Dietary Approaches to Stop Hypertension for Diabetes Trial (DASH4D)

PROTOCOL
and
STATISTICAL ANALYSIS PLAN (SAP)

Protocol Number: IRB00232059
ClinicalTrials.gov Number: NCT04286555

Funded by: Sheikh Khalifa Stroke Institute at Johns Hopkins University

Table of Contents

PROTOCOL

Version 2.6
November 1, 2024

History of Protocol Changes

The following table summarizes substantive changes between protocol versions. Minor changes, such as minor corrections and clarifications, page numbering, and formatting are not described in the table but can be seen in the track changes versions of the protocol.

Protocol version	Affected section(s)	Brief description of change	Brief rationale for change
v2.0 (03-21-2021)	Version of the protocol approved and in use at the time of enrollment of the 1st DASH4D participant.		
v2.1 (09-29-2021)	6.2 Eligibility Criteria	Cancer exclusion modified to also allow participants with benign tumors to enroll and change the treatment exclusion from systemic therapy to chemotherapy.	Investigator clarifications to better capture the intent of the exclusion criteria.
	6.2 Eligibility Criteria; 8.1 Data Collection Scheduled – Table 3	Weight change exclusion modified to also exclude participants with a large weight change during screening. Participants will be re-weighed prior to randomization, and the dietitians will assess weight change.	Participants whose weight is not stable during screening are not good candidates for a controlled feeding study where we aim to maintain weight.
	6.2 Eligibility Criteria	Exclusion related to participation in another trial modified to indicate exclusion only for a trial that might affect blood pressure or the participant's ability to comply with study procedures.	Investigator clarification to better capture the intent of the exclusion criteria.
	6.2 Eligibility Criteria; Appendix E 21.4 Exclusions	For the CGM ancillary, implantable pacemaker was added as an exclusion criterion.	The CGM sensor's manufacturer has not extensively tested the use of the sensor in conjunction with use of an implantable pacemaker, so this exclusion was added out of an abundance of caution.
	8.1 Data Collection Schedule – Table 3	Food frequency questionnaire replaced with two 24-hour dietary recall interviews.	Investigator decision because the recalls are less burdensome for participants to complete, less prone to errors, and yield more accurate calorie estimates.

Protocol version	Affected section(s)	Brief description of change	Brief rationale for change
v2.1 (09-29-2021)	8.1 Data Collection Schedule – Table 3; Appendix E 21.6 Symptom Reporting Survey	Addition of a symptom reporting survey during each period of CGM sensor wear.	Reporting of symptoms will allow investigators to learn more about the relationship between changes in glucose levels and symptoms experienced.
	8.1 Data Collection Schedule – Table 3; Appendix F: Postprandial Blood Pressure Ancillary Study	Addition of an ancillary study to obtain detailed blood pressure assessments during each feeding period.	Detailed blood pressure assessment around a meal will allow investigators to learn more about how different diets affect blood pressure regulation and occurrence of postprandial orthostatic hypotension.
	10.3 Risks	Addition of information about continuation in the study following hypertensive event.	The protocol was not previously clear on whether participants meeting blood pressure escape levels and referred for medical care should continue in the study. Because blood pressure values can vary widely and because of the regression-to-the-mean phenomenon, decisions about continuation in the study will be made on a case-by-case basis based on any safety concerns.
	10.3 Risks	Addition of information about how hyperkalemia will be handled if detected in the end-of-period laboratory results.	The protocol did not previously include this information, so the investigators have added the plan.
v2.2 (01-11-2023)	6.2 Eligibility Criteria	Updated information on eGFR estimation.	The commercial lab used by the study changed the equation that they were using to estimate GFR. We are still using the same eGFR threshold for exclusion despite the change in how the eGFR is calculated.
	6.2 Eligibility Criteria	Added use of Tirzepatide to the list of medication exclusions	Tirzepatide is a new medication for diabetes that, although not yet approved for weight loss, has been shown to cause significant weight loss in some users, and therefore, its use should exclude participants from this study, similar to other medications on the exclusion list known to affect weight.

Protocol version	Affected section(s)	Brief description of change	Brief rationale for change
v2.2 (01-11-2023)	6.2 Eligibility Criteria	Exclusion related to bariatric surgery modified to indicate that only participants who have had bariatric surgery and are limited in the amount or types of foods they can consume are excluded.	This modification is a clarification of the intent of the exclusion criteria. DASH4D wanted to exclude participants who have had bariatric surgery and who would not be able to comply with the study interventions as a result. Some participants have a history of bariatric surgery but are no longer limited in volume or types of foods that they can consume, and therefore, they would be suitable candidates to participate in the study.
	8.1 Data Collection Schedule – Table 3	Removed spot urine collection from the SV2 visit and reduced the end-of-period (FP W4-W5) spot urine collection from two to one.	Protocol section 8.1 previously stipulated that some data collection items might be dropped based upon participant burden, scientific considerations, available resources, and the results of field testing. The investigators decided that the scientific rationale for collecting two spot urines at each time point no longer outweighed the burden on participants and the study's available resources.
v2.3 (11-02-2023)	2.1 Synopsis, 4 Outcomes and Objectives	Objective updated	The wording of the primary objective has been updated to clarify that the objective is to examine the *mean difference* between the diets previously labeled as the primary contrast. This does not reflect an actual change in the objective.
	2.1 Synopsis	Objectives and outcomes (formerly labeled as endpoints) were updated to only reflect primary and secondary objectives/outcomes where appropriate.	All objectives and outcomes are still listed in Section 4, but the decision was made to keep the synopsis focused on the key information.
	Throughout document	Terminology "washout" changed to "break" or "eating own food".	Participants are still eating food during the time between the intervention feeding periods, so those times are not true "washouts" and are more accurately described as "breaks".
	4 Outcomes and Objectives	Changed from "Objectives and Endpoints" to "Outcomes and Objectives". Updated the descriptions of the primary and secondary outcomes and some other outcomes.	Clarifications of how the outcomes are being assessed.

Protocol version	Affected section(s)	Brief description of change	Brief rationale for change
v2.3 (11-01-2023)	4 Outcomes and Objectives	Added fructosamine, albumin-creatinine ratio, 24-hour urinary albumin excretion, and diet acceptability as outcomes.	These outcomes were previously omitted and have now been added to the protocol document.
	6.2 Eligibility Criteria	Potassium supplementation exclusion clarified.	Clarified that participants are not allowed to use potassium supplementation in any form (e.g., electrolyte drink mixes) if the amount of potassium is greater than 99mg/day (maximum amount allowed by FDA per serving for over-the-counter supplements).
	7.1 Intervention Diets	Added information that when participants need to miss a planned feeding interval, they resume their assigned sequence of diets upon their return.	For logistical reasons, it has been necessary to permit participants to have extended breaks between feeding periods due to the COVID epidemic, personal health issues, family matters, and other reasons.
	8.1 Data Collection and Measurements; 8.2 Measurements	Removed spot urine collection from Screening Visit 2 and second spot urine collection from Feeding Period Weeks 4-5.	Missed during protocol v2.2 updates (see above for rationale).
	8.1 Data Collection Contact Schedule Table 3; Former Appendix F	Removed optional Postprandial Blood Pressure Ancillary Study.	The ancillary study was not funded, and therefore, it was never implemented within the DASH4D Trial.
	8.2 Measurements	Added that fructosamine and glycated albumin, along with HbA1c, will be measured from stored specimens at the end of the trial.	Timing of the HbA1c measurement was not previously included, and the planned measurement of fructosamine and glycated albumin was accidently omitted from the description of measurements.
	10.3 Risks	Added that there is a risk of dental issues.	Due to the nuts and seeds in the study diets, a couple of participants have experienced dental problems, so we are adding this as a risk to the protocol and consent.
	10.3 Risks	Added information on the risk of low BP in the study, and information on how low BP will be monitored and evaluated.	The protocol previously only described the risk of elevated BP. Given that the study diets have been shown to reduce BP in persons without diabetes and that most participants are taking BP-lowering medications, we felt the need to add that low BP is also a risk, along with details of how the low BP will be assessed and managed. The low BP risk is also being added to the consent form.

Protocol version	Affected section(s)	Brief description of change	Brief rationale for change
v2.3 (11-01-2023)	10.3 Risks	Updated description of ascertainment of Adverse Events.	Corrected the description of how adverse events are being ascertained in DASH4D, on an ongoing basis via participant report to staff or participant response to an item on the Daily Diary, rather than just assessing AEs at the end of the feeding period.
	11.1 Statistical Considerations	Removed all prior text and now refer to the full SAP that has been prepared.	The full SAP has now been prepared and incorporated at the end of this document as an Appendix. The overall analytic approach remains the same, but additional details are now provided.
	Appendices	Appendices A-D were consolidated into a single Appendix A; former Appendix E was relabeled Appendix B and its sections were renumbered; former Appendix F was deleted; and a new Appendix C was added.	Updates to the Appendices were made to better represent these materials as separate from but attached to the protocol.
v2.4 (02-28-2024)	4.3 Other Outcomes	Relevant lipid ratios added to the lipid level outcomes.	These lipid ratios were previously omitted and have now been added.
	4.3 Other Outcomes	Added that, contingent upon funding, laboratory testing for diabetes, cardiovascular, and nutrition biomarkers will be completed.	The study team had always planned to complete laboratory testing on the biospecimens collected during the study to examine the effects of the intervention diets on levels of diabetes, cardiovascular, and nutrition biomarkers. This testing is contingent upon securing funding to complete the laboratory assays.
	4.5 Other Objectives	Added language to Other Objective #10 to indicate that the establishment of a repository of biospecimens is for future research beyond any planned assays that have been funded prior to the end of the trial.	This clarifying language was added to distinguish the laboratory assays that were intended as part of the DASH4D research questions/other outcomes, contingent upon funding, that will be conducted as batched analyses at the end of the trial vs. stored specimens for additional future research questions beyond those described in 4.3 Other Outcomes.

Protocol version	Affected section(s)	Brief description of change	Brief rationale for change
v2.4 (02-28-2024)	15 Data Sharing and Biospecimen Transfers 15.1 Biospecimen Transfers	Section renamed from "Data Sharing" to "Data Sharing and Biospecimen Transfers" and expanded to include information on biospecimen transfers.	The planned biomarker assays will involve sending biospecimens collected in DASH4D to external laboratories. As funding is secured for these biomarkers, MTAs will be prepared to ship the de-identified specimens for analysis. The first planned specimen transfer is to Washington University School of Medicine in St. Louis, and details of this collaboration are included in this protocol section. As needed, we will expand this section of the protocol in the future to include other external laboratories and details of those collaborations.
	Appendix B, 1. Ancillary Study Details	New section added with details of the ancillary study.	Although fully embedded in the DASH4D data collection protocol and consistent with the main study's outcomes and objectives, this CGM ancillary study was funded by a separate grant, and those details have been added.
	Appendix B, 2. Rationale	Added a sentence to indicate that the ancillary study also includes examination of biomarkers.	Examination of biomarkers was included in the funded CGM ancillary study.
v2.5 (07-26-2024)	2.1 Synopsis; 4 Outcomes and Objectives; 5.1 Overview; 7.1 Intervention Diets	Clarified that the comparison diet is typical of what many Americans *with diabetes* eat.	Data on what Americans with diabetes eat was used in designing the comparison diet. The McClure et al. (2020) paper with these data has now been cited in the comparison diet rationale.
	4.5 Other Objectives;	Added baseline number of medications used to treat the outcome as a pre-specified subgroup analysis.	Number of medications may be an important factor in examining the effects of the study diets on the outcomes. At the time of addition of this subgroup analysis, no analyses related to baseline medication use have been performed.
	7.2 Food Acquisition, Handling, and Distribution; 7.6 COVID-19 Safety Measures	These sections were updated.	The updates more accurately reflect the COVID modifications to the protocol that were implemented.
	15 Data Sharing and Biospecimen Transfers	Added new subsection 15.1 to address data sharing.	Information on data sharing was not previously included.
	15.2 Biospecimen Transfers	Added a new section describing specimen transfers to University of Colorado.	This specimen sharing is part of an NIH-funded ancillary study to the DASH4D trial, which includes a subcontract to JHU for these specimen transfers.

Protocol version	Affected section(s)	Brief description of change	Brief rationale for change
v2.5 (07-26-2024)	Appendix B	Clarified the timing of the CGM placement and data collection.	Clarified that the CGM sensors are typically placed during the last day of in-person feeding during week 3 and worn for up to 14 days.
	Appendix B, 10. Study Outcomes and Objectives	Section added.	Details on the CGM ancillary study's outcomes and objectives have been added. This information was added prior to the CGM team being unblinded to randomized diet sequence.
	Appendix B, 11. Statistical Analysis	Section added.	Details on the CGM ancillary study's analysis plans have been added. This information was added prior to the CGM team being unblinded to randomized diet sequence.
v2.6 (11-01-2024)	Appendix B, 10. Study Outcomes and Objectives; Appendix B, 11. Statistical Analysis	Updated to reflect that the CGM ancillary study will compare the DASH4D diet to the comparison diet, combining across the sodium levels, rather than focusing on the contrast between the DASH4D diet with lower sodium and the comparison diet with higher sodium.	The CGM ancillary study hypothesized that sodium would not have an effect on glucose, and the grant proposal had indicated that data would be combined across the DASH4D diet with lower sodium and DASH4D diet with higher sodium, and likewise combined for the comparison diet with lower sodium and comparison diet with higher sodium, to increase statistical power. The previous version of the CGM statistical analysis plan had been modeled off of the main trial's SAP inadvertently and is now being updated to match the original planned analytic approach for the ancillary study.
	Appendix B, 10. Study Outcomes and Objectives	The time in range, time above range, and time below range outcomes were more clearly defined for the specific ranges and separated into a few different ranges of interest.	There are multiple glucose ranges of interest for the CGM ancillary study, and the outcomes were updated to more clearly reflect these ranges.
	Appendix B, 10. Study Outcomes and Objectives	Exploratory CGM outcomes were added.	These planned exploratory outcomes were not specified in the previous version of the CGM SAP.
	Appendix B, 11. Statistical Analysis	Exploratory analyses were added.	The planned exploratory analyses were not specified in the previous version of the CGM SAP.

