MECHANISMS DRIVING THE EFFECT OF WEIGHT LOSS ON ARTERIAL STIFFNESS

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Aims Arterial stiffness decreases with weight loss in overweight and obese adults, but the mechanisms by which this occurs are poorly understood. We aimed to elucidate these mechanisms.

Methods We evaluated carotid-femoral pulse wave velocity (cfPWV), a measure of aortic stiffness, and brachial-ankle pulse wave velocity (baPWV), a mixed measure of central and peripheral arterial stiffness, in 344 young adults (mean age 38 yrs, mean body mass index (BMI) 32.9 kg/m^2, 23% male) at baseline, 6 and 12 months in a behavioral weight loss intervention. Linear mixed effects models were used to evaluate associations between weight loss and arterial stiffness and to examine the degree to which improvements in obesity-related factors explained these associations. Pattern-mixture models using indicator variables for dropout pattern and Markov Chain Monte Carlo multiple imputation were used to evaluate the influence of different missing data assumptions.

Results At 6 months (7% mean weight loss from baseline), there was a statistically significant median decrease of 47.5 cm/s (interquartile range (IQR) -44.5, 148) in cfPWV (p<0.0001) and a mean decrease of 11.7 cm/s (standard deviation (SD) 91.4) in baPWV (p=0.049). At 12 months (6% mean weight loss from baseline) only cfPWV remained statistically significantly reduced from baseline (p=0.02). Change in BMI (p=0.01) was statistically significantly positively

associated with change in cfPWV after adjustment for changes in mean arterial pressure (MAP) or any other measured obesity-related factor. Common carotid artery diameter (p=0.003) was associated and heart rate (p=0.08) and MAP (p=0.07) marginally associated longitudinally with cfPWV. Reductions in heart rate (p<0.0001) and C-reactive protein (p=0.02) were associated with reduced baPWV, and each removed the statistical significance of the effect of weight loss on baPWV. Pattern-mixture modeling revealed several differences between completers and non-completers in the models for cfPWV, but marginal parameter estimates changed little from the original models for either PWV measure.

Conclusions The public health importance of this thesis is that firstly, weight loss improves arterial stiffness in overweight and obese young adults. Secondly, its effect on baPWV may be explained by concurrent reductions in heart rate and inflammation. Missing data did not appear to bias these results.

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1.0   INTRODUCTION

Obesity leads to poor vascular health and an increased risk of cardiovascular disease (CVD) (1-3). The metabolic requirements of excess weight necessitate increases in total blood volume and cardiac output, and these hemodynamic changes elevate arterial wall stress, smooth muscle cell proliferation, vessel wall thickness and diameter, and arterial stiffness (1, 4). These hemodynamic alterations work together with other features of obesity, including chronic inflammation and endothelial dysfunction, to impair vascular structure and function in obese individuals (5). Weight loss reverses many adverse vascular changes (6-9) and lowers CVD risk (8, 10, 11). Arterial stiffness, often measured non-invasively as pulse wave velocity (PWV), is an established measure of vascular health. Carotid-femoral pulse wave velocity (cfPWV), a measure of aortic stiffness, and brachial-ankle pulse wave velocity (baPWV), a mixed measure of central and peripheral arterial stiffness, are both predictive of incident vascular events and cardiovascular and all-cause mortality in the general population (12, 13), though cfPWV has been the more frequently reported predictor.

Several studies have shown that improvements in arterial stiffness with weight loss in overweight and obese adults (14-20) may be independent of concurrent reductions in blood pressure (18), though not all studies agree (21). Few studies have evaluated other mechanisms by which weight loss may reduce arterial stiffness, and these studies have included small numbers of either middle-aged and older overweight and obese adults (15, 16) or severely obese adults (18). Because several common factors, such as elevated inflammation (19, 22, 23), insulin resistance (24, 25), and renin-angiotensin-aldosterone system activity (24, 26, 27), have been

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found to be cross-sectionally associated with greater arterial stiffness and have also been found to decrease with weight loss, it is likely that changes in some of these cardiometabolic factors explain the association between weight loss and reduced arterial stiffness. The aim of this study was to determine the mechanisms by which weight loss reduces arterial stiffness in young overweight and obese normotensive adults assessed at 6 and 12 months follow-up during a lifestyle weight loss intervention.

2.0   SUBJECTS AND METHODS

To study the mechanisms linking weight loss and arterial stiffness, we measured cfPWV and baPWV at baseline and 6 and 12 month visits in overweight and obese adults participating in the Slow Adverse Vascular Effects of excess weight study (SAVE), a randomized-controlled trial (NCT00366990) evaluating the effects of weight loss, increased physical activity, and reduced dietary sodium intake on vascular health.

2.1   STUDY POPULATION

Briefly, participants were recruited from June 2007 through May 2009 using mass mailing. Six-month and 12-month data were complete as of February 2009 and July 2010, respectively. The recruitment goal was 350 participants and was based on 84% power to detect a 17 cm/s difference for change in PWV between treatment arms. The study was approved by the University of Pittsburgh IRB and all participants provided written informed consent to participate in the study.

Eligible participants were men and women 20-45 years of age who were overweight or obese (body mass index (BMI) 25-39.9 kg/m^2) and physically inactive (<8 months of physical activity (PA) during the past 12 months). Exclusions included 1) diabetes, 2) hypertension or

average screening blood pressure ≥140/90 mmHg, 3) cholesterol lowering, anti-psychotic, or vasoactive medication use and 4) current pregnancy or lactation.

2.2   INTERVENTION

All eligible participants received a 1-year lifestyle intervention consisting of diet and physical activity (PA). Participants were randomized to either 1) diet and PA alone (Control Na/lifestyle) or to 2) diet and PA plus reduced sodium intake (Low Na/lifestyle). The lifestyle intervention was delivered in group sessions that occurred weekly for months 1-4, biweekly for months 5-8, and monthly for months 9-12. The goal of the intervention was a 10% reduction in body weight over 6 months. Total caloric intake was individualized to promote a 1- to 2-pound/week weight loss. Fat was limited to 25% to 30% of calories. A structured meal pattern provided an initial framework upon which a personalized plan was built as the participant became more informed and skilled. Self-monitoring of dietary intake was encouraged to reinforce the dietary goals. Participants were given a nutrient counter to assist in determining food calorie and fat content.

At the fourth session participants were introduced to progressive PA goals of 150 (minimum PA goal) to 200 (optimum PA goal) min/week (28), beginning with 60 minutes per week and increasing PA by no more than 30 minutes every two weeks until they reached goal. Moderate-intensity aerobic activities, such as brisk walking, were the primary types of PA recommended. We requested that PA be performed in a minimum of three sessions per week and that individual PA bouts last at least ten minutes. Strength training using resistance bands was introduced in the latter portion (month 6) of the intervention to complement rather than replace

aerobic PA. The goal of the sodium reduction intervention (Low Na) was to perform all of the above activities and also to gradually reduce daily sodium intake to approximately 1 mg Na^+/1 kcal/day, an average reduction of about 50% from the participant's usual diet (29).

2.3   CLINIC VISITS

Participants were to complete clinic visits at screening, baseline, 6 months, 12 months and 24 months following randomization. Self-reported demographic information, self- and interviewer-administered questionnaires, anthropometric measurements, fasting blood draw, 24-hour urine collection, and non-invasive tests of vascular structure and function were collected at these visits. The data presented here are from baseline, 6-month and 12-month follow-up visits.

2.3.1   Demographic and Physical Measures

Age, race, and smoking status were self-reported. Race was re-coded as black vs. non-black. Smoking status was assessed as current vs. past or never. Weight was measured in kilograms using a balance scale. Height was measured in centimeters using a stadiometer. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured against the participant's skin at the narrowest part of the torso between the ribs and the iliac crest. Blood Pressure (BP) was measured with a mercury sphygmomanometer after participants sat quietly for 5 minutes with feet flat on the floor. Final BP was the average of the last 2 of 3 readings taken 30 seconds apart.

2.3.2   Blood Assays

Blood analytes were measured at the Heinz Laboratory at the University of Pittsburgh's Graduate School of Public Health. Serum glucose was determined enzymatically with a procedure similar to that described by Bondar and Mead (30). Insulin was measured using an RIA procedure developed by Linco Research, Inc. Total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides and glucose were determined using standard laboratory procedures. Insulin, leptin, adiponectin and total ghrelin were measured using radioimmunoassay (Linco Research, Inc.). C-reactive protein (CRP) was measured using an enzyme-linked immunoassay (Alpha Diagnostic International, Inc.). Aldosterone was measured using an enzyme-linked immunoassay developed by Diagnostic Systems Laboratories (Webster, TX).

2.3.3   Pulse Wave Velocity

Pulse wave velocity measures were generated using the VP2000 system (Omron Health Care Co., Kyoto, Japan), a noninvasive automated waveform analyzer that simultaneously provides measures of carotid-femoral and brachial-ankle PWV. Following ten minutes of rest in a supine position, the participant had occlusion and monitoring cuffs placed around both arms and ankles, ECG electrodes on both wrists and a phonocardiogram on the left edge of the sternum. Occlusion cuffs at the brachial and tibial arteries were connected to pressure sensors that measured blood pressure and pressure waveforms at these peripheral sites as previously described (31). Handheld tonomoters over the right carotid and femoral arterial sites were used to obtain femoral

and carotid pulse waveforms simultaneously. PWV (in cm/sec) was calculated as the path length between arterial sites of interest divided by the time delay between the foot of the respective waveforms. For cfPWV path length, the distance between the carotid and femoral sites was measured (in cm) over the surface of the body with a tape measure. The path length for baPWV was calculated using a height-based formula (31). For baPWV, results for the right and left legs were averaged. For all PWV measures, data were collected twice for each participant, and the values were averaged. Participants with valid PWV measures (defined as between 300 m/s and 2500 m/s) were included in analyses. Intraclass correlation coefficients (ICC) for within technologist replicate measures were 0.76 (cfPWV) and 0.97 (baPWV) and for between technologists replicates were 0.60 (cfPWV) and 0.87 (baPWV).

