disease candidate gene prioritization using mouse phenotype. *BMC Bioinformatics*. 2007;8:392. doi: 10.1186/1471-2105-8-392.

42. Ji H, Ehrlich LI, Seita J, et al. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature*. 2010;467:338–342. doi: 10.1038/nature09367.

43. Zhang X, Ulm A, Somineni HK, Oh S, Weirauch MT, Zhang HX, Chen X, Lehn MA, Janssen EM, Ji H. DNA methylation dynamics during ex vivo differentiation and maturation of human dendritic cells. *Epigenetics Chromatin*. 2014;7:21. doi: 10.1186/1756-8935-7-21.

44. Ji H, Zhang X, Oh S, Mayhew CN, Ulm A, Somineni HK, Ericksen M, Wells JM, Khurana Hershey GK. Dynamic transcriptional and epigenomic reprogramming from pediatric nasal epithelial cells to induced pluripotent stem cells. *J Allergy Clin Immunol*. 2015;135:236–244. doi: 10.1016/j.jaci.2014.08.038.

45. Wang WC, Lin FM, Chang WC, Lin KY, Huang HD, Lin NS. miR-Express: analyzing high-throughput sequencing data for profiling microRNA expression. *BMC Bioinformatics*. 2009;10:328. doi: 10.1186/1471-2105-10-328.

Novelty and Significance

What Is New?

- Study includes youth across the blood pressure (BP) distribution to determine the risk for target organ damage, including BP well below the 90th percentile.
- Combination of auscultatory casual BP measurements with ambulatory BP monitoring data, to better characterize BP and what BP parameter signifies greater risk.
- Inclusion of advanced echocardiographic measures, arterial stiffness, and cognitive function assessment.

What Is Relevant?

- Study allows a better understanding of the determinants of how BP in youth may transition from a risk factor to target organ damage to clinical disease, which may greatly impact the health of patients with primary hypertension.

Summary

SHIP-AHOY (Study of High Blood Pressure in Pediatrics: Adult Hypertension Onset in Youth) is likely to change how hypertension is defined in youth, moving from a statistical definition to one based on intermediate measures of target organ damage. These data should inform development of new treatment guidelines that will identify those at highest risk and modify the cardiovascular disease risk trajectory into adulthood.

SHIP-AHOY (Study of High Blood Pressure in Pediatrics: Adult Hypertension Onset in Youth): Rationale, Design, and Methods

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STUDY OF HIGH BLOOD PRESSURE IN PEDIATRICS: ADULT HYPERTENSION

ONSET IN YOUTH (SHIP-AHOY): RATIONALE, DESIGN AND METHODS

Online data supplement

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Sample Size Estimate: We base our sample size calculation on preliminary data from Urbina, et al.[1] In this study, measures of target organ damage (TOD) were obtained from 150 low-risk BP (NT) (i.e., < 80th percentiles), 25 mid-risk BP (80-89th BP percentiles) and 9 high-risk BP($\geq$ 90th percentile). The following data were obtained:

Preliminary data on LVMI and PWV by BP group									
BP Group		**LVMI (g/m^2)**					**PWV (m/sec)**		
		mean	sd	N			mean	sd	N
Low-risk		29.7	6.9	150		**Low-risk**	5.44	0.73	145
Mid-risk		34.0	8.8	25		**Mid-risk**	5.70	0.56	25
High-risk		35.1	5.6	9		**High-risk**	5.83	0.67	6

If we posit differences of the magnitude seen above, then with samples sizes of 122 for NT, 189 for pre-HT and 189 for HT, we will have the following power estimates:

Power estimates for LVMI and PWV in SHiP AHOY					
BP Group 1	**N**	**BP Group 2**	**N**	**LVMI**	**PWV**
				power	power
Low-Risk	122	Mid-Risk	189	0.998	0.918
Low-Risk	122	High-Risk	189	1.0	0.997

Thus, we will have adequate power ($\geq$91%) to detect reasonable differences in mean LVMI and PWV between the NT group and each of the pre-HT and HT groups.

[1] Urbina EM, Khoury PR, McCoy C, Daniels SR, Kimball TR and Dolan LM. Cardiac and vascular consequences of pre-hypertension in youth. *J Clin Hypertens*. 2011; 13:332-42.

Table S1. Neurocognitive Measures and associated outcomes.

Test	Cognitive Function Assessed
TONI-4	Nonverbal Problem Solving; Nonverbal Intelligence
Connors CPT-3	Inhibition/Attention Regulation
WJ-IV Verbal Attention	Attention
WJ-IV Numbers Reverse	Working Memory
WJ-IV Object-Number Sequence	
BRIEF-2-Parent	Behavior Regulation, Cognitive Regulation, Emotional Regulation, Global Executive Function
BRIEF-2-Self-Report	

Abbreviations used in table: TONI-4 = Test of Nonverbal Intelligence-4th Edition; Connors CPT-3 = Connors Continuous Performance Test-Third Edition; WJ-IV = Woodcock-Johnson Test of Cognitive Abilities-Fourth Edition; BRIEF-2 = Behavioral Rating Inventory of Executive Functions-Second Edition.

Table S2. Selected Hypertension Candidate Genes[1] and their associated miRNAs.

Gene Symbol	Gene Name	Pathyway	Associated miRNAs
ADRB1	adrenoceptor beta 1	Symp NS	23, 30, 125a, 133a
NPY	neruopeptide Y	Symp NS	30
ACE	angiotensin I converting enzyme	RAAS	133a, 146
AGT	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	RAAS	31
REN	renin	RAAS	146
CYP11B1	cytochrome P450, family 11, subfamily B, polypeptide 1	Aldosterone	24
HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2	Aldosterone	101, 125a, 133a
NPPA	natriuretic peptide A	Na reabs kidney	133
SCNN1A	sodium channel, non-voltage-gated 1 alpha subunit	Na reabs kidney	143
EDN1	endothelin 1	Vasoconstriction (NOS)	125
NOS3	nitric oxide synthase 3 (endothelial cell)	Vasoconstriction (NOS)	21
DRD2	dopamine receptor D2	Heart	125a, 133a, 143, 145, 146, 181
GNB3	guanine nucleotide binding protein (G protein), beta polypeptide 3	Heart	101, 125a, 133a, 143, 145, 146
MYH9	myosin, heavy chain 9, non-muscle	Heart	125a, 133a, 145, 146, 181

[1]Selection of candidate genes was based on enrichment of biologic processes involved in hypertension and target organ damage.

Abbreviations used in table:

miRNA, microRNA; Na, sodium; NOS, nitric oxide synthase; RAAS, renin angiotensin aldosterone system; Symp NS, sympathetic nervous system.