1 STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812). All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent forms, recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

2 PROTOCOL SUMMARY

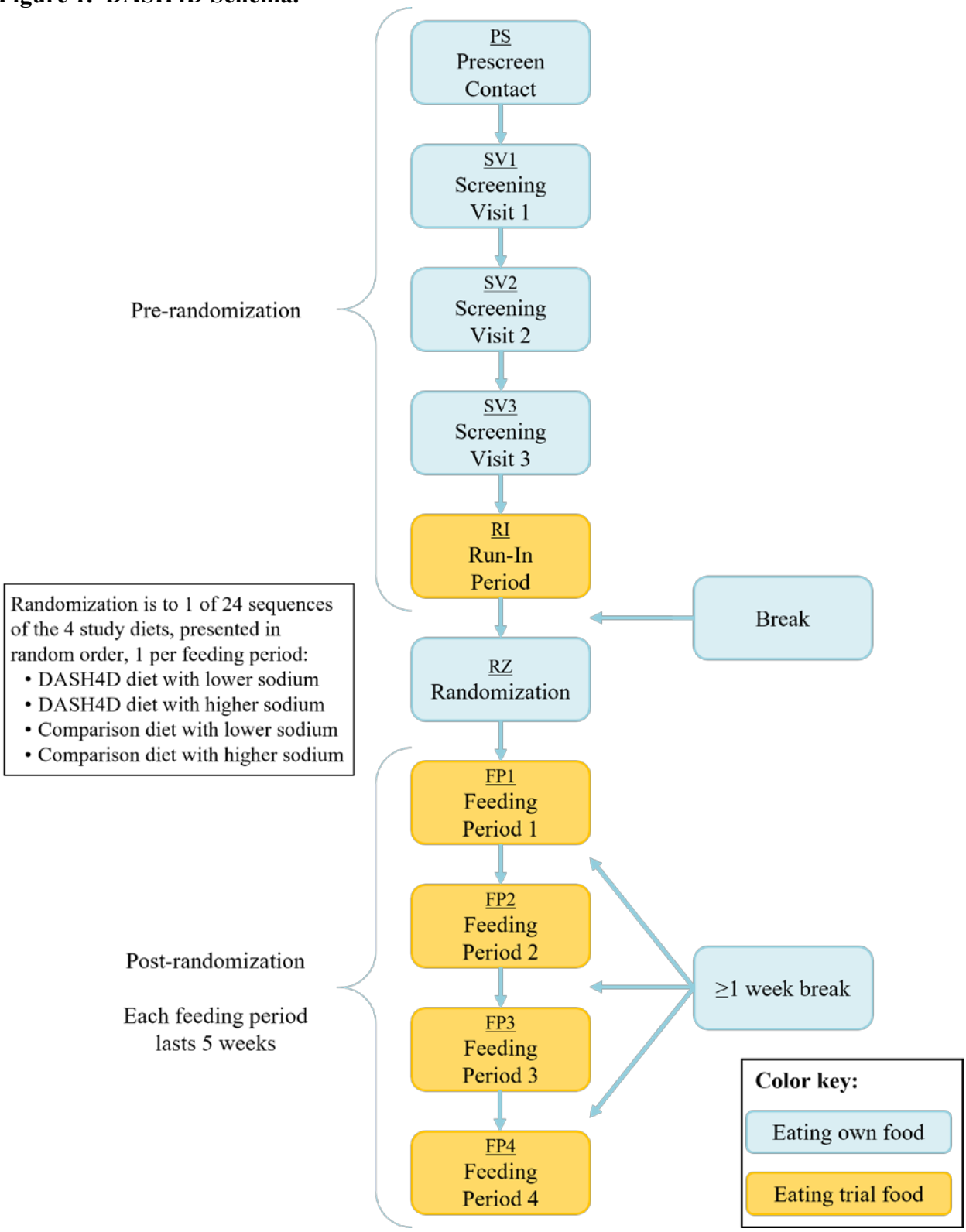
2.1 Synopsis

Title:	Dietary Approaches to Stop Hypertension for Diabetes Trial (DASH4D)
Protocol Number:	IRB00232059
ClinicalTrials.gov:	NCT04286555
Funded by:	Sheikh Khalifa Stroke Institute at Johns Hopkins University
Study Description:	The objective of the DASH4D trial is to determine the effects, alone and combined, of (a) the DASH4D diet (a DASH-style diet modified for people with diabetes) vs. comparison diet that is typical of what many Americans with diabetes eat and (b) lower sodium intake vs. higher sodium intake on blood pressure (BP) among people with Type 2 diabetes and elevated BP. The core design is a single-site, 4-period, crossover feeding study with 5-week periods. Participants are fed each of four isocaloric diets, presented in random order, and outcomes are measured at the end of each feeding period.
Primary objective:	To determine the mean difference between end-of-period systolic BP on the DASH4D diet with lower sodium and the end-of-period systolic BP on the comparison diet with higher sodium. Other objectives are described in section 4 below.

Primary and secondary outcomes:	Primary outcome: End-of-period, office-based systolic BP Seated BP will be measured in triplicate on five separate days during the last 2 weeks of each 5-week feeding period, and the mean of these systolic BP measurements will be used as the primary outcome of the corresponding feeding period. Secondary outcome: End-of-period, office-based diastolic BP Seated BP will be measured in triplicate on five separate days during the last 2 weeks of each 5-week feeding period, and the mean of these diastolic BP measurements will be used as the secondary outcome of the corresponding feeding period. Other outcomes are described in section 4 below.
Study Population:	• Approximately 100 adults, aged 18 and older, who have Type 2 diabetes, a systolic BP of 120-159 mmHg, and diastolic BP <100 mmHg.
Phase:	N/A
Enrollment Site:	This is a single-site study conducted at the ProHealth Clinical Research Unit (ProHealth) of Johns Hopkins University.
Description of Study Intervention:	Participants will be randomized to a sequence of diets, during which they will consume each of the 4 intervention diets listed below for a 5-week period. During each 5-week feeding period, participants will be provided all meals and snacks and most beverages. Each feeding period will be separated by a break of at least 1 week during which participants consume their own food and beverages. We will study the following four intervention diets: 1. DASH4D diet with lower sodium 2. DASH4D diet with higher sodium 3. comparison diet with lower sodium 4. comparison diet with higher sodium
Study Duration:	The intervention portion of the DASH4D trial is expected to last about 3.5 years.
Participant Duration:	Each participant will complete screening, a run-in period, and 4 feeding periods lasting 5 weeks each, separated by a break of at least 1 week during which they eat their own food. The total duration of participation from screening through completion of the study is expected to be about 7-9 months, but could be shorter or longer.

2.2 Schema

Figure 1. DASH4D Schema.



3 INTRODUCTION

3.1 Study Rationale

The Global Pandemic of Diabetes
Approximately 425 million adults worldwide have diabetes (prevalence of 8.8%), most of which is Type 2 diabetes.[1] This number is projected to rise to 629 million by 2045 (prevalence of 9.9%). Type 2 diabetes is increasing in prevalence in most countries around the world.

In the United States (US), there are currently 30.3 million adults (9.4% of US population) with diabetes, and this number is projected to increase to 35.6 million by 2045.[1,2] The prevalence of diabetes is higher among American Indians/Alaska Natives (15.1%), non-Hispanic blacks (12.7%) and people of Hispanic ethnicity (12.1%) compared to non-Hispanic whites (7.4%) and Asians (8.0%).[2]

In the Middle East/North Africa region, approximately 38.7 (27.1-51.4) million people or 9.6% (6.7-12.7%) of adults have diabetes.[1] Furthermore, the proportion of undiagnosed diabetes in this region is 49.0%. Countries in this region with the highest age-adjusted comparative diabetes prevalence include Saudi Arabia (17.7%), Egypt (17.3%), and United Arab Emirates (17.3%).[1]

Diabetes as a Risk Factor for Stroke
Diabetes mellitus is an independent risk factor for stroke, which accounts for approximately 20% of deaths in people with diabetes.[3] Individuals with diabetes have double the risk of stroke compared to the general population.[3] Furthermore, the longer the duration of diabetes, the higher the stroke risk.[4]

Hypertension in People with Diabetes
The prevalence of hypertension among people with diabetes varies based on type and duration of diabetes, age, sex, race/ethnicity, body mass index (BMI), history of glycemic control and presence of kidney disease.[5] Using National Health and Nutrition Examination Survey (NHANES) data from 2011-2014, the Centers for Disease Control and Prevention (CDC) estimates that among adults with diagnosed diabetes, 76% had hypertension defined by a systolic BP of 140 mmHg or higher, or diastolic BP of 90 mmHg or higher, or were on prescription medication for high BP.[2] People with diabetes have nearly two times higher cardiovascular disease death rates and hospitalization rates for heart attack and stroke compared to adults without diabetes.[6]

3.2 Background

Effect of Blood Pressure Reduction on Stroke and CVD in People with Type 2 Diabetes
A substantial body of evidence has documented that lowering BP through anti-hypertensive drug therapy reduces the risk of cardiovascular events and stroke in people with diabetes. A meta-analysis of large-scale randomized controlled trials of blood-pressure lowering treatment in individuals with diabetes showed that each 10-mmHg reduction in systolic BP was associated

with a significant reduction in cardiovascular events (relative risk [RR]=0.89, 95% confidence interval [CI]: 0.83-0.95) and stroke (RR=0.73, 95% CI: 0.64-0.83) among other outcomes.[7] Another systematic review and meta-analysis of trials conducted in people with diabetes found similar results.[8] In the general population, it has been estimated that 54% of all strokes can be attributed to elevated BP.[9]

Effect of DASH Diet on Blood Pressure

Two randomized controlled trials (RCTs), both small (n<50), have studied the effects of the Dietary Approaches to Stop Hypertension (DASH) diet in persons with Type 2 diabetes demonstrating a significant BP reduction with the DASH diet compared to control diet[10,11] (Appendix A-1). One was a 4-week parallel arm study (n=40) conducted in Brazil among patients with diabetes and uncontrolled BP, comparing the DASH diet to the control diet.[11] This study showed a significantly greater reduction in 24-hour ambulatory BP measurement with the DASH diet compared to the control diet (p-value for between-group difference in systolic BP: <0.001, diastolic BP: 0.018); however, this study did not report the actual difference in BP between the two arms.[11] The other trial was an 8-week crossover study (n=31) conducted in Iran among patients with diabetes (average systolic BP at baseline was 134.5-137.4 mmHg) comparing the DASH diet to control diet. This study demonstrated a significant between-group BP reduction in the DASH vs. control diet (p-value for between-group difference in systolic BP: <0.02, diastolic BP: 0.04); again, this study did not report the actual BP difference between the two diets.[10] These two studies were behavioral intervention trials, in which partial adherence prevents an assessment of the maximum potential effect of the DASH diet, which is best assessed in the setting of a controlled feeding study.

Effect of Sodium Reduction on Blood Pressure

Twelve RCTs (total n=371) have studied the effects of sodium reduction in persons with diabetes (Appendix A-2, A-3, A-4). Five of these studies evaluated behavioral interventions (Appendix A-2), and three showed a significant between-group BP reduction with a low sodium diet compared to control or high sodium diet.[12-14]

One 12-week parallel arm study of patients with diabetes and mild hypertension (n=34) compared moderate sodium restriction (not specified) to the control diet.[14] At baseline, the mean BP was 179/98 mmHg in the moderate sodium restriction group and 174/100 mmHg in the control group. At follow-up, there was a significant between-group systolic BP reduction (p<0.03) but not with diastolic BP.[14] A 24-week crossover study (n=45) compared low sodium (50 mmol) to normal sodium diet combined with hydrochlorothiazide or placebo.[12] This study showed a significant systolic and diastolic BP reduction with the low sodium arm compared to baseline treatment (systolic BP change: -5.3 mmHg, 95% CI: -1.5 to -9.1, p=0.008; diastolic BP change: -3.4 mmHg, 95% CI: -1.0 to -5.8, p=0.0067).[12] The third study was a 12-week crossover study (n=115) comparing low sodium (100 mEq) to high sodium (200 mEq) diet combined with paricalcitol or placebo.[13] The baseline mean systolic BP was 146.3 mmHg and diastolic BP was 79.7 mmHg. At follow-up, the study demonstrated a significant BP reduction with the low sodium diet group (systolic BP change: p=0.001; diastolic BP change: p=0.01) compared to no BP change with the high sodium diet group.[13]

Two of the behavioral intervention studies did not show a significant reduction in BP for the lower versus higher sodium intervention.[15,16] Notably, one of these null studies was conducted among young participants with Type 1 diabetes without elevated BP.[16] Five of the 12 studies were feeding studies (Appendix A-3) of one-week duration[17-21] with only one study showing significant BP reduction with a low sodium diet compared to normal sodium diet. This crossover study (n=32) compared low sodium (80 mmol) to normal sodium (200 mmol) diet.[18] The mean baseline systolic BP ranged from 132-144 mmHg and mean baseline diastolic BP ranged from 73-85 mmHg. At follow-up, systolic BP was lower in the low sodium group vs. normal sodium group (p=0.001). Two studies did show a higher BP when switching from the low to high sodium diet but only among those with microalbuminuria; no changes were seen with participants with normoalbuminuria.[17,20] Overall, these studies were very short in duration and primarily enrolled participants without elevated BP, limiting the conclusions that can be drawn.

Two of the 12 studies, both with significant findings, used pills (Appendix A-4) and demonstrated that those assigned to placebo pill without sodium had a lower BP than those taking the sodium supplement.[22,23] One 4-week parallel arm study (n=14) compared 100 mmol sodium supplement with placebo in people with Type 1 diabetes, showing a significant between-group clinic diastolic BP reduction with placebo compared to the supplement (5.3 mmHg, 95% CI: 1 to 9.7, p=0.02).[22] One 12-week crossover study (n=26) compared 90 mmol sodium supplement with placebo among patients with Type 2 diabetes (average BP 134±2/82±1 mmHg). The study demonstrated a significant between-group clinic systolic BP reduction with placebo vs. sodium supplement (p<0.01).[23] The authors of this study noted that the difference in salt intake between groups was small due to the high prevalence of salt in many foods, so maintaining patient compliance with the dietary regimen is a challenge.[23]

Dietary Guidelines for People with Diabetes

Dietary guidelines for persons with diabetes are largely directed at glycemia. Still, some guidelines make recommendations with a goal of lowering BP. The American Diabetes Association (ADA) recommends that lifestyle management of hypertension among patients with diabetes should include reducing sodium intake to 2300 mg/day[24] and a DASH-style dietary pattern.[5] As noted above, evidence from trials on the effects of diet changes on BP is sparse in persons with diabetes.