2.3.4 Carotid Ultrasound

Common carotid artery (CCA) intima-media thickness (IMT) and adventitial diameter (AD) measurements and readings were performed at the Ultrasound Research Laboratory of the Department of Epidemiology, University of Pittsburgh using an Acuson Sonoline Antares high resolution duplex scanner. Detailed methodology using the same protocol has been published (32). Two sets of digitized images for later reading were obtained of both near and far walls of the right and left distal common carotid arteries (1 cm proximal to the carotid bulb). Intima-media thickness measures were obtained by electronically tracing the lumen-intima interface and the media-adventitia interface across these 1-cm segments; one measurement was generated for each pixel over the area, for a total of approximately 140 measures for each segment. CCA inter-adventitial diameters were measured directly as the distance from the adventitial-medial

interface on the near wall to the medial-adventitial interface on the far wall at end-diastole across the same CCA segments used for IMT measurement. The reading software used was the AMS system developed by Dr. Thomas Gustavsson (33). The same reader was used across visits. For these analyses, the mean of the average IMT and AD values was used. Reproducibility of IMT measures was excellent with an ICC between sonographers of >0.87 and within reader of >0.90. For AD measures, the between-sonographer ICC's were >0.83 and the within reader ICC's were >0.90.

2.4   LINEAR MIXED EFFECTS MODELS

The primary goal of longitudinal analysis is to assess within-individual changes in characteristics of interest over time and to determine which factors influence heterogeneity among these within-individual changes. Mixed models are widely used in health studies for longitudinal analysis. Linear mixed effects models can be used to model continuous outcome variables and, as the name implies, these models assume that some of the regression parameters vary randomly between subjects (random effects) and some are common to all subjects (fixed effects). The introduction of random effects induces within-subject correlation among outcomes. Such correlation must be accounted for in order to avoid obtaining biased standard errors for both within- and between-subject factors (34). Another appealing aspect of linear mixed effect models, in addition to their capacity to differentiate within-subject and between-subject sources of variation, is their ability to accommodate imbalanced data. Linear mixed effects models, unlike univariate or multivariate repeated-measures analysis of variance (ANOVA), require

neither the same number of observations nor the same timing of measurement occasions on all subjects. Thus, mixed models are particularly convenient for handling unbalanced longitudinal data.

In the simplest case of a linear mixed effects model, only the intercept is treated as random, thereby assuming that each subject has a latent underlying level of response that persists throughout the study duration:

$$Y_{it} = X'_{it}\beta + b_i + e_{it}$$

In this model, b_i is the random intercept for subject i and e_{it} is the measurement or sampling error for subject i and time t. It is typically assumed that $b_i \sim N(0,\sigma^2_b)$ and $e_i \sim N(0, \sigma^2 I_{ni})$ where I_{ni} is the n_i-dimensional identity matrix, though additional within-subject serial correlation beyond that accounted for by random effects can be investigated. In addition, b_i and e_{ij} are assumed to be independent of one another. In this model, the conditional mean of Y_{ij} given the subject-specific effect, is:

$$E(Y_{it}|b_i) = X'_{it}\beta + b_i$$

and the marginal mean of Y_{ij} in the population (averaged over the subject-specific effects) is:

$$E(Y_{it}) = X'_{it}\beta$$

Linear mixed effect models can also include random coefficients. For example, in longitudinal studies time is often treated as a random effect. In general, a linear mixed effect model is any model that satisfies the four properties below:

$$Y_i = X_i\beta + Z_ib_i + e_i$$

$$b_i \sim N(0, D)$$

$$e_i \sim N(0, \Sigma_i)$$

$$b_1,\ldots, b_N, e_1,\ldots,e_N \text{ independent}$$

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where Y_i is the n_i-dimensional response vector for subject i, $1 \leq i \leq N$, N is the number of subjects, X_i and Z_i are (n_i x p) and (n_i x q) dimensional matrixes of known covariates, β is a p-dimensional vector containing the fixed effects, b_i is a q-dimensional vector containing the random effects, and e_i is an n_i-dimensional vector of residual components. D is a general (q x q) covariance matrix with (i, j) element $d_{ij} = d_{ji}$ and Σ_i is a (n_i x n_i) covariance matrix which depends on i only through its dimension n_i (35).

Though the main goal of a longitudinal study is to investigate within-individual changes in responses over time, longitudinal studies provide both longitudinal and cross-sectional information. These two sources of information can sometimes be at odds. Care must be taken in model specification in order to avoid the confounding of longitudinal effects with cross-sectional effects when the two differ. This can be accomplished by including separate parameters for the cross-sectional (between-subject) and longitudinal (within-subject) effects of time-varying variables in the model, as shown below:

$$Y_{ij} = Z'_i \beta_0 + X'_{i1} \beta^{(C)} + (X'_{ij} - X'_{i1}) \beta^{(L)} + e_{ij}$$

where X'_{ij} is the row vector of q time-varying covariates for the jth response on the ith subject and Z'_i is the row vector of p – q time-stationary covariates. This model allows the simultaneous estimation of both cross-sectional effects, $\beta^{(C)}$, and longitudinal effects, $\beta^{(L)}$. When investigating the associations between time-varying covariates and an outcome of interest during an intervention, such as in the present study, it is mainly $\beta^{(L)}$ that is of interest (34).

2.5   MISSING DATA

Although mixed models for longitudinal data have many advantages, they are not guaranteed to produce unbiased parameter estimates in studies with missing data. Missing data are ubiquitous in longitudinal biomedical research, in which missing data usually occur in the form of dropouts. Since the form of the non-response process can never be fully known, assumptions must be made in any analysis of available data (36). According to widely used terminology first conceived by Rubin (37), missing data are missing completely at random (MCAR) if missingness is independent of both unobserved and observed outcome and covariate data, and missing at random (MAR) if, conditional on the observed outcome and covariate data, missingness is independent of the unobserved data. Missing data that is neither MCAR nor MAR is termed missing not at random (MNAR). In the context of likelihood inference, which is used in linear mixed effects modeling, when the parameters describing the measurement process are independent of the parameters describing the missingness process, MCAR and MAR processes are ignorable whereas an MNAR missingness process is non-ignorable. Thus, as long as the observed outcome and covariate data included in a linear mixed effects model are sufficient to bring about a MAR mechanism for the missing data, the parameter estimates of the model will be unbiased. This is not the case for frequentist methods such as repeated-measures ANOVA, which require the missing data to be MCAR (36). In the past, simple methods for dealing with missing data, such as last observation carried forward (LOCF), single imputation, and complete case analysis have been popular. However, given the commercial software available today, there is little reason to use these simple, typically biased methods (36).

To examine the non-response process, one must first assume that the outcome vector, Y_i = $(Y_{i1}, \ldots, Y_{in})$, contains a sequence of responses designed to be measured at occasions $j = 1, \ldots, n$ for all subjects $i = 1, \ldots, N$. Next, one can define a dropout indicator D_i for the occasion at which dropout occurs and assert that $D_i = n + 1$ for a complete sequence. Y_i can be split into observed (Y_{oi}) and missing (Y_{mi}) components. Generally the aim is to examine the full data density $f(y_i, d_i | \theta, \psi)$, in which the parameter vectors θ and ψ describe respectively the measurement and missingness processes. To examine the full data, several different methods can be used. One method is selection modeling, which uses the following factorization of the full data density:

$$f(y_i, d_i | \theta, \psi) = f(y_i | \theta) f(d_i | y_i, \psi)$$

in which the first factor is the marginal density of the measurement process and the second factor is the density of the missingness process, conditional on the observed and unobserved outcomes. A second method to examine the full data is pattern-mixture modeling, which is based on the reversed factorization:

$$f(y_i, d_i | \theta, \psi) = f(y_i | d_i, \theta) f(d_i | \psi)$$

Pattern-mixture models can easily be seen to be a mixture of subpopulations each characterized by a distinct non-response pattern. Both of these methods assume that covariates included in the analysis are fully observed, often not the case for time-varying covariates in longitudinal studies (36). Using multiple imputation, however, both missing covariate and outcome data can be imputed consistent with an a priori hypothesis for the missing data process, often called an identifying restriction, for pattern-mixture modeling.

An important problem with pattern-mixture models is that they are always under-identified. There are two main strategies used for pattern-mixture modeling, and they handle the

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problem of under-identification quite differently (38). Little, Thijs, and others have advocated the use of identifying restrictions, in which data that are unavailable for a particular pattern are borrowed from a pattern or patterns in which such data are available (38, 39). Alternatively, model simplification can be used to identify parameters. With this technique, parameters are made to vary across patterns in a controlled parametric way by including pattern as a covariate in the pattern-mixture model. Though the second strategy is computationally simple, it requires the untestable assumption that it is appropriate to extrapolate time trends beyond the point of dropout. The first strategy, on the other hand, can accommodate a greater variety of hypotheses about the missing data mechanism through the use of multiple imputation (38).

Multiple imputation is a valuable tool for longitudinal biomedical research studies, especially in the area of sensitivity analysis. In multiple imputation, the imputation model can be easily changed to reflect hypothesized departures from the MAR assumption and the analytical model subsequently refitted to the imputed data (40). With a general (non-monotone) pattern of missingness, such as occurs in many clinical trials and observational epidemiologic studies, Bayesian methods based on Markov Chain Monte Carlo (MCMC) can be used to impute missing covariate and outcome data. This method assumes that the missing data, given the observed data, follows a multivariate normal distribution. The method constructs a Markov chain long enough for the distributions of the imputed variables to stabilize to a stationary distribution (40). Finally, one issue that applies to both pattern-mixture modeling strategies is that the models do not always yield marginal parameter estimates and standard errors. Hogan and Laird provide a method of averaging over the missing data patterns that involves using the delta method to approximate standard errors of marginal quantities of interest (41).

2.6 STATISTICAL ANALYSIS

Descriptive statistics were calculated to summarize study variables at baseline, 6 months, and 12 months and were presented as median/inter-quartile range (IQR) or mean (SD) for continuous variables and frequency and percentages for categorical variables. Whether the changes in body size, cardiometabolic factors, and PWV were statistically significantly different from zero at each follow-up visit was determined by testing the coefficient for time, as a nominal variable, in a linear mixed model for the measure of interest. Non-normally distributed variables were transformed as necessary before mixed modeling. Though the additional sodium reduction intervention did not statistically significantly affect any outcome in the trial except urinary sodium excretion, intervention arm was included as a covariate in every model for consistency with trial design. Interaction between intervention arm and time since baseline were tested and included only if statistically significant at $p<0.10$. The main analysis began with a separate mixed model for each PWV measure at all three time points, with age, sex, race (black/non-black), smoking status (current vs. past/never), and time (in years since baseline) evaluated for inclusion in the model and kept if statistically significant at $p<0.10$. Time since baseline and all possible second order interactions were evaluated and kept if statistically significant at $p<0.10$ in this and subsequent models. The statistical significance of each fixed effect parameter estimate was determined using the Satterthwaite-type approximate t-test. Random intercepts and slopes were included if found to be statistically significant at $p<0.10$ using likelihood ratio tests under the appropriate mixture of chi-squared distributions.

Next, baseline BMI and change in BMI (or weight or waist circumference) were added to the mixed model for each PWV measure to determine the cross-sectional and longitudinal

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relationships between measures of body size and arterial stiffness. The measure of change in body size showing the most statistically significant association with each PWV measure was kept in the model for additional analyses. Baseline mean arterial pressure (MAP) and change in MAP were then added to the model to determine whether the changes in PWV that occurred with weight loss could be explained by blood pressure change. Next, other factors that could explain the relationship between weight loss and arterial destiffening were added individually to the model for each PWV measure. Variables of interest included cardiovascular and metabolic risk factors known to be associated with subclinical cardiovascular disease that also showed changes that were associated with the amount of weight lost during the intervention. Linear mixed models for these factors of interest were used to determine whether their changes were associated with changes in BMI or waist circumference after adjustment for age, sex, race (black/non-black), and intervention arm; only those factors showing longitudinal associations statistically significant at p<0.10 with one or both measures of body size were evaluated in subsequent models. The separate within-subject and between-subject effects of each of these factors were evaluated by including the baseline level and change from baseline as distinct variables. The determination of the degree to which various within-subject changes explained the relationship between body size reduction and PWV reduction was accomplished by establishing which factors removed the statistical significance of the change in body size variable when included in each mixed model for PWV.

Because participants who were not as successful with weight loss during the intervention may have been more likely to drop-out, non-ignorable mechanisms for the missing data were considered by evaluating pattern-mixture models as a sensitivity analysis. Linear mixed effects pattern-mixture models were used to evaluate the influence of non-completion of the intervention

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on the analysis (42). This method required the stratification of study subjects by their missing data pattern, then the evaluation of the influence of each missing data pattern on the outcome. A dichotomous variable for completion versus non-completion was included as a covariate in the mixed models. This variable's interactions with within-subject factors in the final mixed models were then evaluated (pattern-mixture model 1). The parameter estimates of the pattern-mixture model were averaged over the missing data patterns to obtain marginal estimates that accounted for missing data patterns. Because this simple extrapolation method of pattern-mixture modeling may be inaccurate, identifying restrictions were also used to enable the estimation of effects that were under-identified for non-completers (38). Markov Chain Monte Carlo (MCMC) multiple imputation was used to impute missing data for time-varying covariates and outcomes using two different identifying restrictions for the imputation. First, the available case missing values (ACMV) restriction (38) was used (pattern-mixture model 2) and second, the assumption was made that the conditional multivariate distribution of missing data for all continuous time-varying variables, given the observed data, followed the corresponding distribution in the subgroup of subjects who either 1) had some missing follow-up data or 2) had no missing data but achieved less than the 6 month mean weight loss percentage in the total sample (pattern-mixture model 3). Finally, using these "complete" datasets derived from multiple imputation, the marginal parameter estimates from each pattern-mixture model were evaluated by averaging over the missing data patterns and using the delta method to approximate standard errors (41). In all sensitivity analyses, subjects with intermittent missing data (PWV data missing at 6 months only, n=6) were treated as if they had complete data. Values of $p < 0.05$ were considered statistically significant. All analyses were performed using SAS (Statistical Analysis Software release 9.2, Cary, NC, USA).

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3.0   RESULTS

The study population consisted of 344 participants in the SAVE clinical trial who had baseline cfPWV and/or baPWV data. The sample had a mean age of 37.9 years (SD 6.1) at baseline and consisted of 23% males and 16% African-Americans. Nine percent of the study population identified themselves as current smokers at baseline. Mean values of key study measures over the course of the intervention are shown in Table 1. After 6 months an average weight loss of 7.1% was achieved and there were statistically significant mean and median decreases in cfPWV of 58.1 cm/s (SD 233) and 47.5 cm/s (IQR -44.5, 148) respectively (p<0.0001). Carotid-femoral PWV was log transformed to normalize its skewed distribution for further analyses. At 6 months there was a mean reduction of 11.7 cm/s (SD 91.4) in baPWV (p=0.049), which showed a normal distribution. At the conclusion of the one year intervention, average weight loss was 6.4% and the decrease from baseline was statistically significant for cfPWV (mean 44.0 cm/s (SD 274), median 32.5 cm/s (IQR -84.5, 140.5), p=0.02) but not baPWV (mean 2.6 cm/s (SD 97.4)) (Figure 1). The only measured changes over time that differed at least marginally statistically significantly by intervention arm were 24-hour urinary sodium and serum aldosterone. Mean urinary sodium was 186.1 meq/day at baseline in the total sample and decreased by 48.9 meq/day (SD 80.4) at 6 months and 38.3 meq/day (SD 78.4) at 12 months in the Low Na/lifestyle arm but decreased by only 8.6 meq/day (SD 78.2) at 6 months and 21.1

meq/day (SD 85.7) at 12 months in the Control Na/lifestyle arm (p<0.001 and p=0.20 respectively for between-arm comparisons). Median serum aldosterone was 107.0 pg/mL at baseline in the total sample and increased by 15.0 pg/mL (IQR -28.0, 50.0) at 6 months and 5.2 pg/mL (IQR -30.7, 38.0) at 12 months in the Low Na/lifestyle arm but changed by only 1.0 pg/mL (IQR -32.0, 28.4) at 6 months and 2.1 pg/mL (IQR -37.0, 24.4) at 12 months in the Control Na/lifestyle arm (p=0.06 and p=0.37 respectively for between-arm comparisons).

Those participants with missing PWV data at 6 months were more likely than those with non-missing 6 month data to be male and in the low sodium intervention arm (p<0.05 for both). Study subjects missing PWV data at 12 months were more likely to be male, to have higher baseline BMI, and to have achieved lesser weight and BP reductions at 6 months than those with available 12 month data (p<0.05 for all). All evaluated associations between changes in body size and changes in cardiometabolic and vascular parameters were statistically significant with the exception of those involving aldosterone, ghrelin, and carotid intima-media thickness (Table 2).

In linear mixed models for log cfPWV, reductions in weight (p<0.0001), waist circumference (p=0.002), and BMI (p<0.0001) were all associated with decreases in log cfPWV during the lifestyle intervention. BMI was kept in subsequent models for log cfPWV because its longitudinal association with log cfPWV had the greatest statistical significance of the three body size measures. When baseline MAP (p<0.0001) and change in MAP (p=0.02) were added to the model including BMI, reduction in BMI (p=0.0003) remained a statistically significant determinant of decrease in log cfPWV during the intervention. When each factor hypothesized to partially explain the relationship between BMI reduction and cfPWV reduction was added to the model, only change in heart rate (p=0.08) and change in common carotid artery (CCA)

adventitial diameter (AD) (p=0.003) were individually associated at p<0.10 with change in log cfPWV. However, neither of these factors removed the statistical significance of the association between change in BMI and change in log cfPWV (Table 3).

In linear mixed models for baPWV, reductions in weight (p=0.002), waist circumference (p=0.0009), and BMI (p=0.005) were all associated with decreases in baPWV during the lifestyle intervention. Waist circumference was kept in subsequent models for baPWV because its longitudinal association with baPWV showed the greatest statistical significance of the three body size measures. When baseline MAP (p<0.0001) and change in MAP (p<0.0001) were added to the model including waist circumference, reduction in waist circumference (p=0.02) remained a statistically significant determinant of decrease in baPWV during the intervention. When other variables potentially explaining the relationship between waist circumference reduction and baPWV reduction were added to the model, only change in heart rate (p<0.0001) and change in CRP (p=0.005) were individually associated with change in baPWV. Both of these factors, separately or together, removed the statistical significance of the association between change in waist circumference and change in baPWV (Table 4). Change in BMI was similarly not statistically significant (p=0.99) in place of change in waist circumference in the fully-adjusted model for baPWV. No statistically significant interactions were detected in any model.

As a sensitivity analysis, linear mixed effects pattern-mixture models were used to examine the influence of several hypothesized non-ignorable missing data mechanisms on the results. In the first pattern-mixture model for log cfPWV, in which a dichotomous variable for completion versus non-completion of the study as well as its interactions with within-subject changes were added to the fully adjusted model, it appeared that participants who dropped out of the study after the six month visit showed a stronger positive association between change in BMI

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and change in log cfPWV than participants who completed the study. In this model, it also appeared that non-completers showed a negative association between change in MAP and change in log cfPWV whereas completers showed a positive association (Table 5). In the second pattern-mixture model for log cfPWV, in which an available case missing values identifying restriction was evaluated using multiple imputation, it appeared that participants who dropped out of the study after either the baseline or six month visit did not differ from completers with regard to their parameter estimates in the fully adjusted model for log cfPWV (Table 6). In the third pattern-mixture model, the conditional multivariate distribution of missing data for all continuous time-varying variables, given the observed data, was assumed to follow the corresponding distribution in the group of subjects who had some missing data or achieved less than the mean weight loss at six months. Under this pessimistic hypothesis for the missing data mechanism, participants who dropped out of the study did not differ statistically significantly from completers in terms of their parameter estimates in the fully adjusted model except that subjects who dropped out after the six month time point showed a negative association between change in MAP and change in log cfPWV whereas completers showed a positive association (Table 6). Marginal parameter estimates from each of the pattern-mixture models for log cfPWV differed little from those in the original fully-adjusted model for log cfPWV with the exception of those for change in heart rate and change in MAP, which remained marginally significant only in the second pattern-mixture model (Table 7).

In the first pattern-mixture model for baPWV, when a dichotomous variable for completion versus non-completion of the study as well as its interactions with within-subject changes were added to the final model for baPWV, there were no significant differences between completers and non-completers in terms of their parameter estimates in the fully adjusted model

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(Table 8). In the second pattern-mixture model for baPWV, which invoked an available case missing values identifying restriction, it appeared that participants who dropped out of the study after either the baseline or six month visit did not differ from completers with regard to their parameter estimates in the fully adjusted model for baPWV (Table 9). The third pattern-mixture model, in which missing data was imputed under the assumption that it came from subjects who achieved less weight loss success than completers, appeared to show that participants who dropped out of the study did not differ from completers with regard to their parameter estimates in the fully adjusted model (Table 9). Marginal parameter estimates from each of the pattern-mixture models for baPWV differed little from those in the original fully-adjusted model for baPWV (Table 10).