The recommendation for reducing sodium intake to 2300 mg/day is extrapolated from guidelines for the general population. A Cochrane review of RCTs found that sodium intake reduction lowers BP in those with diabetes.[25] The ADA has recommended that persons with diabetes not consume less than 1500 mg of sodium per day given evidence from some observational studies demonstrating increased mortality associated with very low sodium intake;[26,27] however, the updated Dietary Reference Intakes for Sodium and Potassium from the National Academy of Medicine concluded that these observational studies had substantial methodological flaws and that better evidence was needed about the health effects of sodium intakes below 2300 mg/day.[28]

The ADA recommends the DASH eating plan based on evidence that it can help control BP and lower CVD risk in the general population.[29] However, as noted above, there is extremely limited evidence on the effects of the DASH diet in individuals with diabetes, and no trial has assessed the combined and separate effects of the DASH diet and sodium reduction in the same trial.

4 OUTCOMES AND OBJECTIVES

The objective of the DASH4D trial is to determine the effects, alone and combined, of (a) the DASH4D diet (a DASH-style diet modified for people with diabetes) vs. comparison diet (typical of what many Americans with diabetes eat) and (b) lower sodium intake (vs. higher sodium intake) on BP in adults with Type 2 diabetes. Outcomes (unless otherwise specified) are measured at the end of each feeding period, with "end-of-period" assessments captured during the final 2 weeks of each 5-week feeding period. In rare instances when extenuating circumstances require it, feeding and outcome assessments may be extended briefly into a 6th week to complete the end-of-period measures.

4.1 Primary Outcome

End-of-period, office-based systolic BP (mmHg)

- *Description:* Seated BP will be measured in triplicate on five separate days during the last 2 weeks of each 5-week feeding period, and the mean of these systolic BP measurements will be used as the primary outcome of the corresponding feeding period.

4.2 Secondary Outcome

End-of-period diastolic BP (mmHg)

- *Description:* Seated BP will be measured in triplicate on five separate days during the last 2 weeks of each 5-week feeding period, and the mean of these diastolic BP measurements will be used as the secondary outcome of the corresponding feeding period.

4.3 Other Outcomes

Other end-of-period outcomes, each from a single assessment during the final 2 weeks of each 5-week feeding period, include:

- Glycemia measures, including fasting glucose (mg/dL), glycated albumin (%), fructosamine (µmol/L), and HbA1c (%). Of these, our principal measures of glycemia are fructosamine and HbA1c, both of which are used clinically as integrated measures of glycemia. Fructosamine represents the average level of glycemia over 2 to 3 weeks prior to the test date, while HbA1c represents average level of blood glucose level over the preceding 3 months.
- Lipid levels (mg/dL), including total cholesterol, LDL cholesterol (Martin-Hopkins calculation[30]), HDL cholesterol, and triglycerides, and relevant lipid ratios
- Estimated CVD risk (%): current 10-year atherosclerotic cardiovascular disease risk, using the ACC/AHA ASCVD equation
- Orthostatic hypotension outcomes, including presence of orthostatic hypotension (yes, no), postural change in systolic BP (mmHg), and postural change in diastolic BP (mmHg)
- 24-hour urinary albumin excretion (mg/24hr), which is our principal measure of urinary protein excretion; the albumin-creatinine ratio, used clinically, will also be reported
- Patient-reported symptoms (any; any moderate or severe)
- Diet acceptability, as rated by participant on two questions with a 9-point Likert scale for how much the participant liked the diet (1=disliked extremely, 9=liked extremely) and how hungry the participant felt while eating the diet (1=not at all hungry, 9=extremely hungry)

Other outcomes assessed throughout the intervention include:

- Safety outcomes, including hypoglycemia, hypokalemia, hyperkalemia, elevated BP, low BP, SAEs, and adverse medical events

Contingent upon securing sufficient funding, and consistent with the informed consent form that participants signed, we are also planning to complete laboratory testing of biospecimens collected at the end of each feeding period, to examine:

- Additional diabetes biomarkers (e.g., 1,5-anhydroglucitol)
- Cardiovascular biomarkers (e.g., cardiac troponins, NT-pro-BNP, inflammatory markers)
- Nutrition biomarkers (e.g., metabolomics, proteomics, analysis of microbiome and associated fatty acids)

4.4 Primary Objective

1. To determine the mean difference between end-of-period systolic BP on the DASH4D diet with lower sodium and the end-of-period systolic BP on the comparison diet with higher sodium ("primary testing contrast").

4.5 Other Objectives

2. To determine the mean difference between end-of-period systolic BP for the lower and higher sodium intakes in (a) the comparison diet and (b) the DASH4D diet.
3. To determine the mean difference between end-of-period systolic BP for the DASH4D diet and comparison diet at (a) higher sodium intake and (b) lower sodium intake.
4. To examine whether there is an interaction between the effects of diet and sodium intake on systolic BP.
5. To determine the time course over 5 weeks of lower and higher sodium intakes on systolic BP in the DASH4D and comparison diets.
6. To determine the time course over 5 weeks of the DASH4D and comparison diets on systolic BP at lower and higher sodium intakes.
7. To conduct corresponding analyses (repeat above objectives) with diastolic BP as the outcome.
8. To determine the effects, alone and combined, of the DASH4D diet (vs. comparison diet) and lower sodium intake (vs. higher sodium intake) on the other outcomes.
9. To conduct subgroup analyses by gender, race, age, baseline level of the outcome variable, baseline number of medications used to treat the outcome, and CKD status, as appropriate, corresponding to the above objectives.
10. To establish a repository of biospecimens (plasma, serum, urine, stool, PAXgene) for future research beyond planned assays that have been funded prior to the end of the trial.

5 DESIGN

5.1 Overview

In persons with and without diabetes, elevated BP is the leading cause of stroke and a major risk factor for other CVD, including coronary heart disease and heart failure. Strategies that effectively lower BP include drug therapy and lifestyle modification. Lifestyle modifications,

particularly dietary approaches, have been shown to lower BP in persons without diabetes. However, there is a striking dearth of evidence on BP-lowering, lifestyle modifications, other than weight loss, in persons with diabetes. The DASH4D trial is designed to provide this evidence.

The DASH4D trial builds upon our experience in four NIH-sponsored, feeding studies (DASH,[31] DASH-Sodium,[32] OmniHeart,[33] and OmniCarb[34]). The trial will enroll approximately 100 adults with Type 2 diabetes, systolic BP 120-159 mmHg, and diastolic BP <100 mmHg, to determine the effects, alone and combined, of (a) the DASH4D diet (a DASH-style diet modified for people with diabetes) vs. comparison diet (typical of what many Americans with diabetes eat) and (b) lower sodium intake vs. higher sodium intake on BP.

The core design is a 4-period, single-site, crossover feeding study with 5-week periods. Participants are fed each of 4 isocaloric diets, presented in random order:

1. DASH4D diet with lower sodium
2. DASH4D diet with higher sodium
3. comparison diet with lower sodium
4. comparison diet with higher sodium.

Detailed descriptions of each of the dietary patterns are included in section 7.1, but in brief, the DASH4D diet is similar to the original DASH diet, but is lower in carbohydrates and higher in unsaturated fat than the original DASH diet, and therefore, is more consistent with dietary recommendations for persons with diabetes than the original DASH diet. The comparison diet reflects what many persons with diabetes currently consume. The lower sodium intake of approximately 1500 mg/day (at 2000 kcal) has been shown to lower BP in persons without diabetes, and has been recommended in some dietary guidelines.[35] The higher sodium intake of approximately 3700 mg/day (at 2000 kcal) is based on estimated average intake in the US.

Outcomes are measured at the end of each feeding period. The primary outcome is end-of-period, office-based systolic BP. The secondary outcome is end-of-period diastolic BP. Other outcomes are measures of glycemia, plasma lipid levels, patient symptoms, and estimated CVD risk.

A participant is considered to have completed the study if he or she has completed all phases of the trial, including the last visit or the last scheduled procedure shown in **Table 3** (**Schedule of Activities and Measurements**). The end of the trial is defined as completion of the last visit or procedure in the trial globally.

Similar to our prior feeding studies (DASH,[31] DASH-Sodium,[32] OmniHeart,[33] and OmniCarb[34]), we expect that the results of the DASH4D trial will be immediately applicable to public health and clinical guidelines and will influence nutrition policy. Furthermore, the trial will provide a rigorous platform to assess the impact of diet and sodium intake on a diverse array of other outcomes in persons with Type 2 diabetes.

6 STUDY POPULATION AND ELIGIBILITY

6.1 Study Population

The study population will consist of approximately 100 adults, ages 18 and older, who have Type 2 diabetes, a systolic BP of 120-159 mmHg, and diastolic BP <100 mmHg. We will attempt to enroll approximately one-third of participants from each of 120-129, 130-139, and 140-159 mmHg systolic BP ranges, about 50% women, and about 50% African-Americans.

6.2 Eligibility Criteria

Table 1 presents the eligibility criteria for the study. In addition to safety considerations, eligibility criteria were selected to exclude individuals with medical conditions, treatments, and special dietary requirements, or medications that would affect BP or the ability to complete the protocol. Eligibility will be determined over a series of contacts, as detailed in section 8.1 (Data Collection Contact Schedule).

Table 1. DASH4D Eligibility Criteria.

Inclusion Criteria

▪ Age 18 or older
▪ Diabetes Mellitus Type 2 defined by:
  ▪ HbA1c ≥6.5%, or
  ▪ Treatment of diabetes with diabetes medication(s)
▪ Baseline systolic BP of 120-159 mmHg (based on average across 3 screening visits)
▪ Baseline diastolic BP <100 mmHg (based on average across 3 screening visits)
▪ Willing and able to eat on site for one meal per day, 3 days per week, and eat only and all food provided as part of the study diets during the controlled feeding periods (run-in and four 5-week feeding periods). *Note that actual frequency of on-site dining may be fewer than 3 days per week due to COVID-related restrictions, but participants will still need to be on site to pick up food and be weighed 3 days per week, and will still be expected to have meals monitored (in-person or remotely) for one meal per day, 3 days per week.*
▪ Willing and able to complete required measurement procedures
▪ Have access to a mobile device or computer with video conferencing capabilities, or be willing to use a device for video conferencing provided by the study

Exclusion Criteria

▪ <u>Laboratory Exclusions</u>
  ▪ Serum potassium ≥5.2 mmol/L or <3.5 mmol/L
  ▪ Estimated GFR <30 mL/min/1.73m^2 by commercial lab result (note that prior to 7/12/22, the lab was using the race-based CKD Epi equation, and on/after 7/12/22, the lab switched to using the CKD-Epi 2021 equation, which does not provide different estimated GFR by race)
  ▪ HbA1c >9.0%

▪ <u>Medication Exclusions</u>
  ▪ Unstable dose (i.e., change in the 2 months prior to screening or prior to randomization) of any of the following:
  ▪ Anti-hypertensive medications
  ▪ Sodium-glucose co-transporter 2 (SGLT2) inhibitors or glucagon-like

peptide-1 (GLP-1) receptor agonists
        - Stimulants, including oral medications for asthma or chronic obstructive pulmonary disease (COPD)
        - Hormone replacement therapy or thyroid hormone
        - Weight-increasing psychotropic agents
            - Antipsychotic agents
            - Lithium
            - Mirtazapine
    - Use of any of the following medications:
        - Potassium supplementation in any form, including a multivitamin or electrolyte drink mix, with a dose >99 mg/day, which is the allowable amount in over-the-counter products
        - Prandial or short-acting insulin
        - GLP-1 receptor agonist if on weight loss dose
        - Warfarin (Coumadin)
        - Chronic oral corticosteroid (intermittent use is okay)
        - Weight loss medications
        - Tirzepatide (Mounjaro™)
    - Unwillingness to keep same dose of vitamin, mineral, and botanical supplements
    - Any medication not compatible with participation as determined by the investigators

- Medical History Exclusions
    - Type 1 diabetes
    - Hypoglycemia requiring hospitalization or the assistance of another person in the last 12 months
    - Active CVD or any event in the prior 6 months, including coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA), myocardial infarction (MI), cerebrovascular accident (CVA), or congestive heart failure (CHF) exacerbation requiring hospital admission
    - Cancer diagnosis or treatment in the last 2 years (benign tumors or non-melanoma skin cancer or localized breast or prostate cancer not requiring chemotherapy is acceptable)
    - Active inflammatory bowel disease, bowel resection, malabsorptive syndrome, pancreatitis (episode within past year), history of Roux-en-Y gastric bypass, or history of other bariatric surgery that limits food intake volume or that requires a specific diet plan
    - Pregnancy or lactation or planned pregnancy
    - Any emergency department (ED) visit for asthma or COPD in the last 6 months
    - Any other serious illness or condition not compatible with participation as determined by the investigators

- Physical Exclusions
    - Body weight >420 pounds
    - Arm circumference >50cm
    - Weight loss or gain of >5.0% of body weight during 2 months prior to screening, or large weight change during screening prior to randomization

▪ Lifestyle and Other Exclusions ▪ Significant food allergies, preferences, intolerances, or dietary requirements that would interfere with diet adherence ▪ Not able to self-monitor glucose if needed ▪ Consumption of more than 14 alcoholic drinks per week or consumption of more than 6 drinks on one or more occasion per week ▪ Active substance use disorder that would interfere with participation ▪ Participation in or planning to start weight loss program ▪ Current participation in another clinical trial that might affect blood pressure or ability to comply with study procedures ▪ Planning to leave area prior to end of study ▪ Investigator discretion
Continuous Glucose Monitoring (CGM) Ancillary Study Exclusions
▪ History of allergic skin reaction to adhesive ▪ Implantable pacemaker *Note: Participants excluded from, or who do not wish to participate in, the CGM ancillary study are still allowed to participate in the DASH4D trial*

6.3 Screen Failures and Rescreening

Screen failures are defined as participants who consent to be screened for the trial or to participate in the trial but are not subsequently randomized to the study intervention. A minimal set of screen failure information will be collected to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and to respond to queries from regulatory authorities.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a modifiable exclusion criteria, including initial laboratory value, may, at the discretion of a study clinician, be rescreened. The participant will be permitted to be randomized if the subsequent value falls within the eligible limits.

6.4 Recruitment

The investigative team has an exceptional track record in recruiting and retaining participants. In the NHLBI-sponsored DASH, DASH-Sodium, OmniHeart, and OmniCarb feeding studies, which enrolled individuals without diabetes, the Hopkins center exceeded its overall recruitment goals in each study. In the NIDDK-sponsored Look AHEAD trial, a behavioral intervention trial in persons with Type 2 diabetes, the Hopkins center likewise exceeded its recruitment goal. As in previous recruitment drives, we will implement several approaches simultaneously, with a focus on strategies that were successful in the Look AHEAD trial.

Initially, we plan to send invitational letters and brochures to prior study participants, use print and digital media (e.g., advertisements and stories in community-based newspapers), implement targeted mass mailings of brochures to persons with diabetes, and word-of-mouth.

- *Prior study participants* – in some of our prior trials, we enrolled persons with diabetes. In other studies, we screened out persons with diabetes who still expressed interest in our studies. We will send invitational letters and/or brochures to those screenees and participants who have expressed interest in our studies.

- *Mass media* – advertisements and/or stories in community newspapers and other print and digital media.
- *Targeted mass mailings* – commercial lists of individuals with Type 2 diabetes. These individuals have consented to receive materials related to diabetes.
- *Word-of-mouth* – individuals often learn about our studies informally, through other screenees, participants, and staff.

In addition, we will consider other strategies as listed below:

- *Targeted mailings and/or MyChart invitations based on computerized searches of outpatient databases at Johns Hopkins* – at Hopkins, the investigators will introduce the trial to the leaders of clinics at which patients with diabetes are managed. After securing approval from clinic leadership, we will apply to the Core for Clinical Research Data Acquisition (CCDA). As part of the process, we will use search criteria that match our enrollment criteria. With appropriate approvals, we will send invitations via MyChart. We will also consider sending mailed brochures and/or invitational letters to persons identified through the database searches. We will consider the following clinics: General Internal Medicine and Diabetes clinics at Johns Hopkins Hospital, and affiliated clinics, potentially, Johns Hopkins Community Physicians, as well as General Internal Medicine and Diabetes clinics at Bayview.
- *Targeted mailings to patients at medical facilities other than Johns Hopkins* – similar to the approach at Johns Hopkins, we will secure approval from clinic leadership and then follow prevailing guidelines at these institutions. Potential centers include Greater Baltimore Medicine Center, Good Samaritan Hospital, and Jai Medical Clinics.

6.5 Retention

The investigative team recognizes the vital importance of participant retention, which is critical to the internal validity of the trial. While feeding studies have substantial requirements, we have developed policies and procedures which reduce participant burden and enhance the participant experience. Specifically, we offer convenient times for appointments and meals at our facility in Woodlawn, which has plentiful parking and easy access. We also provide participant reimbursement and modest non-financial incentives. Finally, we make a point of sharing study results with participants at a celebratory closeout event. All of these factors promote goodwill and enhance retention.

7 INTERVENTION

7.1 Intervention Diets

We will study the following four diets (two dietary patterns each with two levels of sodium intake):

1. DASH4D diet with lower sodium
2. DASH4D diet with higher sodium
3. comparison diet with lower sodium
4. comparison diet with higher sodium.

Rationale

Blood pressure is an important determinant of outcomes in diabetes, but evidence is lacking on the effects of overall dietary pattern and sodium intake on BP in persons with diabetes.

DASH4D dietary pattern

The composition of the DASH4D dietary pattern is guided by prior evidence of BP lowering within the general population without diabetes. The DASH dietary pattern, which emphasizes fruits, vegetables, and low-fat dairy products, includes whole grains, poultry, fish, and nuts, and is reduced in red meat, sweets, and sugar-containing beverages, is currently recommended by the ADA for persons with diabetes and hypertension.[5] However, there are no prior feeding studies testing the efficacy of the DASH diet for BP lowering in a population with diabetes. The DASH4D diet is based on the original DASH diet[31,32] and the subsequent DASH-style diet, reduced in carbohydrate and higher in unsaturated fat, which was tested in the OmniHeart study.[33] The target intake of potassium in the DASH4D diet is somewhat lower than that used in DASH because some participants in the DASH4D trial will have early CKD. The target potassium level is the same as that used in the CKD-K feeding study, which was a trial of higher vs. lower potassium diets among persons with CKD (https://clinicaltrials.gov/ct2/show/NCT00949585).

Comparison dietary pattern

The comparison dietary pattern is based on a typical American diet,[36,37] which is also similar to what Americans with diabetes eat.[38] The macronutrient distributions are generally at the average of typical US intakes. The DASH studies[31,32] suggest that specific nutrients (e.g., micronutrients and fiber) are important for BP lowering in the general population, and therefore, we evaluate a larger contrast between the DASH4D and comparison diets for those nutrients. Micronutrient targets for the comparison diet in this study are generally near the 25th percentile of usual US intake (with the exception of sodium which is discussed below).

Sodium levels

The ADA recommends that persons with diabetes consume fewer than 2300 mg of sodium per day, consistent with the recommendation for the general population.[39,40] In DASH-Na,[32] BP was lowered further for the low sodium diet which resulted in daily urinary sodium excretion of 1500 mg. The 75th percentile of dietary sodium intake in the US is estimated at 3700 mg/day. Therefore, we have selected a level of 3700 mg/day for the higher sodium diet and a level of 1500 mg/day for the lower sodium diet.

Nutrient Goals

Table 2 displays the nutrient targets of the two dietary patterns at the 2000 kcal level. The four study diets were creating by adjusting only sodium within each diet pattern (DASH4D diet higher sodium, DASH4D diet lower sodium, comparison diet higher sodium, comparison diet lower sodium).

Table 2. Nutrient Targets for the DASH4D and Comparison Diets.

Diet Patterns based on 2000 kcal	DASH4D Diet	Comparison Diet
Carbohydrate (% kcal)	45%	48%
Fat (% kcal)	37%	37%
Saturated	<10%	<10%
Protein (% kcal)	18%	15%
Fiber (g/day)	>30	<13
Sodium (mg/day)	Lower: 1500 Higher: 3700	Lower: 1500 Higher: 3700
Potassium (mg/day)	3900	1950
Magnesium (mg/day)	476	238
Calcium (mg/day)	1200	600

Calorie Levels

Participants are fed sufficient calories to maintain their weight during each feeding period. Participants will be provided with one of 5 calorie-level diets (1500, 2000, 2500, 3000, or 3500) with additional 100 calorie unit foods to supplement. For the run-in period, the initial calorie level is estimated using both the Mifflin St. Jeor equation to estimate resting energy expenditure and an activity factor to obtain an estimate of total energy requirements. Participants' weights are monitored by a registered dietitian 3 times per week. Weight gain of more than 3% or weight loss of more than 2% from baseline weight will prompt calorie adjustments.

Menu Cycle

Each of four feeding periods lasts 5 weeks, during which time participants are provided all of their food, snacks, and most caloric beverages (see section on beverages below). The anticipated break between feeding periods will last 1 or more weeks; the break allows ad libitum food intake. We attempt to have participants complete their sequence of four diets during consecutive intervention feeding periods, but in some cases, participants may have a "gap" (due to the COVID epidemic, personal illness, personal circumstances, etc.) where they miss a feeding period. In situations with a missed intervention period, participants resume their randomized sequence of diets upon returning to finish out their four diets as assigned. Below is an example illustrating feeding with a one-period gap:

	Study Intervention Period 1	Study Intervention Period 2	Study Intervention Period 3	Study Intervention Period 4	Study Intervention Period 5
As planned	Assigned Diet #1 (Participant's FP1)	Assigned Diet #2 (Participant's FP2)	Assigned Diet #3 (Participant's FP3)	Assigned Diet #4 (Participant's FP4)	
With gap	Assigned Diet #1 (Participant's FP1)	Missed due to illness	Assigned Diet #2 (Participant's FP2)	Assigned Diet #3 (Participant's FP3)	Assigned Diet #4 (Participant's FP4)

Menu Development & Strategy

During the planning phase, a 7-day menu cycle is developed for each diet (DASH4D diet higher sodium, DASH4D diet lower sodium, comparison diet higher sodium, comparison diet lower

sodium). In brief, the planning process involves preparation of 7 full-day menus (breakfast, lunch, dinner, and snacks) for each diet at each of 5 calorie levels (1500, 2000, 2500, 3000, and 3500); each full-day menu is designed to meet the nutrient targets as displayed in **Table 2**. The difference between the nutrient target and the corresponding estimate from the Food Processor® software is assessed. For each of the four 7-day menu cycles, acceptable variation (the difference as a percent of the target) is a difference of ±2% kcal for total fat, saturated fat, carbohydrates, and protein. Acceptable variation is ±5% for sodium, potassium, and calcium and ±10% for magnesium and fiber. In addition to the full-day menus, diet-specific unit foods are developed to meet caloric needs between the calorie levels. The nutrient distribution of the unit foods corresponds to nutrient targets of the diet.

Beverages

Participants are permitted to consume up to three (3) servings per day of non-caloric coffee, tea, or soda (caffeinated, decaffeinated, or caffeine-free), where one serving is equal to 8 fluid ounces of coffee or tea or 12 fluid ounces of diet soda. Participants are also permitted to consume up to one (1) alcoholic beverage per day, equal to 12 fluid ounces of beer, 5 fluid ounces of wine, or 1.5 fluid ounces of distilled spirits. Participants are permitted to have an unrestricted amount of designated non-caloric, caffeine-free, unsweetened or artificially sweetened beverages that are sodium- and potassium-free (e.g., water, certain types of Crystal Light and sparkling waters, etc.).

7.2 Food Acquisition, Handling, and Distribution

Quality control procedures developed by ProHealth's research kitchen staff during prior studies are used to monitor food procurement, preparation, and distribution. Detailed food preparation procedures and standardized recipes are developed to ensure that participants receive the same diets within each group.

Purchasing & Acquisition

Once menus are formulated, all foods are identified and selected to promote consistency throughout the study. Food will be obtained from local vendors or using online shipping for items not available in store. Specific national brands are selected for purchase, and for meats and produce, purchasing specifications are provided. When foods arrive on site, they are inspected by trained kitchen staff to ensure proper quality and that specifications have been met. Any foods not meeting the specifications are returned and replaced with the correct item. Foods are properly stored until ready to use.

Production and Storage

Food production and storage is conducted according to state and county public health guidelines. The process of preparing foods is labor intensive. In this process, kitchen staff weigh, portion, and package individual food items for the four diets, according to the calorie levels of the participants being fed. Ingredients are weighed on electronic balances to the nearest 0.2 grams. Table salt (sodium chloride) is weighed to the exact number. Mixed foods are prepared in batch quantities, individually portioned, weighed, sealed, labeled, and frozen until ready to use. Freezers are maintained at 0°F, and refrigerators are maintained at 40°F. The cooks prepare all cooked items (e.g., casseroles, meats); in the process, they measure raw product, cook the items

following standard hygienic procedures, and store the individualized portions. Some portion-controlled packaged food items are used for production efficiency.

Trays are assembled for on-site meals, and to-go bags and coolers are assembled for distribution of off-site meals, by diet and calorie level. Kitchen staff independently recheck the assembled foods to confirm the accuracy of food delivery.

Feeding Logistics

The feeding protocol is similar to that used in the DASH,[31] DASH-Sodium,[32] OmniHeart,[33] and OmniCarb[34] trials. All food is provided to participants for each of the four 5-week feeding periods (i.e., controlled feeding days) of the study. Participants are instructed to eat all of the food provided for each controlled feeding day and to consume no additional food, other than select approved beverages (under 7.1 above). On all controlled feeding days, participants are expected to complete a daily food diary (see Measures of Compliance under 7.4 below).

Approach to on-site and remote feeding

In prior feeding studies (DASH,[31] DASH-Sodium,[32] OmniHeart,[33] and OmniCarb[34]), participants were required to eat one on-site meal in the ProHealth dining area each weekday. During more recent feeding studies, the frequency of on-site eating was reduced to three days per week.[41] During the period when Johns Hopkins is restricting clinical research involving group activities due to COVID-19, we may minimize on-site feeding, replacing this with off-site feeding with remote monitoring using video conference. In addition, we will implement protocols to minimize the risk to participants while engaging in on-site activities, as detailed below. Based on experience with prior feeding studies at ProHealth, we expect the vast majority of participants will have access to a mobile device or computer with video conference capabilities, and thus will be able to participate in remote monitoring. If participants do not have access to video conferencing, we may provide participants a study device.

In brief, while there are COVID-related restrictions on group gatherings, participants will eat three on-site meals during their first week of the study, which is necessary to ensure that participants understand the feeding protocols and can receive feedback from study staff. Participants will also eat at least one remote-monitored meal during the run-in period. A hybrid approach may be used, where participants will still have three monitored meals per week, but those meals may include a mix of on-site or remote monitoring. When COVID-related restrictions on group gatherings are fully lifted, we will ask participants to eat three meals per week on-site, as we originally intended. We recognize that COVID restrictions are a fluid situation, and we will be prepared to adapt between on-site and remote-monitored feeding as needed.

On-site feeding

Participants will be scheduled to eat either lunch or dinner at the ProHealth study site. On each day of on-site feeding, a kitchen staff member, typically a study dietitian, meets with the participant to elicit general feedback, address any participant concerns, track weight, and adjust calorie level to ensure eucaloric diet, if needed. Staff review the daily diary for the days since the last on-site visit for completeness and address any compliance concerns. At the end of the meal, a kitchen staff member checks the tray for compliance, and then provides take-home meals

for off-site consumption until the next scheduled visit (either on-site feeding day or on-site visit including meal pick up).

To address participant safety when there are COVID-related restrictions on research involving group gatherings, participants will be scheduled to eat separately (one person per room) in the ProHealth dining area and other rooms close to the metabolic kitchen, in 1-hour shifts, with 30 minutes between shifts for cleaning. If restrictions are gradually lifted to allow more than one person per room, we will follow prevailing guidelines related to number of participants and physical spacing (e.g., seating 2 participants at one time in the ProHealth 625 sq. ft. dining room, and 3 participants in the adjacent 1218 sq. ft. conference room would allow participants to be seated at their own table on opposite ends of the rooms, at least 18 feet apart). Participants will be asked to wear a mask while not eating, leave the building as soon as they are done with study procedures, and minimize interaction with other participants. Study staff monitoring participants will wear masks and face shields and remain physically distant to the extent possible.

Remote-monitored feeding

If remote monitoring is required, participants may be scheduled for one meal to be remote-monitored up to three days per week. They will be asked to prepare their food and then to sign on to the remote monitoring video conference. Video conferencing will be conducted using a Johns Hopkins-approved HIPAA compliant platform (e.g., Johns Hopkins Medicine Zoom Web Conferencing, a cloud-hosted online collaboration platform enabling real-time communication by computer video). All participants eating the remote-monitored meal will sign on to the same group video conference, allowing participants to interact with each other and allowing staff to monitor multiple participants at once. A kitchen staff member, typically a study dietitian, will host these meetings and monitor participants' meals to ensure adherence, elicit general feedback, and address any participant questions or concerns. If participants have questions or concerns that they would prefer to discuss privately, these will be addressed by kitchen staff in an individual "break-out room" or by individual communication after the video conference. During remote-monitoring, participants will come to the study center three days per week for brief individual visits, where study staff will weigh the participant, review the daily diary for days since the last on-site visit, and give the participant their food until the next scheduled on-site visit. There will be no interaction among study participants for these brief visits.