4.0   DISCUSSION

This study showed that weight loss is statistically significantly associated with aortic PWV reduction in normotensive overweight and obese young adults, and that this association is independent of concurrent improvements in obesity-associated cardiometabolic and hemodynamic factors. In addition, diameter reduction of the elastic common carotid artery is strongly associated with aortic stiffness reduction; this longitudinal association is statistically stronger than those between changes in aortic stiffness and either blood pressure or heart rate. In contrast, the associations between weight loss or waist circumference reduction and reduction in baPWV, a mixed measure of aortic and peripheral arterial stiffness, appear to be explained not by concurrent blood pressure changes but by changes in heart rate and inflammation.

Obesity has been found to impair arterial structure and function in numerous studies (1, 4, 43). Weight loss, through either lifestyle modification or bariatric surgery, can reverse these vascular alterations (6-9, 14-20), but the precise mechanisms by which this occurs are poorly understood. Only a few studies have evaluated mechanisms other than blood pressure reduction by which weight loss may reduce arterial stiffness, and these studies have not focused on young adults (15, 16, 18). In a sample of middle-aged obese adults who achieved dietary or surgical weight loss, Rider et al. found that only BMI reduction, not concurrent hormonal or metabolic factor changes, independently correlated with aortic PWV reduction (15). In a study of middle-

aged and older overweight and obese adults, only BMI reduction, total weight loss, or total fat loss were independently associated with cfPWV reduction when other adiposity-related measures were included in a multivariable model (16). Similarly, Ikonomidis et al. found that BMI reduction was the strongest independent determinant of reduction in thoracic aortic stiffness index in severely obese young and middle-aged adults who underwent bariatric surgery; concurrent changes in blood pressure, lipids, glucose, or indices reflecting elevated circulating blood volume were not as strong determinants (18). However this study, similarly to our study, found a significant association between BMI reduction and reduction in diastolic aortic diameter, suggesting that a reduction in circulating blood volume with weight loss may have some effect on arterial destiffening (18). Evidence from these studies and the present investigation point to weight loss having an impact on aortic destiffening that is independent of concurrent changes in established cardiometabolic and hemodynamic risk factors.

In addition to the cardiometabolic and hemodynamic factors and vascular geometry measures reported in this study, micro-structural properties of the aortic wall, for example the extent of cross-linking of extracellular matrix proteins or the balance between matrix protein synthesis and degradation, may be altered by weight loss and aerobic exercise and in turn influence arterial stiffness (14, 44). However, an understanding of whether such relationships exist will require additional animal studies. Furthermore, neurohumoral modulation of vascular smooth muscle tone plays a role in short term changes in arterial stiffness, and improvements in sympathovagal balance that occur with weight loss may influence arterial destiffening (45). Improvements in other obesity-associated characteristics, such as nitric oxide bioavailability and local and/or circulating angiotensin II levels, may also contribute to improvements in aortic stiffness with weight loss (45). Though changes in serum aldosterone were not found to be

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associated with PWV changes in this study, changes in serum aldosterone were mainly driven by changes in dietary sodium intake and moreover, some of the effects of angiotensin II on the arterial wall are independent of aldosterone (45). Finally, the finding that weight loss drove aortic destiffening independently of concurrent risk factor changes in this and other studies might be partially explained by the heterogeneous effects of obesity and obesity-related factors on different segments of the aorta (15, 46). Some studies have shown that the effect of weight loss on aortic stiffness in obese adults is greater in the abdominal aorta than in more proximal segments (15, 46). Thus, although changes in cardiometabolic factors and carotid artery geometry did not explain the association between weight loss and aortic stiffness reduction in this study, it is possible that these factors might better explain the association in the abdominal aorta specifically.

In addition to the possible segment-specific effects of obesity on aortic stiffness, there appear to be differences in the effects of obesity on peripheral as compared to central arterial stiffness. In the present study, weight loss produced a greater and more sustained reduction in cfPWV than baPWV, and the effect of weight loss was independent of concurrent risk factor changes for cfPWV only. This finding is similar to that of another study in which a small group of healthy middle-aged males participated in an aerobic exercise intervention that promoted weight loss; in that study cfPWV decreased statistically significantly (mean = 58 cm/s) whereas the decrease in baPWV was not significant (47). In contrast, a longitudinal observational study of healthy middle-aged Japanese men found that weight gain over a three year period led to a significant increase in baPWV in those who were overweight at the start of the study (48). Cross-sectionally, greater BMI or total body fat has been found to be associated with greater arterial stiffness of both muscular and elastic arteries, and the association for elastic arteries may be

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strongest in young adults (1, 49). However, the direction of the association between excess weight and arterial stiffness has been found to be negative for the muscular brachial artery in at least one study (49). Our findings, together with these studies, seem to suggest that obesity plays a role in arterial stiffening throughout the arterial tree, but that in young overweight and obese adults, weight loss may be particularly beneficial for reducing central arterial stiffness. This is not unexpected, given that cfPWV is considered by many to be the gold-standard measure of arterial stiffness and has been repeatedly associated with incident vascular events, cardiovascular mortality, and all-cause mortality in the general population (12).

Brachial-ankle PWV is highly correlated with cfPWV and exhibits similar associations with cardiovascular risk factors in some studies (47, 50, 51). Brachial-ankle PWV has also been found to predict cardiovascular and total mortality in community-dwelling older Japanese adults (13) and total mortality in a general population of middle-aged and older Japanese adults (52), though larger studies with longer follow-up times are needed to substantiate these findings. There have been few longitudinal studies using baPWV, and to our knowledge none of these studies have attempted to determine the mechanisms by which weight change may influence baPWV (47, 48). However, similar to the present findings for baPWV, an observational cohort study that followed over 800 older men found that, besides blood pressure, cumulative exposure to CRP and long term cyclic stress, defined as the product of heart rate and pulse pressure, are important predictors of cfPWV at 20 years of follow-up (53). It has been found that functional polymorphisms in the gene encoding CRP are not associated with aortic PWV in the general population, which seems to indicate that CRP is not a causal factor in arterial stiffening (54). However, it remains possible that the chronic inflammation present in obese individuals plays a causal role in arterial stiffening, and it is possible that this role may be more evident in peripheral

arteries given the known effects of elevated circulating CRP levels on vascular endothelial and smooth muscle cells (55). One recent observational cohort study found that higher baseline heart rate and an increase in heart rate were associated with a greater increase in baPWV over 5-6 years (56). The relationship between reduced heart rate and reduced PWV may reflect concurrent decreases in sympathetic activation, improvements in physical fitness, and reduced cyclic stretching of the arteries, or it may simply reflect the frequency dependence of the viscoelasticity of the arterial walls (57). In light of the associations between reductions in inflammation or heart rate and reduced baPWV, additional studies should investigate whether these factors play causal roles in arterial stiffening in overweight and obese individuals.

In sensitivity analysis using pattern-mixture modeling, there were few differences from the original results. Using the extrapolation method of pattern-mixture modeling, those participants who dropped out after the six month visit showed a stronger association between weight loss and cfPWV reduction, which is not unexpected given that most of the weight loss during the intervention occurred during the first six months. In this pattern-mixture model and the third pattern-mixture model, in which it was supposed that dropouts achieved less weight loss than completers, individuals who dropped out after the six month visit showed an unexpectedly negative association between MAP change and cfPWV change. This finding appeared to be partially due to the influence of measurement error on this small subgroup, but it also suggests that weight loss and other concurrent changes independently influenced arterial destiffening more strongly than blood pressure changes in this group. The differences in parameter estimates in the pattern-mixture models as compared to those from the original mixed model for cfPWV indicated that, under each of the assumptions for non-ignorable missing data, the longitudinal associations between cfPWV and MAP or heart rate were no longer statistically significant

whereas the longitudinal associations between cfPWV and either BMI or common carotid artery adventitial diameter remained significant. These results indicated that weight loss and reduced circulating blood volume may be the strongest drivers of aortic destiffening during lifestyle modification, regardless of the degree of weight loss. The negligible differences in parameter estimates from all pattern-mixture models for baPWV as compared to those from the original mixed model indicate that, regardless of the degree of success with weight loss, changes in blood pressure, heart rate, and inflammation are strongly associated with changes in baPWV during lifestyle modification.

This study had several strengths. First, many obesity-associated cardiometabolic risk factors and vascular parameters were measured, such that numerous mechanisms for the reduction in arterial stiffness with weight loss could be explored. Still, some additional measurements may have proven useful in this study. For example, we did not measure aortic diameter, which has been found to increase markedly with increased blood flow (58). It could be that the association between weight loss and aortic destiffening is better explained by aortic diameter reduction than common carotid artery diameter reduction. A second strength of this study was the stability of the results under various hypothesized mechanisms for the missing data. Though the amount of missing data was substantial (17% and 26% dropout at 6 months and 12 months respectively), the sensitivity analyses indicated that this missing data likely did not influence the validity of the results. Another strength of this study was that all participants were normotensive and not on any antihypertensive or vasoactive medications, thus ensuring that these findings were not confounded by treatment effects. One limitation of this study, in addition to the missing data, was the relatively small number of males and non-white participants, which limited the power available to detect subgroup effects by sex or race, though no significant

interactions were detected with these factors. Another limitation was that no adjustment was made for multiple comparisons. However, this study included only two outcomes and was mainly an exploratory analysis to find any potential mechanisms by which weight loss might reduce arterial stiffness.

5.0   CONCLUSIONS

In conclusion, we have shown that weight loss reduces aortic stiffness independently of concurrent changes in cardiometabolic factors and common carotid artery geometry in young normotensive overweight and obese adults. However, reduced common carotid artery diameter, an indicator of reduced circulating blood volume, is a strong independent determinant of decreased aortic stiffness. In addition, reductions in heart rate and inflammation are strongly associated with reduced baPWV, and these factors may drive the effect of weight loss on baPWV. Though PWV reductions were not as large in this study as some others (15, 16), likely due to the young age and overall good health of the participants, the predictive power of both cfPWV and baPWV for cardiovascular and overall mortality in the general population suggests that these small reductions made early in adulthood may be clinically meaningful in the long-term.