7.3 Measures to Minimize Bias

Randomization

Each participant will receive each of the four study diets for a 5-week period, but the order in which they will receive the diets will vary across participants. Prior to the end of run-in feeding, participants will be randomized to a sequence of diets via a computer-generated assignment. The order of the diets across the sequences will be balanced, and there will be equal allocation of participants to the sequences. The Statistical Analysis Plan will include additional details on randomization.

Randomization occurs prior to the end of run-in feeding in order to allow kitchen staff adequate time to assemble and prepare the foods that are needed for the start of intervention feeding since they need to know each participant's dietary assignment. Participants who exhibit non-

compliant behavior after randomization are retained in the study. However, randomized participants who drop out of the study before starting intervention feeding are replaced provided that they have not been told their randomized sequence of diets.

Blinding

Due to the nature of feeding studies, kitchen staff involved in meal preparation need to have knowledge of participant diet assignment; hence, kitchen staff are unblinded. The assignment is communicated to the kitchen personnel in confidence. However, the data collection staff and outcome assessors are blinded to diet sequence assignment. The ProHealth clinic is organized, in terms of space and personnel, to accomplish blinding of data collectors. Additionally, participants are not told the sequence of diets to which they have been assigned and are masked to post-randomization BP readings.

Until the end of the trial, all investigators, staff, and participants are masked to all trial outcome data, with the exception of the trial statisticians, the data manager, and the Data and Safety Monitoring Board (DSMB). However, a mean of all BP readings, across diets, is provided to each participant at the end of his/her participation.

7.4 Dietary Compliance

Efforts to promote compliance begin at the earliest stages of the study. During screening and orientation, participants are repeatedly provided with information about the demands of participation in the study. At the first screening visit (SV1), participants are given a detailed list of foods provided in the diets. Individuals must be willing to eat each of these foods. If a person cannot eat a key study food, the participant will be excluded, unless a satisfactory substitute for the food is available. Key contacts with dietary staff include an orientation session during screening and an evaluation by a dietitian. The intent of these efforts is to identify and exclude participants who are unwilling or unable to comply with the feeding protocol. During COVID-related restrictions for clinical research involving group gatherings, the orientation session will be done individually on-site and/or virtually, and the evaluation by the dietitian will be completed over the phone.

Prior to randomization, participants will complete a 6-8 day run-in period, during which they get a chance to experience the demands of the study protocol, including eating sample full-day menus from the intervention diets, completing the daily diary, and participating in monitored meals. During the run-in period, participants will be expected to eat one meal with on-site monitoring on three of the days and to eat at least one meal with remote monitoring, while COVID-related restrictions on group activities are in place. When COVID-related restrictions are lifted, participants will be expected to eat three on-site meals during the run-in period (no remote monitoring required). Participants may be excluded from study participation for non-compliance with the protocol during the run-in period. Participants meet with a study dietitian to review progress and assess their continued interest in the trial. Towards the end of run-in, a team that includes the clinical center dietitian, study coordinator, and principal investigator (or clinician investigator) confirms suitability for randomization.

Efforts to promote compliance center on making the foods palatable and convenient to participants' lifestyles; maintaining easy access to staff; providing supportive contacts; and providing a variety of items. Acceptance of the controlled feeding protocol is increased by allowing participants to consume certain beverages (see section 7.1).

Measures of Compliance

During run-in and for each day of each 5-week controlled feeding period, participants complete a daily diary. The daily diary asks about study foods not eaten, non-study foods eaten, and beverages consumed. On on-site feeding days, the daily diaries are returned to and reviewed by kitchen staff. During remote-monitored feeding, daily diaries will be turned in when participants come to pick up their food three times per week. Kitchen staff will review the records and discuss any participant questions individually in person or by phone. If there are questions or concerns regarding a food diary, kitchen staff are to report to one of the study's registered dietitians.

Measures of urinary electrolyte excretion (primarily, sodium and potassium excretion) will be used as objective measures of adherence.

7.5 Food Safety

The safety of our research participants is of primary importance. To that end, food safety and sanitation will be an important concern of the food production team. All Registered Dietitians and Dietary Managers hired in the study will be required to possess a current Serve Safe certificate or make plans to become Serve Safe certified within 2 months of hire. They will also be required to possess a Baltimore County Certified Food Service Manager-Level 1 card, in compliance with Baltimore County regulations. At least one person possessing a current Serve Safe certification and Baltimore County Certified Food Service Manager-Level 1 card will be present whenever food is prepared in the research kitchen. All other staff in the kitchen and dining room will be trained on basics of food safety and will be supervised by certified staff.

Kitchen staff will be trained on proper handling, storing, cooking, cooling, and reheating of foods. They will also be trained on storage, use, and disposal of chemicals used in the kitchen. Kitchen staff will be prepared to manage participants who may have a food allergy that did not exclude the participant from the study, as well as how to manage a food recall, should one occur during the study. Equipment will be maintained to assure proper cooling, storage, and sanitation. As required by the state of Maryland, a Hazard Analysis of Critical Control Points (HACCP) plan will show how food is protected at each potential food contamination point in the process.

Food Transportation and Home Care of Foods

All participants will be given guidelines for transporting and handling of study food once it leaves the site. They will be offered ice packs and coolers, if needed, and instructed to get foods to their home (or work) and refrigerated as soon as possible. Participants will also be instructed not to eat any suspicious foods, and to report any foods that appear spoiled to the study dietitians. They will be asked to keep sufficient space in their refrigerators to accommodate research foods.

7.6 COVID-19 Safety Measures

As the ongoing COVID-19 pandemic continues to evolve, study staff will keep up-to-date on the latest JHU COVID-19 restrictions, guidelines, and best practices. Some of the initial safety measures we will implement to protect study participants during the pandemic are described below. Over the course of the study, as vaccination becomes more widespread and the pandemic is hopefully brought under control, we anticipate an easing or elimination of some of these safety procedures.

- Study staff will adhere to all JHU COVID-19 safety policies, including daily electronic symptom screening, PPE, hand hygiene practices, and physical distancing as much as possible
- Study staff will wear masks and face shields when there is direct interaction with participants
- Duration of interactions with study staff will be minimized wherever feasible
- Participants will be screened for COVID-19 symptoms or exposures before entering the ProHealth facility
- Participants will be required to wear a mask at all times while in the facility except while eating or drinking. They will be provided a mask if they did not bring one.
- No visitors will be permitted, with the exception that one caregiver may accompany a participant with a disability
- Participants in the facility will follow one-way flow to minimize interaction with other participants
- As discussed in 7.2 above, on-site eating may be minimized in favor of remote-monitored eating
- As discussed in 7.2 above, participants eating on site will do so for the minimum time necessary and be appropriately distanced
- Food surface areas will be thoroughly cleaned between each participant contact using hospital-grade sanitizer
- High touch surfaces will be disinfected a minimum of once per day/shift and at end of each day/shift

8 DATA COLLECTION AND MEASUREMENTS

8.1 Data Collection Contact Schedule

Eligibility, baseline, and follow-up data will be collected by phone, through mailings, and at in-person visits. In-person data collection visits will primarily be conducted at the Johns Hopkins ProHealth Clinical Research Unit in Woodlawn, MD. In general, we try to be as flexible as possible to meet the needs of our participants. For example, we might divide or bundle data collection across visits. See **Table 3** below for an overview of proposed data collection items by visit. Some items might be dropped based upon participant burden, scientific considerations, available resources, and the results of field testing. On the pages following **Table 3**, the principal data collection visits for participant-level data are outlined.

Table 3. Schedule of Activities and Measurements.

Activities/Measurements	PS	Screening Visits			RI	RZ	Each Feeding Period (FP1, FP2, FP3, & FP4)				
		SV1	SV2	SV3			W1	W2	W3	W4	W5
Informed consent	o	o+w				w					
Randomization to diet order						✓					
Feeding activities incl. diary					daily		daily----------				
Clinical Assessments & Physical Measurements											
Blood pressure (seated)		✓	✓	✓			✓[1]	✓	✓	✓[2]	
Weight		✓		✓	✓[3]	✓[3]	✓[4]	✓[4]	✓[4]	✓[4]	✓[4]
Height		✓									
Orthostatic blood pressure				✓						✓	
Dietitian evaluation			✓								
Biospecimen Collection & Laboratory Assessments											
HbA1c point-of-care testing		✓									
Non-fasting blood draw			✓[5]					✓[6]			
Fasting blood draw[7]				✓						✓	
Spot urine collection[8]				✓						✓[9]	
24-hour urine collection[8]			✓							✓	
Stool sample			✓							✓	
Self-monitoring blood glucose					✓[10]		✓[10]	✓[10]	✓[10]	✓[10]	✓[10]
Questionnaires/Forms											
Prescreening	✓										
Demographic		✓									
Medical history		✓									
Medications		✓		✓		✓	✓	✓	✓	✓	✓
Dietary information		✓									
Contact information		✓									
Dietary recall			✓	✓							
Symptoms				✓						✓	
Diet acceptability										✓	
CGM Ancillary Study (optional)[11]											
Continuous glucose monitoring			✓[12]---------							✓[13]---------	
Symptom reporting survey			✓[14]---------							✓[14]---------	

o=oral consent, w=written consent

[1]W1 BP is to be measured on day 1 of each feeding period (FP)

[2]W4-W5 BP measured on 5 days during the last 2 weeks of each FP, including ≥2 in W5

[3]Weight measured on each day of on-site feeding during RI and re-measured prior to RZ if not measured within the past 7 days

[4]Weight measured 3 times per week (typically on each day of on-site feeding or food pick-up)

[5]CMP assayed, including serum potassium and creatinine to calculate eGFR for eligibility

[6]Only for participants at-risk for hyperkalemia (see section 10.3): serum potassium assayed for safety monitoring; should occur ~W2 D1

[7]CMP with lipids assayed, and stored specimens (serum, plasma, whole blood, PAXgene)

[8]Sodium, potassium, creatinine, and albumin assayed, and stored specimens

[9]One spot urine sample will be collected during W4-W5, typically early in W4 prior to the start of the 24-hour urine collection

[10]Only for participants at-risk for hypoglycemia (see section 10.3)

[11]Offered to all participants, but optional (see section 0 Appendix B: Continuous Glucose Monitoring (CGM) Ancillary Study for details)

[12]The CGM device will be placed on the participant's arm at SV2 and worn for 14 days or until the start of Run-In, whichever comes first

[13]The CGM device will be placed on the participant's arm between W3 D4 and W4 D1 of each FP and worn for 14 days

[14]The symptom reporting survey should be completed at least daily during each period of CGM device wear

Pre-Randomization

Participants must complete a prescreening evaluation, three in-person screening visits, and a run-in feeding period in order to be randomized (see **Figure 1**). Each screening visit includes questions and procedures designed to determine eligibility for the trial, and the sequence and timing of data collection during these screening visits is designed to maximize efficiency in excluding ineligible participants. Some screening visit procedures may be shifted to a different screening visit as needed. Screening may be stopped as soon as the participant is determined to be ineligible, even if the exclusionary information was obtained out of sequence. For instance, if during the prescreening contact a screenee mentions having a medical condition that is exclusionary, the screenee will be excluded from further participation even though medical eligibility is normally not reviewed until a later visit. The run-in period is designed to identify and exclude those individuals not likely to comply with the dietary requirements of the trial and to verify the caloric level needed to maintain weight. **Table 3** (**Schedule of Activities and Measurements**) summarizes the elements of each screening visit, as well as the procedures done during run-in and intervention.

Prescreening Contact (PS)

PS is typically a telephone contact. In some instances, PS may be conducted in person, such as at a community health fair. PS involves a brief questionnaire intended as a fast, efficient way to eliminate ineligible participants prior to formal screening visits. Individuals who participate in PS are either excluded from further participation or scheduled for SV1.

Screening Visit 1 (SV1)

SV1 is intended as a brief, in-person visit to identify major exclusionary criteria. SV1 will be divided into two parts. The first part will include oral consent for BP measurement. It is our experience that more than half of screened participants will be ineligible based on low BP, and this approach minimizes participant and staff burden. If the participant meets the SV1 BP eligibility cut points (shown in **Table 4**), based on the average of the three SV1 BP measurements, the participant will proceed to the second part of SV1, starting with written informed consent for the remaining screening procedures and visits, and the run-in period. The remaining SV1 questionnaires and procedures will then be completed: (1) demographic questionnaire, (2) medical history questionnaire, (3) review of medications, (4) dietary information questionnaire, (5) height and weight measurement, and (6) finger-stick for point-of-care HbA1c testing (non-fasting).

During SV1, study staff will review the completed forms to preliminarily assess eligibility. During or after SV1, for participants not already documented as meeting exclusion criteria, a study clinician will review the completed medical history questionnaire and medications to determine eligibility. The study clinician will discuss with the participant any items requiring clarification (in person or by telephone). Additionally, a study dietitian will review the completed dietary information questionnaire to determine eligibility. The study dietitian will discuss with the participant any items or potential issues with study participation requiring clarification (in person or by telephone).

Screening Visit 2 (SV2)
SV2 is an in-person visit and should generally occur seven days or more after SV1. SV2 includes (1) triplicate BP measurement and (2) a non-fasting blood draw for serum K and eGFR eligibility. A 24-hour dietary recall will be completed during SV2. Participants will be given instructions and supplies for a collecting a stool sample and 24-hour urine collection at home, to be brought back prior to or at SV3.

SV2 BP eligibility cut points are listed in **Table 4** and are determined by averaging the three BPs from SV1 and the three BPs from SV2 (six total measurements).

During or after SV2, participants will meet in-person or by phone with a study dietitian to review the dietary information they provided and determine if they will be able to tolerate the study diets and adhere to the feeding study procedures. This time will also be used by the dietitian to discuss the participants' usual activity and assign them an activity factor for determining their calorie levels as discussed in 7.1 above.

Screening Visit 3 (SV3)
SV3 is an in-person visit and should generally occur seven days or more after SV2. Participants should bring their 24-hour urine collection and stool sample with them to SV3 (if not already returned prior to that). SV3 includes: (1) triplicate BP measurement, (2) weight measurement, (3) an orthostatic hypotension assessment, (4) a fasting blood draw, (5) a review of medications, (6) a symptoms questionnaire, and (7) spot urine collection. A second 24-hour dietary recall will be completed either remotely between SV2 and SV3 or at SV3.