Table 1. Body size, cardiometabolic factors, and common carotid artery geometry across the intervention

Characteristic	Baseline (N=344)	6 Months (N=284)	12 Months (N=255)
Weight (kg)	92.2 (14.9)	85.7 (15.0)*	85.5 (15.1)*
BMI (kg/m^2)	32.9 (3.8)	30.4 (4.2)*	30.4 (4.5)*
Waist Circumference (cm)	100.4 (11.2)	95.4 (11.5)*	95.4 (12.2)*
SBP (mmHg)	113.5 (10.5)	110.2 (9.6)*	110.1 (9.9)*
DBP (mmHg)	72.9 (8.7)	71.1 (8.4)*	72.0 (8.2)
Glucose (mg/dL)	97.7 (8.0)	98.0 (8.5)	98.0 (8.3)
Insulin (µU/mL)	12.5 (9.6, 17.4)	11.6 (9.0, 15.6)*	11.7 (9.4, 15.2)*
LDL-C (mg/dL)	123.3 (33.2)	121.6 (30.4)	124.4 (30.9)
HDL-C (mg/dL)	52.7 (13.5)	53.4 (13.1)	55.9 (14.2)*
Triglycerides (mg/dL)	115.5 (78.0, 170.0)	93.0 (68.0, 138.0)*	88.0 (69.0, 137.0)*
CRP (mg/dL)	2.6 (1.3, 5.8)	2.0 (1.0, 4.4)*	2.0 (0.9, 4.1)*
Leptin (ng/mL)	25.9 (13.3)	18.5 (11.8)*	20.7 (13.3)*
Adiponectin (µg/mL)	11.8 (5.9)	11.9 (5.3)	12.0 (5.4)
Ghrelin (pg/mL)	672 (547, 874)	746 (608, 1011)	832 (639, 1102)
Heart Rate (bpm)	64.1 (9.1)	62.3 (8.3)*	63.9 (9.0)
Mean IMT (mm)	0.60 (0.08)	0.61 (0.08)	0.61 (0.09)*
Mean AD (mm)	6.91 (0.53)	6.86 (0.52)*	6.83 (0.56)*

Mean (SD) or median (IQR) are shown. *P<0.05 versus baseline in a linear mixed model with time since baseline as a nominal variable and with adjustment for intervention arm. Insulin, triglycerides, CRP, and ghrelin were log transformed. IMT = common carotid artery intima-media thickness. AD = common carotid artery adventitial diameter.

Table 2. Longitudinal associations between changes in body size and changes in cardiometabolic factors and common carotid artery geometry

Dependent Variable	Change in BMI			Change in Waist Circumference		
	Estimate	Standard Error	P	Estimate	Standard Error	P
MAP	0.66	0.12	<0.0001	0.21	0.04	<0.0001
LDL-C	2.12	0.43	<0.0001	0.74	0.14	<0.0001
HDL-C	-0.39	0.15	0.01	-0.12	0.05	0.02
Triglycerides	0.05	0.007	<0.0001	0.02	0.002	<0.0001
Insulin	0.06	0.006	<0.0001	0.02	0.002	<0.0001
Aldosterone	-0.006	0.009	0.53	-0.001	0.003	0.68
Adiponectin	-0.41	0.06	<0.0001	-0.13	0.02	<0.0001
Leptin	2.45	0.14	<0.0001	0.71	0.05	<0.0001
Ghrelin	-0.009	0.007	0.18	-0.004	0.002	0.09
CRP	0.11	0.01	<0.0001	0.03	0.005	<0.0001
24-hour Urinary Sodium*	5.84	1.34	<0.0001	1.37	0.46	0.003
IMT	0.001	0.0009	0.20	5.9×10^{-6}	0.0003	0.98
AD	0.03	0.005	<0.0001	0.006	0.002	0.0001

Each dependent variable is the outcome in a linear mixed effects model. Random intercept and years since baseline effects were included in each model. Intervention arm, baseline age, race (black/non-black), sex, and baseline BMI or waist circumference were included as fixed effects. Triglycerides, insulin, aldosterone, CRP, and ghrelin were log transformed. MAP = mean arterial pressure. IMT = common carotid artery intima-media thickness. AD = common carotid artery adventitial diameter. Number of subjects = 344. Number of observations = 882. *Number of subjects = 324. Number of observations = 674.

Table 3. Fully- adjusted multivariable linear mixed effects model for log cfPWV

Variable	Estimate	Standard Error	P
Between-subject factors			
Age	0.009	0.001	**<0.0001**
Race (black vs. non-black)	0.03	0.02	0.17
Sex (male vs. female)	-0.002	0.02	0.95
Baseline MAP	0.004	0.001	**<0.0001**
Baseline Heart Rate	0.005	0.001	**<0.0001**
Baseline BMI	0.01	0.002	**<0.0001**
Baseline AD	0.03	0.02	0.18
Within-subject factors			
Change in MAP	0.002	0.001	0.07
Change in Heart Rate	0.002	0.001	0.08
Change in BMI	0.01	0.004	**0.01**
Change in AD	0.11	0.03	**0.001**
Years	-0.001	0.02	0.93

Random intercept and years since baseline effects were included in this model. Intervention arm was included as a fixed effect. Between-subjects factors were determined at baseline. Within-subject factors were evaluated as changes from baseline at the time of the subject's follow-up measurements. MAP = mean arterial pressure. AD = common carotid artery adventitial diameter. Number of subjects = 326. Number of observations = 804.

Table 4. Fully-adjusted multivariable linear mixed effects model for baPWV

Variable	Estimate	Standard Error	P
Between-subject factors			
Age	7.32	0.95	**<0.0001**
Race (black vs. non-black)	59.87	15.96	**0.0002**
Sex (male vs. female)	55.24	17.50	**0.002**
Baseline MAP	3.82	0.72	**<0.0001**
Baseline Heart Rate	3.73	0.66	**<0.0001**
Baseline Waist Circumference	-0.22	0.68	0.74
Baseline CRP	18.87	6.13	**0.002**
Within-subject factors			
Change in MAP	2.59	0.55	**<0.0001**
Change in Heart Rate	2.42	0.50	**<0.0001**
Change in Waist Circumference	0.38	0.60	0.53
Change in CRP	10.43	4.68	**0.03**
Years	6.28	5.47	0.25

A random intercept was included in this model. Intervention arm was included as a fixed effect. Between-subjects factors were determined at baseline. Within-subject factors were evaluated as changes from baseline at the time of the subject's follow-up measurements. MAP = mean arterial pressure. CRP = C-reactive protein. CRP was log transformed. Number of subjects = 335. Number of observations = 832.
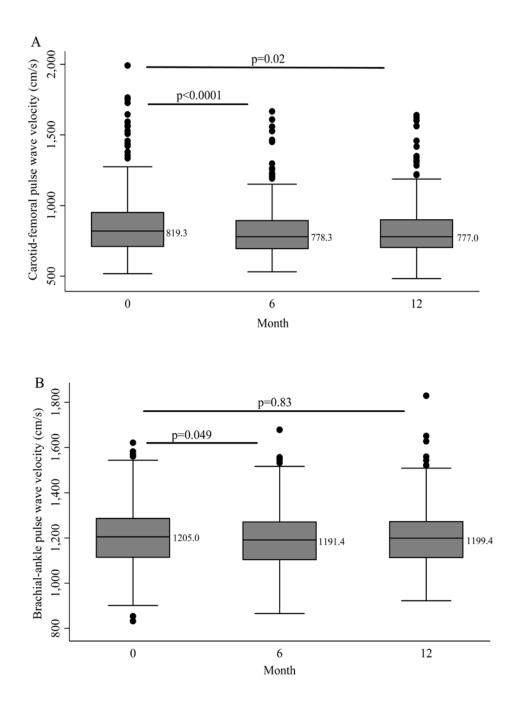
Figure 1. Boxplots of Pulse Wave Velocity Across the Intervention for (A) cfPWV and (B) baPWV

P values are for follow-up versus baseline measures in linear mixed models with adjustment for age, sex, race (black/non-black), and intervention arm. Median values are shown at each time point.

Table 5. Multivariable pattern-mixture linear mixed effects model for log cfPWV using simple extrapolation

Variable	Pattern Mixture Model 1		
	Estimate	Standard Error	P
Between-subject factors			
Age	0.01	0.001	<0.0001
Race (black vs. non-black)	0.03	0.02	0.14
Sex (male vs. female)	0.001	0.02	0.96
Baseline MAP	0.004	0.001	<0.0001
Baseline Heart Rate	0.005	0.001	<0.0001
Baseline BMI	0.01	0.002	<0.0001
Baseline AD	0.02	0.02	0.29
Dropout (Non-completers vs. Completers)	-0.006	0.02	0.80
Within-subject factors			
Change in MAP	0.003	0.001	0.01
Change in MAP x Dropout (reference: Completers)	-0.02	0.005	0.0005
Change in Heart Rate	0.003	0.001	0.04
Change in Heart Rate x Dropout (reference: Completers)	-0.005	0.004	0.29
Change in BMI	0.007	0.004	0.09
Change in BMI x Dropout (reference: Completers)	0.04	0.02	0.01
Change in AD	0.11	0.03	0.001
Change in AD x Dropout (reference: Completers)	0.07	0.13	0.61
Years	-0.006	0.02	0.71
Years x Dropout (reference: Completers)	0.12	0.08	0.12

Random intercept and years since baseline effects were included in this model. Intervention arm was included as a fixed effect. Between-subjects factors were determined at baseline. Within-subject factors were evaluated as changes from baseline at the time of the subject's follow-up measurements. AD = common carotid artery adventitial diameter. MAP = mean arterial pressure. Number of subjects = 326. Number of observations = 804.

Table 6. Multivariable pattern-mixture linear mixed effects models for log cfPWV using multiply imputed datasets

Variable	Pattern-Mixture Model 2			Pattern-Mixture Model 3		
	Estimate	Standard Error	P	Estimate	Standard Error	P
Between-subject factors						
Age	0.009	0.001	<0.0001	0.01	0.001	<0.0001
Race (black vs. non-black)	0.04	0.03	0.15	0.03	0.02	0.14
Sex (male vs. female)	-0.01	0.02	0.69	-0.01	0.02	0.60
Baseline MAP	0.004	0.001	0.0002	0.004	0.001	<0.0001
Baseline Heart Rate	0.005	0.001	<0.0001	0.005	0.001	<0.0001
Baseline BMI	0.01	0.002	<0.0001	0.01	0.003	<0.0001
Baseline AD	0.03	0.02	0.14	0.03	0.02	0.14
Dropout (reference: Completers) Baseline only	-0.007	0.03	0.82	-0.007	0.03	0.81
Baseline and 6 months only	-0.005	0.03	0.88	0.05	0.05	0.84
Within-subject factors						
Change in MAP	0.003	0.001	0.01	0.003	0.001	0.01
Change in MAP x Dropout (reference: Completers)						
Baseline only	-0.0009	0.004	0.83	-0.003	0.003	0.32
Baseline and 6 months only	-0.008	0.004	0.07	-0.01	0.004	0.01
Change in Heart Rate	0.002	0.001	0.06	0.002	0.001	0.048
Change in Heart Rate x Dropout (reference: Completers) Baseline only	-0.0009	0.004	0.81	-0.001	0.004	0.80
Baseline and 6 months only	-0.002	0.004	0.64	-0.004	0.004	0.28
Change in BMI	0.007	0.004	0.09	0.008	0.004	0.07
Change in BMI x Dropout (reference: Completers)						
Baseline only	0.006	0.01	0.71	0.003	0.01	0.86

Table 6 continued

Baseline and 6 months only	0.01	0.01	0.27	0.02	0.01	0.13
Change in AD	0.12	0.04	0.0007	0.12	0.03	0.001
Change in AD x Dropout (reference: Completers)						
Baseline only	-0.01	0.10	0.93	-0.01	0.09	0.87
Baseline and 6 months only	0.005	0.12	0.97	-0.002	0.12	0.99
Years	-0.005	0.02	0.75	-0.006	0.02	0.73
Years x Dropout (reference: Completers)						
Baseline only	0.01	0.05	0.84	0.008	0.04	0.86
Baseline and 6 months only	-0.005	0.03	0.61	0.05	0.05	0.35

Random intercept and years since baseline effects were included in this model. Intervention arm was included as a fixed effect. Between-subjects factors were determined at baseline. Within-subject factors were evaluated as changes from baseline at the time of the subject's follow-up measurements. AD = common carotid artery adventitial diameter. MAP = mean arterial pressure. Number of subjects = 326. Number of observations = 978.

Table 7. Marginal parameter estimates from multivariable pattern-mixture linear mixed effects models for log cfPWV

Variable	Pattern-Mixture Model 1			Pattern-Mixture Model 2			Pattern-Mixture Model 3		
	Estimate	Standard Error	P	Estimate	Standard Error	P	Estimate	Standard Error	P
Between-subject factors									
Age	0.01	0.001	<0.0001	0.009	0.001	<0.0001	0.01	0.001	<0.0001
Race (black vs. non-black)	0.03	0.02	0.14	0.04	0.03	0.15	0.03	0.02	0.14
Sex (male vs. female)	0.001	0.02	0.96	-0.01	0.02	0.69	-0.01	0.02	0.60
Baseline MAP	0.004	0.001	<0.0001	0.004	0.001	0.0002	0.004	0.001	<0.0001
Baseline Heart Rate	0.005	0.001	<0.0001	0.005	0.001	<0.0001	0.005	0.001	<0.0001
Baseline BMI	0.01	0.002	<0.0001	0.01	0.002	<0.0001	0.01	0.003	<0.0001
Baseline AD	0.02	0.02	0.29	0.03	0.02	0.14	0.03	0.02	0.14
Within-subject factors									
Change in MAP	-0.001	0.002	0.44	0.002	0.001	0.12	0.002	0.001	0.22
Change in Heart Rate	0.001	0.002	0.51	0.002	0.001	0.10	0.002	0.001	0.20
Change in BMI	0.02	0.006	0.0007	0.01	0.004	0.02	0.01	0.004	0.01
Change in AD	0.13	0.05	0.003	0.12	0.04	0.0006	0.11	0.03	0.0004
Years	0.03	0.03	0.28	0.0001	0.02	0.99	0.002	0.02	0.91

Random intercept and years since baseline effects were included in this model. Intervention arm was included as a fixed effect. Between-subjects factors were determined at baseline. Within-subject factors were evaluated as changes from baseline at the time of the subject's follow-up measurements. AD = common carotid artery adventitial diameter. MAP = mean arterial pressure. Number of subjects for model 1 = 326. Number of subjects for models 2 and 3 = 326. Number of observations for model 1 = 804. Number of observations for models 2 and 3 = 978.

Table 8. Multivariable pattern-mixture linear mixed effects model for baPWV using simple extrapolation

Variable	Estimate	Standard Error	P
		Pattern-Mixture Model 1	
Between-subject factors			
Age	7.34	0.95	<0.0001
Race (black vs. non-black)	60.49	15.96	0.0002
Sex (male vs. female)	55.62	17.72	0.002
Baseline MAP	3.79	0.72	<0.0001
Baseline Heart Rate	3.75	0.66	<0.0001
Baseline Waist Circumference	-0.26	0.68	0.70
Baseline CRP	19.30	6.13	0.002
Dropout (Non-completers vs. Completers)	0.42	13.95	0.98
Within-subject factors			
Change in MAP	2.79	0.57	<0.0001
Change in MAP x Dropout (reference: Completers)	-2.75	2.34	0.24
Change in Heart Rate	2.40	0.51	<0.0001
Change in Heart Rate x Dropout (reference: Completers)	0.54	2.03	0.79
Change in Waist Circumference	0.54	0.62	0.39
Change in Waist Circumference x Dropout (reference: Completers)	-1.84	2.84	0.52
Change in CRP	9.74	4.87	0.046
Change in CRP x Dropout (reference: Completers)	12.69	18.34	0.49
Years	6.73	5.68	0.24
Years x Dropout (reference: Completers)	4.85	30.31	0.87

A random intercept was included in this model. Intervention arm was included as a fixed effect. Between-subjects factors were determined at baseline. Within-subject factors were evaluated as changes from baseline at the time of the subject's follow-up measurements. CRP was log transformed. MAP = mean arterial pressure. CRP = C-reactive protein. Number of subjects = 335. Number of observations = 832.

Table 9. Multivariable pattern-mixture linear mixed effects models for baPWV using multiply imputed datasets

Variable	Pattern-Mixture Model 2			Pattern-Mixture Model 3		
	Estimate	Standard Error	P	Estimate	Standard Error	P
Between-subject factors						
Age	7.23	0.96	<0.0001	7.17	0.95	<0.0001
Race (black vs. non-black)	57.05	15.80	0.0003	58.20	15.84	0.0002
Sex (male vs. female)	60.88	17.65	0.0006	58.55	17.78	0.001
Baseline MAP	3.94	0.71	<0.0001	3.82	0.72	<0.0001
Baseline Heart Rate	3.72	0.65	<0.0001	3.77	0.65	<0.001
Baseline Waist Circumference	-0.45	0.69	0.51	-0.31	0.69	0.65
Baseline CRP	19.48	6.07	0.001	19.00	6.18	0.002
Dropout (reference: Completers) Baseline only	-4.91	16.22	0.76	-6.24	16.66	0.71
Baseline and 6 months only	6.80	18.96	0.72	8.08	19.39	0.68
Within-subject factors						
Change in MAP	2.60	0.59	<0.0001	2.68	0.59	<0.0001
Change in MAP x Dropout (reference: Completers) Baseline only	0.02	1.78	0.99	-1.8	2.06	0.39
Baseline and 6 months only	-1.07	2.01	0.60	-2.15	2.23	0.34
Change in Heart Rate	2.40	0.53	<0.0001	2.48	0.53	<0.0001
Change in Heart Rate x Dropout (reference: Completers) Baseline only	0.07	1.62	0.96	-0.11	2.03	0.96
Baseline and 6 months only	0.31	1.76	0.86	0.23	1.86	0.90
Change in Waist Circumference	0.40	0.64	0.53	0.42	0.64	0.51
Change in Waist Circumference x Dropout (reference: Completers)	-0.52	2.00	0.80	-1.12	2.29	0.63

Table 9 continued

Baseline only						
Baseline and 6 months only	-1.35	2.34	0.56	0.62	2.35	0.79
Change in CRP	11.40	5.04	0.02	9.75	5.06	0.05
Change in CRP x Dropout (reference: Completers)						
Baseline only	4.50	13.42	0.74	3.67	16.02	0.82
Baseline and 6 months only	6.19	16.02	0.70	6.15	15.65	0.70
Years	4.78	5.68	0.40	5.15	5.76	0.37
Years x Dropout (reference: Completers)						
Baseline only	4.74	16.43	0.77	8.53	15.63	0.59
Baseline and 6 months only	2.12	21.77	0.92	6.24	24.00	0.80

A random intercept was included in this model. Intervention arm was included as a fixed effect. Between-subjects factors were determined at baseline. Within-subject factors were evaluated as changes from baseline at the time of the subject's follow-up measurements. CRP was log transformed. MAP = mean arterial pressure. CRP = C-reactive protein. Number of subjects = 339. Number of observations = 1017.

Table 10. Marginal parameter estimates from multivariable pattern-mixture linear mixed effects models for baPWV

Variable	Pattern-Mixture Model 1			Pattern-Mixture Model 2			Pattern-Mixture Model 3		
	Estimate	Standard Error	P	Estimate	Standard Error	P	Estimate	Standard Error	P
Between-subject factors									
Age	7.34	0.95	<0.0001	7.23	0.96	<0.0001	7.17	0.95	<0.0001
Race (black vs. non-black)	60.49	15.96	0.0002	57.05	15.80	0.0003	58.20	15.84	0.0002
Sex (male vs. female)	55.62	17.72	0.002	60.88	17.65	0.0006	58.55	17.78	0.001
Baseline MAP	3.79	0.72	<0.0001	3.94	0.71	<0.0001	3.82	0.72	<0.0001
Baseline Heart Rate	3.75	0.66	<0.0001	3.72	0.65	<0.0001	3.77	0.65	<0.001
Baseline Waist Circumference	-0.26	0.68	0.70	-0.45	0.69	0.51	-0.31	0.69	0.65
Baseline CRP	19.30	6.13	0.002	19.48	6.07	0.001	19.00	6.18	0.002
Within-subject factors									
Change in MAP	1.99	0.78	0.01	2.48	0.55	<0.0001	2.11	0.67	0.002
Change in Heart Rate	2.56	0.68	0.0002	2.45	0.51	<0.0001	2.48	0.60	<0.0001
Change in Waist Circumference	0.00	0.93	1.00	0.16	0.63	0.80	0.29	0.82	0.72
Change in CRP	13.45	6.25	0.03	12.91	4.66	0.006	11.11	5.27	0.04
Years	8.14	9.58	0.40	5.86	5.56	0.29	7.38	5.70	0.20

A random intercept was included in this model. Intervention arm was included as a fixed effect. Between-subjects factors were determined at baseline. Within-subject factors were evaluated as changes from baseline at the time of the subject's follow-up measurement. CRP was log transformed. MAP = mean arterial pressure. CRP = C-reactive protein. Number of subjects for model 1 = 335. Number of subjects for models 2 and 3 = 339. Number of observations for model 1 = 832. Number of observations for models 2 and 3 = 1017.