SV3 BP eligibility cut points are listed in **Table 4** and are determined by averaging the three BPs from SV1, the three BPs from SV2, and the three BPs from SV3 (nine total measurements).

Table 4. Blood Pressure Eligibility Criteria by Screening Visit.

Visit	Systolic BP Eligibility Range[a] (mmHg)	Diastolic BP Eligibility Range[a] (mmHg)
SV1[b]	118-170	<105
SV2[c]	119-165	<103
SV3[d]	120-159	<100

[a]Participants must meet BOTH the systolic AND diastolic BP criteria.
[b]Based on average of SV1 BP measurements (3 total).
[c]Based on average of SV1 and SV2 BP measurements (6 total).
[d]Based on average of SV1, SV2, and SV3 BP measurements (9 total).

Run-In (RI) Period
All participants who are eligible based on the three screening visits, including laboratory results, undergo a run-in period, during which they will be provided sample study menus from the 4 intervention diets for a total of 6-8 days. The run-in phase has two main objectives: 1) to identify and exclude individuals who will not comply with the eating requirements of the trial, and 2) to determine the appropriate caloric level for each participant that is needed to maintain weight. Run-in feeding typically will begin within 90 days after the completion of SV1; no minimum length is required between SV3 and the start of run-in.

During the run-in period, participants receive all of their food from the clinic and are required to attend the clinic for at least one meal per day (lunch or dinner) for 3 days during the 6-8 day period. During the period of COVID-related restrictions on group gatherings, participants will also complete at least one remote-monitored meal during run-in. During the run-in period, participants will have their weight measured each day of on-site feeding. Participants will also be asked to complete a daily diary (see Measures of Compliance under 7.4 above).

Participants may be excluded during run-in for non-compliance with the protocol or for unusually large weight change. Non-compliance may include missed meals, poor clinic attendance or remote-monitored meal participation, and over- or under-consumption of food. Unusually large weight change is defined as 5% or greater change between SV3 and the first day of run-in. Additionally, the study team will subjectively evaluate each participant's overall compliance and attitude just prior to randomization and may exclude participants on the basis of this assessment as well.

Randomization (RZ)
Towards the end of the run-in period, eligibility is confirmed and interested participants are asked to sign an informed consent statement that covers the main portion of the trial (randomization and the 4 feeding periods). See 7.3 Measures to Minimize Bias for additional details related to randomization.

Intervention Feeding Periods (FP1, FP2, FP3, & FP4)
For each day of controlled feeding, participants complete a daily diary (see Measures of Compliance under 7.4). On each day of on-site feeding or on-site pick-up of food, weight will be measured. Medication usage will be reviewed every week. For participants who require it, SMBG safety monitoring is conducted every week.

Feeding Period Weeks 1-3 (FP#W1-3)
BP will be measured on day 1 of the first week (FP#W1D1) and then weekly during weeks 2 (FP#W2) and 3 (FP#W3).

Feeding Period Weeks 4-5 (FP#W4-5; “End-of-period” measures)
During the last two weeks of each feeding period, BP will be measured on 5 days, at least 2 of which will be during the last week of the intervention period. In addition, participants will be asked to collect a 24-hr urine and a stool sample at home and return the specimens to the clinic. A fasting blood specimen will be drawn and a spot urine will be collected. Participants will also complete (1) a symptom questionnaire, (2) a diet acceptability questionnaire, and (3) an orthostatic hypotension assessment.

8.2 Measurements
The following sections describe the specific measurements to be collected from participants, in accordance with the above **Schedule of Activities and Measurements.**.

Office-based BP measurement (for the primary outcome)
Systolic and diastolic BP will be determined by the Omron HEM 907XL device which records BP using an oscillometric technique. The Omron device has been validated.[42] BP is obtained by trained and certified data collectors according to a standard protocol, adapted from that used in the Omni-Heart, Sprint, and other trials. Three consecutive measurements (each separated by 30 seconds) are obtained on the right arm of participants after they rest quietly in the seated position for at least 5 minutes. Heart rate and average BP will also be reported by the device.

Other measurements
The trial will collect laboratory specimens, perform physical assessments, and administer questionnaires to participants throughout the study. These data will be used for a variety of purposes—baseline data to describe participants, outcome data to assess the effects of the trial interventions, and mediating variables to assess potential causal pathways.

Laboratory Assessments

- *Hemoglobin A1c (HbA1c):* In order to establish eligibility, a finger-stick for point-of-care HbA1c testing will be performed at SV1.
- *Non-fasting blood draw:* Blood drawn at SV2 will be sent for a comprehensive metabolic panel with lipids, including gamma-glutamyl transferase (GGT), lactic acid dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, total protein, albumin, globulin, albumin to globulin ratio, glucose, blood urea nitrogen (BUN), creatinine, BUN to creatinine ratio, uric acid, calcium, phosphorus, sodium, potassium, chloride, carbon dioxide, cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, calculated low-density lipoprotein (LDL) cholesterol, calculated very-low-density lipoprotein (VLDL) cholesterol, and iron. Results of these labs will be used to establish serum potassium level for eligibility, as well as creatinine level for calculation of eGFR for eligibility.
- *Fasting blood draw:* Participants will be required to fast for a minimum of 8 hours prior to the blood draw at SV3 and at the end of each feeding period. A comprehensive metabolic panel with lipids will be performed in a local, commercial laboratory shortly after collection; laboratory tests include GGT, LDH, AST, ALT, ALP, total bilirubin, direct bilirubin, total protein, albumin, globulin, albumin to globulin ratio, glucose, BUN, creatinine, BUN to creatinine ratio, uric acid, calcium, phosphorus, sodium, potassium, chloride, carbon dioxide, cholesterol, triglycerides, HDL cholesterol, calculated LDL cholesterol, calculated VLDL cholesterol, and iron. HbA1c, fructosamine, and glycated albumin will also be measured from stored specimens at the end of the trial. Aliquots of serum, plasma, whole blood, and a PAXgene tube to study the effects of diet on gene expression, will also be stored for subsequent measurement.
- *Spot urine collection:* Participants will be asked to provide a spot urine sample during screening (SV3), as well as to provide a spot urine sample at the end of each feeding period. Sodium, potassium, creatinine, and albumin will be assayed, and aliquots of urine will also be stored for future measurement.
- *24-hour urine collection:* Participants will be given supplies to collect their urine for a 24-hour period prior to SV3 and prior to the end of each feeding period. Excretion of sodium, potassium, creatinine, and albumin, and total urine volume will be measured to

determine the effects of the diets on urine excretion of the above biomarkers. These measures will provide an objective assessment of diet compliance. Aliquots of urine will also be stored for subsequent measurement.

- *Stool sample:* Participants will be given supplies to collect a stool sample at home prior to SV3 and prior to the end of each feeding period to determine the effects of diet on microbiota. They will also be asked to complete a questionnaire related to the stool specimen collection and recent medications.

Clinical Assessments & Physical Measurements

- *Height:* Height will be measured at SV1 using a stadiometer. Height will be used in the calculation of BMI.
- *Weight:* Weight will be measured using a calibrated scale during SV1, SV3, and each day of on-site feeding or food pick-up during run-in and the four intervention feeding periods.
- *Orthostatic hypotension:* Supine and standing BP will be measured with an Omron HEM 907XL. Participants will lie in the supine position for 5 minutes and undergo 3 BP measurements separated by 30 seconds. Then they will stand with their arm rested on an adjacent Mayo table at 70-80 degrees from their torso. They will then undergo another set of 3 BP measurements separated by 30 seconds each. Participants will also be asked a question about lightheadedness/dizziness upon standing.

Questionnaires

When there are COVID-related guidelines to minimize in-person interactions, questionnaires, wherever possible, will not be administered face-to-face by a staff member. We will use several possible approaches for questionnaire completion to allow maximum flexibility depending on participant preference and needs, while minimizing risks to participants and staff. Possible methods of questionnaire completion include self-administration of forms on-site in a separate room from staff, at-home self-administration of paper-based forms to be returned to ProHealth, staff-administered interviews via telephone or video call, or web-based survey completion using REDCap.

- *Prescreening Questionnaire:* This is used to determine eligibility and includes details related to diabetes history, cardiovascular disease, gastrointestinal conditions, cancer, pregnancy, alcohol consumption, weight, and dietary preferences.
- *Demographic Questionnaire:* A self-reported assessment of basic participant characteristics. It assesses age, gender, race, ethnicity, income, employment, marital status, and education level. This will be collected at SV1.
- *Medical History Questionnaire:* Administered during SV1 to gather details related to past medical history for eligibility and to describe participants at baseline.
- *Medication Use:* A self-reported assessment of current medications. This will be collected at SV1, SV3, during run-in, and during the intervention periods to determine and track medication changes.
- *Dietary Information Questionnaire:* A self-reported assessment of participants' ability and willingness to eat all study foods and comply with the intervention. This will be completed at SV1 and subsequently reviewed by a study dietitian.

- *Food Frequency Questionnaire:* The *Diet History Questionnaire (DHQIII)* will be completed by participants prior to the start of the run-in period to estimate usual nutrient intake for use in exploratory analyses and to describe usual eating patterns.
- *Symptoms Questionnaire:* A list of symptoms will be queried as a Likert scale to assess effects of the diets on the frequency of commonly experienced symptoms.
- *Diet Acceptability Questionnaire:* This questionnaire is administered at the end of each feeding period to determine how much participants liked each diet and how hungry they felt while on each diet.
- *Daily Diary:* These are completed by participants on a daily basis to monitor compliance. See Measures of Compliance under 7.4 for additional details.

9 QUALITY CONTROL AND DATA MANAGEMENT

Principles and Philosophy
The objective of quality control efforts is to ensure that project data and activities are standardized, accurate, and timely, thus minimizing variation not associated with treatment effects. To achieve this, staff are rigorously trained and certified, and key trial activities are monitored routinely.

Staff Training and Certification
Trial staff are trained and certified in three main areas: clinical evaluations, data collection and management, and food preparation and handling. In addition, detailed procedures cover the collection and handling of blood and urine specimens.

Data Collection and Management
All data, including all clinical measurements, eligibility information, and participant questionnaires will be entered into REDCap. Shipments of laboratory specimens and food samples will be regularly logged.

All staff involved in data collection will be trained in the administration each of the questionnaires, as well as data entry into REDCap. At least one data coordinator will be trained in processing and resolving discrepancies. Data coordinators will be trained to understand key concepts of the data management system.

Data Management and Reporting
Data Management System
The official study database (REDCap) will be maintained by a database manager. Staff will enter data into REDCap. Results of central laboratory analyses will be merged with the study database. The database will be monitored regularly for completeness.

Upon prescreening, participants will be assigned a unique study ID. Prior to randomization, the computer checks the database to make sure that all screening activities have occurred, that the participant meets all eligibility criteria, and that all required baseline data have been collected.

Quality Control

The data management system performs range, logic, and missing data checks on all data at the time of data entry. Cross-form edit checks are also performed. Data inconsistencies occurring across forms are summarized in query reports that must be resolved by clinic staff. These audits are re-run periodically to detect unresolved problems. Standardized query reports that summarize problems in the database provide an additional method of assuring data quality.

Reporting

The database manager prepares regular reports summarizing the performance characteristics of the study. These reports are distributed to the members of the study team, appropriate subcommittees, and to the Data and Safety Monitoring Board.

10 SAFETY MONITORING

10.1 Overview

The trial has minimal risks. Still, participant safety is a high priority, and accordingly, we will monitor safety as described in this section. One aspect of this monitoring is to evaluate potential participants during the screening process to determine whether it is safe for them to participate in the interventions. A second aspect is monitoring the safety of enrolled participants. If a participant develops a medical problem, the safety of continuing in the study will be ascertained by a study clinician or designated safety officer, in consultation with the participant's healthcare provider if appropriate.

Substantial effort has been made to identify and minimize potential risks. For instance, those persons with a history of severe hypoglycemia within the past 12 months, with significant kidney disease, or on unstable doses of hypertension or diabetes medications are excluded. We also exclude persons with systolic BP ≥160 mmHg and/or diastolic BP ≥100 mmHg. Such persons are informed of their elevated BP level and advised to consult with their physician. Likewise, persons with active CVD or recent cardiovascular events, eGFR <30 mL/min, a history of high potassium levels, or baseline serum potassium ≥5.2 or <3.5 mmol/L are excluded. See 6.2 Eligibility Criteria for full details on exclusions.

10.2 Interaction with Participants' Healthcare Providers

At the beginning of the study, we will ask participants for permission to contact their healthcare providers. With the participant's permission, a letter will be sent to notify the participant's healthcare provider treating their diabetes of their involvement in the study, and the conditions under which they will be contacted. As detailed below, we will contact the participant's healthcare provider treating their diabetes to inform them of diabetes medication changes. We may also contact their healthcare providers for urgent medical issues that may arise during the study.

10.3 Risks

Risks of Questionnaires, Health History, and Anthropometric Measures
These procedures are routine in clinical research and often clinical practice, and do not pose any significant physical risk. As with all medical information, there is always the risk of psychological distress if personal health information (PHI) is not kept confidential according to the wishes of the participant. In order to minimize this risk, data will be stored in HIPAA-compliant password-protected databases, and written information will be stored in locked files or file-rooms when not attended by research personnel. Data are collected by professional staff who are trained to perform these procedures according to detailed study protocols. The results of clinically study tests will be provided to the patient and treating sources consistent with HIPAA guidelines. Data are used only in aggregate, and no identifying characteristics of individuals will be published or presented.

Risks of Phlebotomy
Blood will be drawn at multiple time points during the study. Risks of phlebotomy include bleeding, swelling, bruising, vasovagal syncope, and pain. The likelihood of bleeding, pain, and/or bruising is high, but this risk is not serious. The risk of vasovagal syncope is low. These are all risks that exist in the course of routine medical care. To minimize risks, blood will be drawn using aseptic technique by trained staff.

Risks of the Intervention
The diet interventions follow dietary patterns of a typical American diet or similar to a healthy diet recommended for the treatment of high BP. The diet interventions have minimal risks; specific risks related to changes in BP, blood glucose, and potassium levels are discussed below in detail.

Discomforts Due to Change in Diet
Participants may experience some bloating and other minor gastrointestinal (GI) discomfort related to the high fruit, dairy, and fiber content of the intervention diets. It has been our experience that GI discomfort is generally minor and resolves soon after changes in diet. For those persons with lactose intolerance, we provide lactase. Participants are monitored for reactions to the diets and, if necessary, the diet can be modified or terminated. Additionally, because the study diets include nuts and seeds, there is a small risk of dental issues (e.g., chipped tooth or broken crown).