APPENDIX B: SAS CODE

SAS v9.2 code used for fully-adjusted linear mixed models, pattern-mixture models, and multiple imputation:

I. Final linear mixed models for log cfPWV and baPWV

```
PROC mixed data= save_longform_2152 covtest method=reml;
class ID randomization_group;
model logcfpwvcol =age racedich male avgmapbl mapdiff bmiBL bmidiff prbl prdiff adavgbl adavgdiff
years / solution ddfm=satterthwaite;
random intercept  years/type=un subject=ID;
run;

PROC mixed data= save_longform_2152 covtest method=reml;
class ID randomization_group;
model bapwv =age randomization_group racedich male avgmapbl mapdiff  waistBL waistdiff  prbl prdiff
logcrpbl logcrpdiff years / solution ddfm=satterthwaite;
random intercept /type=un subject=ID;
run;
```

II. Pattern-mixture model 1 (cfPWV)

```
PROC mixed data= save_longform_2152 covtest method=reml;
class ID randomization_group;
model logcfpwvcol =age racedich male avgmapbl mapdiff mapdiff*cfPWVcoldropout
bmiBL bmidiff bmidiff*cfPWVcoldropout prbl prdiff prdiff*cfPWVcoldropout adavgbl adavgdiff
adavgdiff*cfPWVcoldropout years cfPWVcoldropout years*cfPWVcoldropout randomization_group /
solution ddfm=satterthwaite;
random intercept  years /type=un subject=ID;
ESTIMATE 'avg int' INTERCEPT 1 racedich 0 male 0 avgmapbl 0 mapdiff 0 mapdiff*cfPWVcoldropout
0 bmiBL 0 bmidiff 0
```

bmidiff*cfPWVcoldropout 0 prbl 0 prdiff 0 prdiff*cfPWVcoldropout 0 adavgbl 0 adavgdiff 0
adavgdiff*cfPWVcoldropout 0
years 0 cfPWVcoldropout 0.307 years*cfPWVcoldropout 0;
ESTIMATE 'avg years' INTERCEPT 0 racedich 0 male 0 avgmapbl 0 mapdiff 0
mapdiff*cfPWVcoldropout 0 bmiBL 0 bmidiff 0
bmidiff*cfPWVcoldropout 0 prbl 0 prdiff 0 prdiff*cfPWVcoldropout 0 adavgbl 0 adavgdiff 0
adavgdiff*cfPWVcoldropout 0
years 1 cfPWVcoldropout 0 years*cfPWVcoldropout 0.307;
ESTIMATE 'avg bmidiff' INTERCEPT 0 racedich 0 male 0 avgmapbl 0 mapdiff 0
mapdiff*cfPWVcoldropout 0 bmiBL 0 bmidiff 1
bmidiff*cfPWVcoldropout 0.307 prbl 0 prdiff 0 prdiff*cfPWVcoldropout 0 adavgbl 0 adavgdiff 0
adavgdiff*cfPWVcoldropout 0
years 0 cfPWVcoldropout 0 years*cfPWVcoldropout 0;
ESTIMATE 'avg mapdiff' INTERCEPT 0 racedich 0 male 0 avgmapbl 0 mapdiff 1
mapdiff*cfPWVcoldropout 0.307 bmiBL 0 bmidiff 0
bmidiff*cfPWVcoldropout 0 prbl 0 prdiff 0 prdiff*cfPWVcoldropout 0 adavgbl 0 adavgdiff 0
adavgdiff*cfPWVcoldropout 0
years 0 cfPWVcoldropout 0 years*cfPWVcoldropout 0;
ESTIMATE 'avg prdiff' INTERCEPT 0 racedich 0 male 0 avgmapbl 0 mapdiff 0
mapdiff*cfPWVcoldropout 0 bmiBL 0 bmidiff 0
bmidiff*cfPWVcoldropout 0 prbl 0 prdiff 1 prdiff*cfPWVcoldropout 0.307 adavgbl 0 adavgdiff 0
adavgdiff*cfPWVcoldropout 0
years 0 cfPWVcoldropout 0 years*cfPWVcoldropout 0;
ESTIMATE 'avg adavgdiff' INTERCEPT 0 racedich 0 male 0 avgmapbl 0 mapdiff 0
mapdiff*cfPWVcoldropout 0 bmiBL 0 bmidiff 0
bmidiff*cfPWVcoldropout 0 prbl 0 prdiff 0 prdiff*cfPWVcoldropout 0 adavgbl 0 adavgdiff 1
adavgdiff*cfPWVcoldropout 0.307
years 0 cfPWVcoldropout 0 years*cfPWVcoldropout 0;
run;

III.   Pattern-mixture model 2 and corresponding multiple imputation (cfPWV)

data cfpwv_miss;
set save_longform_2152;
where logcfpwvcolbl ne .;
keep ID randomization_group randgrp_num timeclass logcfpwvcol age racedich male avgmapbl mapdiff
bmiBL bmidiff prbl prdiff adavgbl adavgdiff years logcfpwvcol6 logcfpwvcol12;
run;

data wide;
  set cfpwv_miss;
  array logcfpwvcolt(3);

```
array mapdifft(3);
array bmidifft(3);
array prdifft(3);
array adavgdifft(3);
array yearst(3);
by id;
retain logcfpwvcolt mapdifft bmidifft prdifft adavgdifft yearst;
if first.id then do i = 1 to 3;
  logcfpwvcolt(i) = .;
        mapdifft(i) = .;
         bmidifft(i) = .;
          prdifft(i) = .;
          adavgdifft(i) = .;
                yearst(i) = .;
end;
logcfpwvcolt(timeclass) =  logcfpwvcol;
mapdifft(timeclass) = mapdiff;
bmidifft(timeclass) = bmidiff;
prdifft(timeclass) = prdiff;
adavgdifft(timeclass) = adavgdiff;
yearst(timeclass) = years;
if last.id;
drop timeclass logcfpwvcol mapdiff bmidiff prdiff adavgdiff years i;
run;
proc mi data = wide out=wide_imputed nimpute=20 seed=1213445 ;
var logcfpwvcolt: mapdifft: bmidifft: prdifft: adavgdifft: yearst: age racedich male avgmapbl
bmiBL prbl adavgbl randgrp_num;
mcmc timeplot(mean(logcfpwvcolt2 logcfpwvcolt3)) acfplot(mean(logcfpwvcolt2 logcfpwvcolt3));
mcmc plots=acf(wlf) plots=acf(mean) nbiter=1000 timeplot(mean);
run;
data long_imputed;
 set wide_imputed;
 array logcfpwvcolt(3) logcfpwvcolt:;
 array mapdifft(3) mapdifft:;
 array bmidifft(3) bmidifft:;
 array prdifft(3) prdifft:;
 array adavgdifft(3) adavgdifft:;
 array yearst(3) yearst:;
 do timeclass = 1 to 3;
  logcfpwvcol = logcfpwvcolt(timeclass);
  mapdiff = mapdifft(timeclass);
  bmidiff = bmidifft(timeclass);
  prdiff = prdifft(timeclass);
```

```
  adavgdiff = adavgdifft(timeclass);
  years = yearst(timeclass);
 output;
 end;
 drop logcfpwvcolt: mapdifft: bmidifft: prdifft: adavgdifft: yearst:;
run;
proc sort data = long_imputed;
 by _imputation_ ID timeclass;
run;
data long_imputed;
set long_imputed;
if logcfpwvcol6=. and logcfpwvcol12=. then cfPWVcoldropout=0;
else if logcfpwvcol6 ne . and logcfpwvcol12=. then cfPWVcoldropout=1;
else cfpwvcoldropout=2;
run;
proc mixed data = long_imputed covtest method=reml;
by _imputation_;
class ID cfpwvcoldropout randomization_group;
model logcfpwvcol =age racedich male avgmapbl mapdiff mapdiff*cfPWVcoldropout
bmiBL bmidiff bmidiff*cfPWVcoldropout prbl prdiff prdiff*cfPWVcoldropout adavgbl adavgdiff
adavgdiff*cfPWVcoldropout years cfPWVcoldropout years*cfPWVcoldropout
randomization_group/solution ddfm=satterthwaite;
random intercept years /subject=id type=un;
ESTIMATE 'avg int' INTERCEPT 1 cfPWVcoldropout 0.181 0.120 0.699;
ESTIMATE 'avg years' years 1 years*cfPWVcoldropout 0.181 0.120 0.699;
ESTIMATE 'avg mapdiff' mapdiff 1 mapdiff*cfPWVcoldropout 0.181 0.120 0.699;
ESTIMATE 'avg bmidiff' bmidiff 1 bmidiff*cfPWVcoldropout 0.181 0.120 0.699;
ESTIMATE 'avg prdiff' prdiff 1 prdiff*cfPWVcoldropout 0.181 0.120 0.699;
ESTIMATE 'avg adavgdiff' adavgdiff 1 adavgdiff*cfPWVcoldropout 0.181 0.120 0.699;
ods output CovParms = cov solutionf= mixparms estimates=est;
run;
data mixparms;
set mixparms;
if Effect='bmidiff*cfPWVcoldrop' and cfPWVcoldropout=0 then Effect='bmidiff_cfPWVcoldro0';
if Effect='bmidiff*cfPWVcoldrop' and cfPWVcoldropout=1 then Effect='bmidiff_cfPWVcoldro1';
if Effect='mapdiff*cfPWVcoldrop' and cfPWVcoldropout=0 then Effect='mapdiff_cfPWVcoldro0';
if Effect='mapdiff*cfPWVcoldrop' and cfPWVcoldropout=1 then Effect='mapdiff_cfPWVcoldro1';
if Effect='prdiff*cfPWVcoldropo' and cfPWVcoldropout=0 then Effect='prdiff_cfPWVcoldrop0';
if Effect='prdiff*cfPWVcoldropo' and cfPWVcoldropout=1 then Effect='prdiff_cfPWVcoldrop1';
if Effect='adavgdiff*cfPWVcoldr' and cfPWVcoldropout=0 then Effect='adavgdiff_cfPWVcold0';
if Effect='adavgdiff*cfPWVcoldr' and cfPWVcoldropout=1 then Effect='adavgdiff_cfPWVcold1';
if Effect='years*cfPWVcoldropou' and cfPWVcoldropout=0 then Effect='years_cfPWVcoldropo0';
if Effect='years*cfPWVcoldropou' and cfPWVcoldropout=1 then Effect='years_cfPWVcoldropo1';
```

```
run;
proc mianalyze parms(classvar=full)=mixparms;
class randomization_group cfPWVcoldropout;
modeleffects Intercept age racedich male avgmapbl mapdiff mapdiff_cfPWVcoldro0
mapdiff_cfPWVcoldro1bmiBL bmidiff bmidiff_cfPWVcoldro0 bmidiff_cfPWVcoldro1 prbl prdiff
prdiff_cfPWVcoldrop0 prdiff_cfPWVcoldrop1 adavgbl adavgdiff adavgdiff_cfPWVcold0
adavgdiff_cfPWVcold1 years cfPWVcoldropout years_cfPWVcoldropo0 years_cfPWVcoldropo1
randomization_group;
where estimate ne 0;
run;
proc sort data = est;
by label _imputation_;
run;
ods select parameterestimates;
proc mianalyze data = est;
  by label;
  modeleffects estimate;
  stderr stderr;
run;
```