Risk of Changes in Blood Pressure
We anticipate that the intervention will affect participants' BP, specifically by decreasing BP in the DASH4D and lower sodium diets. However, for many participants, even the comparison diet with higher sodium might be an improvement compared to their usual diet.

Elevated Blood Pressure (Escape Levels)
In this trial, we will implement safety procedures and BP escape criteria, below, that were developed and implemented in our prior feeding studies (DASH,[31] DASH-Sodium,[32] OmniHeart,[33] and OmniCarb[34]). These safety procedures start with regular monitoring of BP.

For high BP values that do occur, we will implement BP escape levels, i.e., values at which participants will be referred for medical care. Two escape levels of BP are applied in this trial:

1) Escape Level #1: systolic BP >180 mmHg or diastolic BP >110 mmHg → refer for medical care
2) Escape Level #2: systolic BP 171-180 mmHg or diastolic BP 106-110 mmHg → repeat BP within 7 days, and if it also is in or above this range, refer for medical care

For participants meeting BP escape levels and referred for medical care, participants will not be withdrawn automatically from the study, given the well-known variability or blood pressure and the phenomenon of regression-to-the-mean. The decision regarding whether a participant should continue in or be withdrawn from the study will be left up to the participant's healthcare provider and/or the study clinician based on safety concerns. These safety procedures and approaches to decision-making are identical to those used successfully in our prior feeding studies.

Low Blood Pressure Reading

The diets being tested have been shown to lower BP in the general population. When combined with anti-hypertensive medications, the dietary interventions being studied in DASH4D might further lower blood pressure, which is often asymptomatic and which, long-term, has been shown to reduce the risk of cardiovascular and kidney disease. Still, some persons might experience symptoms, such as lightheadedness, and other problems (abnormal laboratory tests). Assessment of low BP readings is complicated because BP readings are exceedingly variable, such that frequent monitoring will detect low readings without clinical consequence.

In this context, we will identify randomized participants with low readings, defined by a systolic blood pressure < 90 mmHg. If the average of the triplicate set of systolic BP readings on a given day is < 90 mmHg, the BP form will flag completion of the Hypotension Event form. Staff will ask the participant if he/she is currently feeling lightheaded or dizzy. If yes, staff will contact a study clinician for guidance while the participant is at the clinic; if no, a study clinician will receive an automatic alert from REDCap and will contact the participant within 2 days to assess symptoms of low BP and any possible explanations for the low reading. Repeat BP readings will be obtained within 8 days (or sooner, if appropriate). If the BP remains < 90 mmHg or the participant is determined to be at risk of low blood pressure, the participant will be advised to see their health care provider responsible for treating their BP for evaluation and possible adjustment of anti-hypertensive medications, or, with permission, the study clinician will directly contact the participant's health care provider.

Risk of Changes in Blood Glucose

It is possible that diet interventions will cause changes in blood glucose. However, we expect changes in blood glucose due to the diet interventions to be small, because all diets will be isocaloric, maintaining participants' caloric intake prior to the study. The primary concern is the risk of hypoglycemia for participants using certain diabetes medications. To minimize the risk of hypoglycemia, individuals using multiple daily insulin injections and those with a history of severe or hospitalized hypoglycemia within the past 12 months are excluded.

Participants will be considered at-risk for hypoglycemia if they are using diabetes medications that are known to cause hypoglycemia: insulin, sulfonylureas, or meglitinides. For participants at-risk for hypoglycemia, we will implement a protocol to prevent and manage hypoglycemic

events, which was adapted from the hypoglycemia protocol used successfully in the Look AHEAD trial.[43] This protocol will be used as a general guide; study clinicians will use their clinical judgment to modify these recommendations for specific participant circumstances. All study procedures to prevent hypoglycemia exist in the course of routine medical care for patients with diabetes.

Hypoglycemia Prevention Education
Participants at-risk for hypoglycemia will be educated on hypoglycemia prevention, including the risks of hypoglycemia, hypoglycemia symptoms, monitoring, and management. Participants will be instructed to check and record their blood glucose if any hypoglycemic symptoms occur. Participants will be instructed to carry a small amount of carbohydrate (e.g., glucose tablets or hard candy) in the event that they experience hypoglycemia.

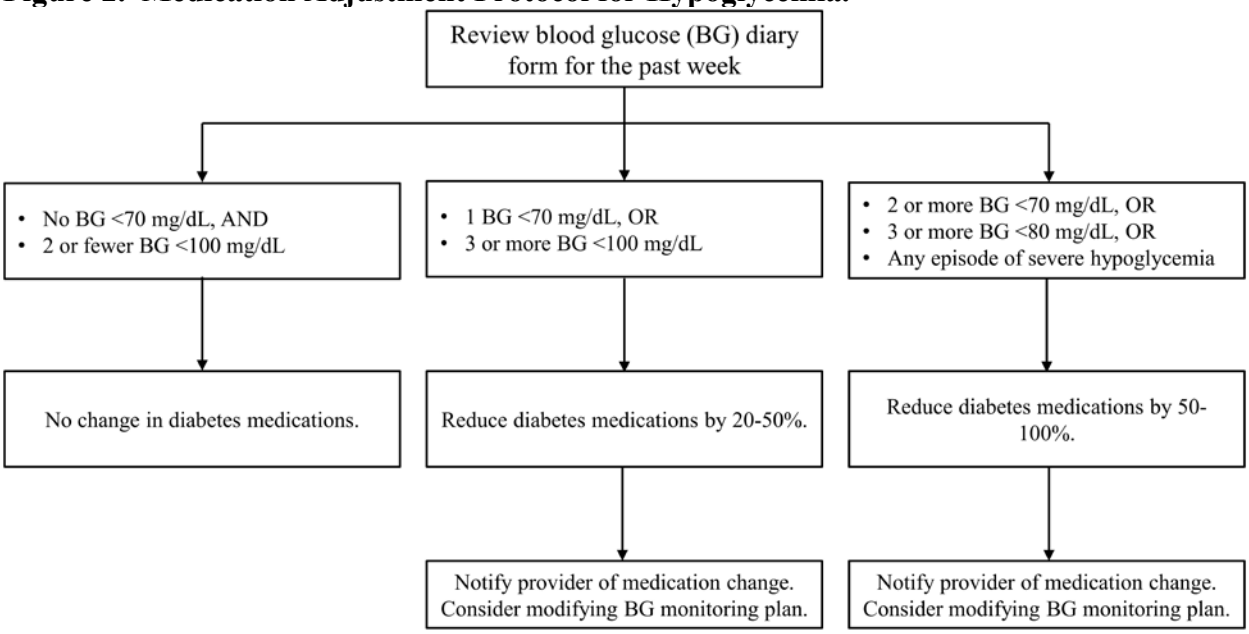
Hypoglycemia Risk Assessment by Study Clinician
Prior to the intervention period, participants at-risk for hypoglycemia will meet with a study clinician, either in-person or by phone, to 1) assess the participant's risk for hypoglycemia during the intervention period, and 2) create a blood glucose self-monitoring plan for the intervention period.

1) **Assessing risk for hypoglycemia** - The study clinician will review the participant's recent home glucose readings and history of hypoglycemic events and symptoms, including any history of hypoglycemia precipitated by changes in diet. If the study clinician determines a participant is at high risk of having hypoglycemic events during the intervention period or may benefit from modifying their diabetes treatment prior to starting the intervention, the study clinician will recommend diabetes medication adjustments (as per **Figure 2** on management of hypoglycemia below) and contact the participant's healthcare provider.
2) **Creating a glucose self-monitoring plan** - The study clinician will review the participant's current glucose self-monitoring practices and recommend whether their self-monitoring should be increased during the intervention period. Participants at risk for hypoglycemia will be required, at minimum, to check a fasting blood glucose two times per week and also with any symptoms that could suggest hypoglycemia or hyperglycemia. Participants who are instructed to increase their glucose self-monitoring during the intervention period will be provided with funds to offset the cost of glucose testing supplies, if needed.

Blood Glucose Self-Monitoring During the Intervention
Participants who are instructed to monitor blood glucose will be given a blood glucose diary form and instructed to record the date, time, and blood glucose values, and any symptoms that could suggest hypoglycemia or hyperglycemia. Blood glucose diary forms will be collected weekly and reviewed by the study clinician on a weekly basis. The study clinician will be notified if there are any blood glucose values <100 mg/dL or >300 mg/dL, or for other blood glucose concerns at the discretion of study staff.

Diabetes Medication Adjustment for Hypoglycemia
We will use the following medication adjustment protocol (**Figure 2**) adapted from the Look AHEAD trial[43] and guidelines from the ADA[44] to modify medications in response to hypoglycemic events, if they occur.

Figure 2. Medication Adjustment Protocol for Hypoglycemia.



Severe hypoglycemia is defined as an event requiring the assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions. If a hypoglycemic event occurs, a study clinician will contact the participant to discuss precipitants (such as skipping a meal), and may recommend behavioral interventions prior to medication change, if appropriate. The participant's healthcare provider treating their diabetes will be notified of any changes in diabetes medications. If frequent or severe hypoglycemic events occur, the frequency of the participant's blood glucose self-monitoring may be increased.

Management of Hypoglycemic Episodes by Study Staff
Study staff interacting with participants will be trained in the basic management of hypoglycemic events including recognizing hypoglycemia symptoms, safe blood glucose ranges, and provision of an appropriate carbohydrate snack. A study physician will be available on-site or by phone at all times to assist with hypoglycemia or other medical issues.

Returning Diabetes Medications to Baseline Levels
Participants whose diabetes medications were reduced during the study period due to hypoglycemia will be instructed to continue the glucose self-monitoring plan instituted during the intervention period for two weeks following the end of the intervention. If they have no blood glucose values <70 mg/dL and two or fewer blood glucose <100 mg/dL per week, increasing their diabetes medications to pre-intervention levels will be considered. Subsequently, the participant will be informed that their healthcare provider should resume

management of their diabetes medications. The participant's healthcare provider will be informed of their diabetes medication regimen at the end of the study.

Risk of Hyperglycemia
The study intervention is not anticipated to increase the risk of hyperglycemia because all study diets are isocaloric and follow the macronutrient balance of a typical American diet. If a participant develops substantial hyperglycemia (blood glucose >350 mg/dL) or hyperglycemic symptoms, the study clinician will inform the participant's healthcare provider. Increases in diabetes medications will be performed by the participants' healthcare provider.

Risk of Changes in Potassium Level
The intervention diet is a DASH-style diet which is higher in potassium than a typical American diet, but within the range of recommended healthy eating patterns. In a general, healthy population, a DASH-style diet should not increase blood potassium levels outside the normal range. As such, previous studies of the DASH diet and similar dietary interventions[31-34] have not monitored potassium levels during the intervention.

Unlike these previous studies, this study includes some individuals who may be at a higher risk for hyperkalemia: those with chronic kidney disease and those who are using certain medications that raise potassium levels. Even among these individuals, there is a low risk of clinically significant hyperkalemia because: (1) we will exclude individuals with a history of hyperkalemia, or with elevated potassium levels during screening, (2) renal potassium excretion is not substantially impaired until the GFR is severely decreased (<20 mL/min/1.73 m^2),[45-49] and we will exclude those with eGFR <30, (3) hyperkalemia as an adverse effect of medications is uncommon, even among individuals with chronic kidney disease,[50-55] (4) in a feeding study enrolling 29 adults with eGFR < 60 mL/min/1.73 m^2 who were randomized to a 4-week dietary intervention with potassium level of 3900 mg/day,[45-49] there were two confirmed cases of hyperkalemia (and both were taking medications or combinations of medications that are exclusions in this trial).

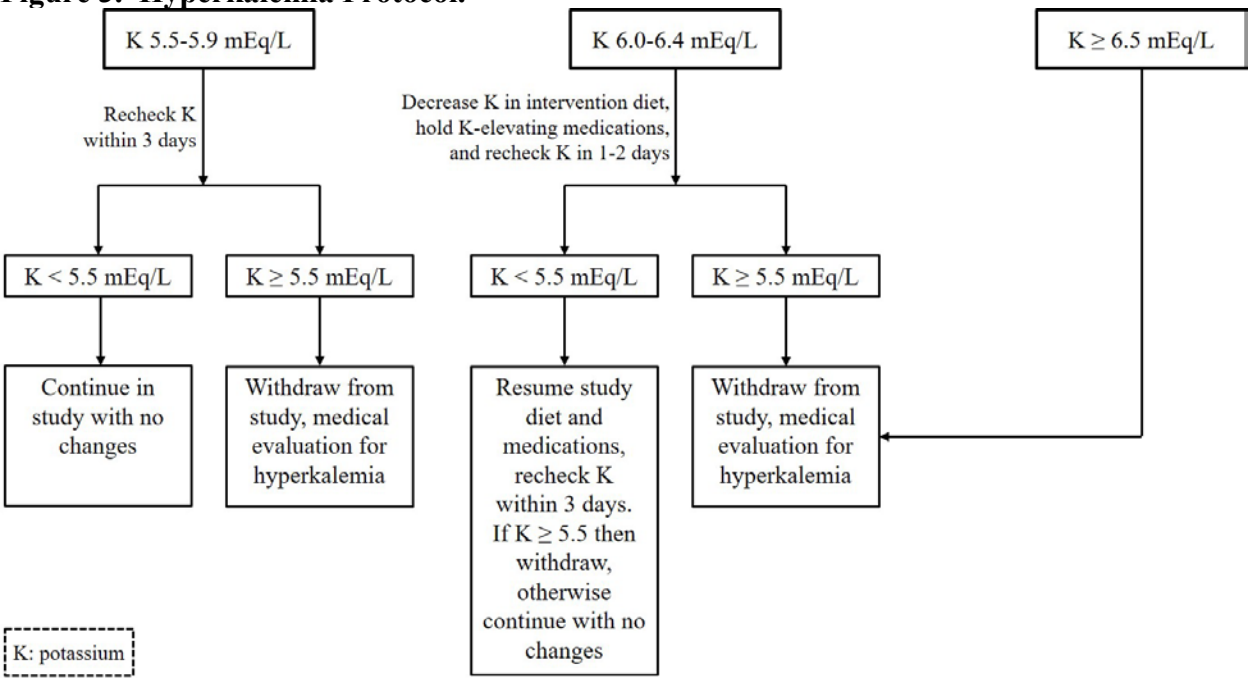
To mitigate the risk of hyperkalemia during this study, we will implement a protocol for potassium monitoring and management of hyperkalemia in at-risk participants. In this trial, participants considered at-risk for hyperkalemia will be those with chronic kidney disease stage 3 or greater (eGFR <60 mL/min/1.73 m^2),[45,47,48] or using aldosterone antagonists (spironolactone or eplerenone) which are associated with increased risk of hyperkalemia in clinical trials.[50] We will not consider participants to be at-risk if they are using medications which cause only minimal elevations in potassium and rarely cause clinically significant hyperkalemia among individuals with GFR > 60, such as beta blockers, ACE inhibitor and angiotensin receptor blockers.[50] Those trial participants who do not meet our 'at risk' criteria for hyperkalemia will not undergo potassium monitoring during the intervention period.