IV.   Pattern-mixture model 3 and corresponding multiple imputation (cfPWV)

```
data cfpwv_miss1;
set save_longform_2152;
if logcfpwvcol6 ne . and logcfpwvcol12 ne . and (weight6-weightbl)/weightbl lt -0.072 then delete;
keep ID randomization_group randgrp_num timeclass logcfpwvcol age racedich male avgmapbl mapdiff
bmiBL bmidiff prbl prdiff adavgbl adavgdiff years logcfpwvcolbl logcfpwvcol6 logcfpwvcol12;
run;

data cfpwv_miss2;
merge save_longform_2152(in=ina) cfpwv_miss1(in=inb);
by ID timeclass;
if ina and not inb;
keep ID randomization_group randgrp_num timeclass logcfpwvcol age racedich male avgmapbl mapdiff
bmiBL bmidiff prbl prdiff adavgbl adavgdiff years logcfpwvcolbl logcfpwvcol6 logcfpwvcol12;
run;

data wide;
  set cfpwv_miss1;
  array logcfpwvcolt(3);
  array mapdifft(3);
  array bmidifft(3);
```

```
array prdifft(3);
array adavgdifft(3);
array yearst(3);
by id;
retain logcfpwvcolt mapdifft bmidifft prdifft adavgdifft yearst;
if first.id then do i = 1 to 3;
   logcfpwvcolt(i) = .;
         mapdifft(i) = .;
           bmidifft(i) = .;
            prdifft(i) = .;
            adavgdifft(i) = .;
                 yearst(i) = .;
end;
logcfpwvcolt(timeclass) =  logcfpwvcol;
mapdifft(timeclass) = mapdiff;
bmidifft(timeclass) = bmidiff;
prdifft(timeclass) = prdiff;
adavgdifft(timeclass) = adavgdiff;
yearst(timeclass) = years;
if last.id;
drop timeclass logcfpwvcol mapdiff bmidiff prdiff adavgdiff years i;
run;
proc print data = wide (obs=10) noobs;
run;

proc mi data = wide out=wide_imputed nimpute=20 seed=1213445;
  var logcfpwvcolt: mapdifft: bmidifft: prdifft: adavgdifft: yearst: age racedich male avgmapbl
bmiBL prbl adavgbl randgrp_num;
mcmc plots=acf(wlf) nbiter=1000;
run;
data long_imputed;
  set wide_imputed;
  array logcfpwvcolt(3) logcfpwvcolt:;
  array mapdifft(3) mapdifft:;
  array bmidifft(3) bmidifft:;
  array prdifft(3) prdifft:;
  array adavgdifft(3) adavgdifft:;
  array yearst(3) yearst:;
  do timeclass = 1 to 3;
   logcfpwvcol = logcfpwvcolt(timeclass);
   mapdiff = mapdifft(timeclass);
   bmidiff = bmidifft(timeclass);
   prdiff = prdifft(timeclass);
```

```
  adavgdiff = adavgdifft(timeclass);
  years = yearst(timeclass);
 output;
 end;
 drop logcfpwvcolt: mapdifft: bmidifft: prdifft: adavgdifft: yearst:;
run;
data cfpwv_miss2imp1;
set cfpwv_miss2;
_imputation_=1;
run;
data cfpwv_miss2imp2;
set cfpwv_miss2;
_imputation_=2;
run;
data cfpwv_miss2imp3;
set cfpwv_miss2;
_imputation_=3;
run;
data cfpwv_miss2imp4;
set cfpwv_miss2;
_imputation_=4;
run;
data cfpwv_miss2imp5;
set cfpwv_miss2;
_imputation_=5;
run;
data cfpwv_miss2imp6;
set cfpwv_miss2;
_imputation_=6;
run;
data cfpwv_miss2imp7;
set cfpwv_miss2;
_imputation_=7;
run;
data cfpwv_miss2imp8;
set cfpwv_miss2;
_imputation_=8;
run;
data cfpwv_miss2imp9;
set cfpwv_miss2;
_imputation_=9;
run;
data cfpwv_miss2imp10;
```

```
set cfpwv_miss2;
_imputation_=10;
run;
data cfpwv_miss2imp11;
set cfpwv_miss2;
_imputation_=11;
run;
data cfpwv_miss2imp12;
set cfpwv_miss2;
_imputation_=12;
run;
data cfpwv_miss2imp13;
set cfpwv_miss2;
_imputation_=13;
run;
data cfpwv_miss2imp14;
set cfpwv_miss2;
_imputation_=14;
run;
data cfpwv_miss2imp15;
set cfpwv_miss2;
_imputation_=15;
run;
data cfpwv_miss2imp16;
set cfpwv_miss2;
_imputation_=16;
run;
data cfpwv_miss2imp17;
set cfpwv_miss2;
_imputation_=17;
run;
data cfpwv_miss2imp18;
set cfpwv_miss2;
_imputation_=18;
run;
data cfpwv_miss2imp19;
set cfpwv_miss2;
_imputation_=19;
run;
data cfpwv_miss2imp20;
set cfpwv_miss2;
_imputation_=20;
run;
```

```
data long_imputed;
set long_imputed cfpwv_miss2imp1 cfpwv_miss2imp2 cfpwv_miss2imp3 cfpwv_miss2imp4
cfpwv_miss2imp5 cfpwv_miss2imp6 cfpwv_miss2imp7
cfpwv_miss2imp8 cfpwv_miss2imp9 cfpwv_miss2imp10 cfpwv_miss2imp11 cfpwv_miss2imp12
cfpwv_miss2imp13 cfpwv_miss2imp14
cfpwv_miss2imp15 cfpwv_miss2imp16 cfpwv_miss2imp17 cfpwv_miss2imp18 cfpwv_miss2imp19
cfpwv_miss2imp20;
run;
proc sort data = long_imputed;
  by _imputation_ ID timeclass;
run;
data long_imputed;
set long_imputed;
if logcfpwvcol6=. and logcfpwvcol12=. then cfPWVcoldropout=0;
else if logcfpwvcol6 ne . and logcfpwvcol12=. then cfPWVcoldropout=1;
else cfpwvcoldropout=2;
run;

proc mixed data = long_imputed method=reml;
by _imputation_;
class ID cfpwvcoldropout randomization_group;
model logcfpwvcol =age racedich male avgmapbl mapdiff mapdiff*cfPWVcoldropout
bmiBL bmidiff bmidiff*cfPWVcoldropout prbl prdiff prdiff*cfPWVcoldropout adavgbl adavgdiff
adavgdiff*cfPWVcoldropout
years cfPWVcoldropout years*cfPWVcoldropout randomization_group/solution ddfm=satterthwaite;
random intercept years /subject=id type=un;
ESTIMATE 'avg int' INTERCEPT 1 cfPWVcoldropout 0.187 0.120 0.693;
ESTIMATE 'avg years' years 1 years*cfPWVcoldropout 0.187 0.120 0.693;
ESTIMATE 'avg mapdiff' mapdiff 1 mapdiff*cfPWVcoldropout 0.187 0.120 0.693;
ESTIMATE 'avg bmidiff' bmidiff 1 bmidiff*cfPWVcoldropout 0.187 0.120 0.693;
ESTIMATE 'avg prdiff' prdiff 1 prdiff*cfPWVcoldropout 0.187 0.120 0.693;
ESTIMATE 'avg adavgdiff' adavgdiff 1 adavgdiff*cfPWVcoldropout 0.187 0.120 0.693;
ods output CovParms = cov solutionf= mixparms estimates=est;
run;
data mixparms;
set mixparms;
if Effect='bmidiff*cfPWVcoldrop' and cfPWVcoldropout=0 then Effect='bmidiff_cfPWVcoldro0';
if Effect='bmidiff*cfPWVcoldrop' and cfPWVcoldropout=1 then Effect='bmidiff_cfPWVcoldro1';
if Effect='mapdiff*cfPWVcoldrop' and cfPWVcoldropout=0 then Effect='mapdiff_cfPWVcoldro0';
if Effect='mapdiff*cfPWVcoldrop' and cfPWVcoldropout=1 then Effect='mapdiff_cfPWVcoldro1';
if Effect='prdiff*cfPWVcoldropo' and cfPWVcoldropout=0 then Effect='prdiff_cfPWVcoldrop0';
if Effect='prdiff*cfPWVcoldropo' and cfPWVcoldropout=1 then Effect='prdiff_cfPWVcoldrop1';
if Effect='adavgdiff*cfPWVcoldr' and cfPWVcoldropout=0 then Effect='adavgdiff_cfPWVcold0';
```

```
if Effect='adavgdiff*cfPWVcoldr' and cfPWVcoldropout=1 then Effect='adavgdiff_cfPWVcold1';
if Effect='years*cfPWVcoldropou' and cfPWVcoldropout=0 then Effect='years_cfPWVcoldropo0';
if Effect='years*cfPWVcoldropou' and cfPWVcoldropout=1 then Effect='years_cfPWVcoldropo1';
run;
proc mianalyze parms(classvar=full)=mixparms;
  class randomization_group cfPWVcoldropout;
  modeleffects Intercept age racedich male avgmapbl mapdiff mapdiff_cfPWVcoldro0
mapdiff_cfPWVcoldro1
bmiBL bmidiff bmidiff_cfPWVcoldro0 bmidiff_cfPWVcoldro1 prbl prdiff prdiff_cfPWVcoldrop0
prdiff_cfPWVcoldrop1
adavgbl adavgdiff adavgdiff_cfPWVcold0 adavgdiff_cfPWVcold1 years cfPWVcoldropout
years_cfPWVcoldropo0
years_cfPWVcoldropo1 randomization_group;
where estimate ne 0;
run;
proc sort data = est;
 by label _imputation_;
run;
ods select parameterestimates;
proc mianalyze data = est ;
  by label;
  modeleffects estimate;
  stderr stderr;
run;
```

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