Hyperkalemia Monitoring and Management Protocol
For at-risk participants, potassium levels will be checked at the end of week 1 of each intervention period. It is well-recognized that serum potassium may be falsely elevated due to issues with hemolysis of the lab specimen, so if the laboratory reports hemolysis, potassium will be rechecked within 3 days.

Hyperkalemia is defined as a serum potassium ≥ 5.5 mEq/L. If hyperkalemia is detected in the absence of hemolysis, investigators will follow the protocol outline in **Figure 3**. Mild hyperkalemia (potassium 5.5-5.9 mEq/L) will be rechecked within 3 days. Moderate hyperkalemia (potassium 6.0-6.4 mEq/L) will be rechecked in 1-2 days, and while awaiting results the participant's potassium-elevating medications will be held and their dietary potassium reduced to a low potassium diet (≤ 2400 mg of potassium per day in total). Participants with moderate hyperkalemia whose potassium is normal on repeat testing will be tested again after reintroducing their study diet and held medications. Any participant with hyperkalemia on repeat testing, or a single potassium value ≥ 6.5 mEq/L, will be withdrawn from the study. Any participant withdrawn from the study for hyperkalemia will receive medical evaluation for hyperkalemia by a study clinician which may include referral to the emergency department, or making dietary or medication changes in coordination with the patient's healthcare provider.

Additionally, all participants will have potassium levels checked as part of their fasting laboratory panel at the end of each feeding period. If hyperkalemia occurs at this time in a participant at-risk for hyperkalemia, we will follow the hyperkalemia protocol as described below. If hyperkalemia occurs in a participant not at-risk, the laboratory will be rechecked and subsequent action will be at the discretion of the study clinician.

Figure 3. Hyperkalemia Protocol.



Hypokalemia

We do not anticipate clinically significant hypokalemia as a result of study interventions. If a potassium level < 3.0 mEq/L occurs, the participant will be withdrawn from the study, they will be given a potassium supplement, and a follow-up serum K level will be arranged with a primary provider within 2-3 days. If the K is 3.0-3.4 mEq/L, it will be repeated. If it is still in this range, then the patient will be given K supplementation and a follow-up K level will be checked. If the

participant frequently or persistently has a K < 3.5 mEq/L despite supplementation, then we will withdraw that participant from the study.

Adverse Events
Other than abnormal laboratory values or high/low BP values, adverse events are ascertained through participant direct report to study staff or via an item on the Daily Diary that participants are expected to complete for each day of intervention feeding. The item asks "Is there anything you would like us to know regarding your participation in this study, including any new medications/pills, vitamins/supplements, or any health problem or issue?" with a yes/no option and a space to describe. Serious adverse events, although extremely unlikely, can be reported by participants at any visit. Because of the frequent protocol-mandated contacts of participants during the feeding phase, ascertainment of adverse events is high and likely complete. The following types of events will be considered serious adverse events and will be recorded using the study's adverse event form:

- Is fatal or life threatening
- Results in significant or persistent disability (lasted at least 1 month & changed life)
- Requires or prolongs hospitalization
- Results in congenital abnormality or birth defect or
- Represents other significant hazards or potentially serious harm to research subjects or others

Legal Risks
There are no unique legal risks to participation in this study.

Financial Risks
There are no financial risks to participants. All aspects of the study (feeding intervention and data collection) will be provided by the study without any financial costs to participants.

10.4 Data and Safety Monitoring Plan
This study is a single-center, clinical trial with minimal risk to participants. Still, a Data and Safety Monitoring Board (DSMB) will be appointed by the trial leadership to review the protocol prior to field work and to monitor trial progress and safety. DSMB members will include experts in biostatistics, CVD prevention, nutrition studies, and clinical trials. During fieldwork, the DSMB will convene every 6 months, either in person or by phone. Adverse events will be reviewed initially by one of the study clinicians and then reported to DSMB and IRB, following Johns Hopkins policies. A DSMB charter will be prepared. This plan should ensure the safety of participants.

11 STATISTICAL CONSIDERATIONS
The full Statistical Analysis Plan (SAP) has now been prepared and incorporated into this combined protocol-SAP document. Please refer to the Appendix C: Statistical Analysis Plan at the end of this document, which supersedes the text previously included in this protocol section, for details on power and sample size and the analytic plan.

12 TIMELINE

The trial consists of three main phases: planning, implementation (recruitment, feeding, and data collection), and data analysis/dissemination (see **Figure 4**). This timeline was developed prior to the COVID pandemic, which halted trial implementation in March 2020, prior to enrollment of participants. Following Johns Hopkins guidelines, the investigators will restart the trial. However, the timeline of the trial is uncertain.

Planning: the focus of the planning period is finalization of the protocol and menu development. The Manual of Operations, instruments and forms are then prepared, and data entry/management systems developed. Meal cycles are developed for each of 4 diets at each calorie level. Menus are prepared, analyzed, and taste-tested. Recruitment planning also occurs.

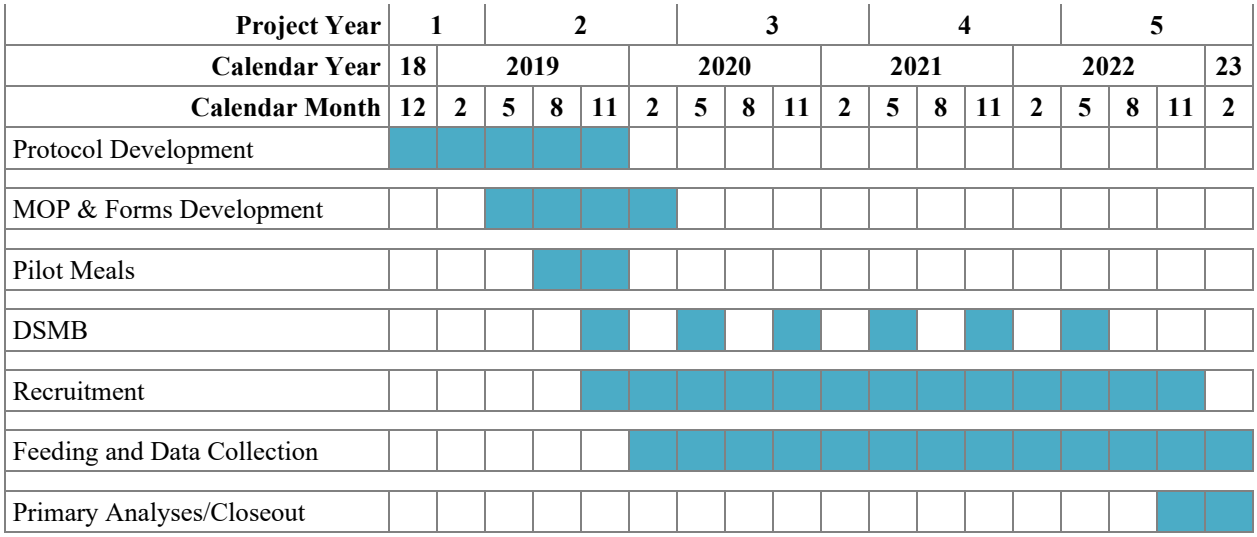
Implementation: In contrast to DASH and DASH-Sodium, in which individuals were enrolled in non-overlapping cohorts, participants in this trial are recruited in smaller waves that overlap, as was done in the OmniHeart and OmniCarb trials. In this fashion, we avoid the wide swings in activities that complicated the conduct of the DASH and DASH-Sodium trials. Feeding for each wave typically lasts 22 weeks (approximately 1 week of run-in plus four 5-week periods) with at least 1 week separating each period. We anticipate 15-20 cohorts, each with 5-7 participants. Recruitment for the initial cohort commences during the first quarter of 2020. Feeding should end early in 2023.

Analyses/closeout/dissemination: Clinic closeout occurs in study year 5. During this period, clinical centers complete all data entry, respond to data edits, and prepare summary reports of trial data for participants. During year 5, analyses are performed, and main results are prepared for publication and presentation.

Figure 4. General Timeline of DASH4D Clinical Trial.

Project Start Date: 12/1/2018

Project End Date: 4/30/2023



13 TRIAL ORGANIZATION

This single center trial will be conducted at Johns Hopkins ProHealth in Woodlawn. A separate data analysis and coordinating unit will be located in the Welch Center for Prevention, Epidemiology, and Clinical Research in Baltimore, MD. Drs. Appel, Maruthur, and Yeh will serve as Principal Investigators, similar to multiple PI studies sponsored by NIH. Dr. Appel, the contact PI, will bear overall responsibility for administrative and financial aspects of the trial. Dr. Maruthur will lead clinical center operations at ProHealth, and Dr. Yeh will lead the data analysis and coordinating unit. All three PIs contributed substantively to the design of the trial. A Steering Committee, comprised of all study investigators, is the primary decision-making body. For decisions in which there is no consensus, the three Principal Investigators will vote. There are two standing subcommittees (Diet, and Design and Measurements). Ad hoc working groups will be assembled, as needed. An independent DSMB will be established and will be advisory to the investigative team.

14 HUMAN SUBJECTS RESEARCH

This study will be conducted in accordance with the policies of and under the oversight of the Johns Hopkins University School of Medicine Institutional Review Board (IRB).

14.1 Informed Consent Process

Participation in this study is voluntary and will occur in the setting of informed consent. Informed consent will take place in a private area. We will only include adults who have capacity to provide informed consent in this study. Participants will be provided ample time to consider participating in this study, including to consult with others. Understandable language will be used during the consent processes for this study, and all questions will be answered prior to provision of consent. Participants will not waive their legal rights in the consent processes of this study. All consent designees will be approved by the IRB.

The consent process will take place in four phases during this study. First, oral consent will be obtained prior to telephone screening. Information collected as part of prescreening will be used to determine if the participant should be invited for an in-person screening visit. Participants will also be asked (if willing) to provide information on gender, race, and ethnicity to help us refine our recruitment strategies as needed to achieve our enrollment targets.

At the start of the first in-person screening visit (SV1), each participant will be asked to provide oral consent for BP measurement. Participants who meet the SV1 BP eligibility levels will then be asked to provide written informed consent for the screening phase of the study (rest of SV1, SV2, SV3, and RI) before any additional data are collected. At the time of randomization (prior to the randomization procedure), participants will provide written consent to be randomized and for the intervention phase of the study. This staged consent process was implemented in each of our prior feeding studies and is appropriate for such studies given that most screenees do not enroll in the whole trial and that the amount of information about the trial is substantial.

Elements of Informed Consent
The oral consent script and written consent forms will include these following elements required by the US Department of Health and Human Services:

- A statement that the study involves research;
- An explanation of the purposes of the research;
- The expected duration of the participant's participation;
- A description of the procedures to be followed;
- Identification of any procedures which are experimental;
- A description of any reasonably foreseeable risks or discomforts to the participant;
- A description of any benefits to the participant or to others which may reasonably be expected from the research;
- A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant;
- A statement describing the extent, if any, to which confidentiality of records identifying the participant will be maintained;
- An explanation as to whether any compensation is available;
- An explanation as to whether any medical treatments are available if injury occurs;
- An explanation of whom to contact for answers to pertinent questions about the research and research participants' rights;
- Whom to contact in the event of a research-related injury to the participant;
- A statement that participation is voluntary;
- A statement that refusal to participate will involve no penalty or loss of benefits to which the participant is otherwise entitled; and
- A statement that the participant may discontinue participation at any time without penalty or loss of benefits to which the participant is otherwise entitled.

14.2 Confidentiality
Confidentiality of study data is of utmost importance. All study staff and investigators are trained in privacy procedures, and approved study personnel will only access data if needed. All hardcopies of forms are stored in locked file cabinets. All computer files are password protected. Participant results will only be released to the participant unless he/she provides written approval to release data (e.g. laboratory results to personal provider).

Data will be only be presented and published in aggregate, i.e. no identifying characteristics of participants will be published or presented.

All computer files pertaining to the study are maintained on a secured server at Johns Hopkins Medicine with firewall protection. The server is subject to daily backup. All computer files are stored in password-controlled accounts at Johns Hopkins ProHealth computers.

14.3 Risks
Risks and risk mitigation are described in section 10 SAFETY MONITORING.

14.4 Benefits

Participants may enjoy participating in studies that relate diet to health. Participants will be provided with a copy of their weight, BP, and clinically-relevant laboratory studies after the study is complete. Participants will be provided with an estimate of their daily caloric needs to maintain current weight at completion of the study.

15 DATA SHARING AND BIOSPECIMEN TRANSFERS

The field of data sharing is rapidly evolving. We will follow prevailing guidelines for data sharing set by the JHSOM and by journals.

15.1 Data Sharing

External Collaborators

Study data (de-identified or limited datasets) may be shared with external collaborators in accordance with the terms of the Data Use Agreement with each external collaborator's institution.

15.2 Biospecimen Transfers

DASH4D collected biospecimens from participants, with plans to conduct laboratory assays for biomarkers related to diabetes, cardiovascular disease, and nutrition (as noted in the consent form), if sufficient funding to perform these assays could be secured. As funding is secured and appropriate subcontracts or agreements are established, we will prepare Material Transfer Agreements (MTAs) to transfer biospecimens to laboratories with expertise in the relevant research areas and biomarker assays, and key details related to each collaboration will be added to this protocol section.

All biospecimens collected in the trial are labeled with the study-assigned participant ID, visit code, and information about the draw tube, specimen type, and tube/aliquot number for that draw. No PHI/PII (e.g., names, MRNs, dates) are included on the specimen tubes, and no data that could lead to identification of the individual participants will be provided to the laboratories.

Transfer to Washington University School of Medicine in St. Louis

Blood specimens (serum and whole blood) collected and stored during the trial will be transferred to Washington University School of Medicine in St. Louis (WashU), upon completion of specimen collection, for each participant-visit where a blood draw was completed (i.e., for all participants who completed the blood draw at SV3, and for each randomized participant who completed a blood draw at the end of each FP).

WashU will conduct a set of standard clinical (FDA cleared) laboratory tests on these specimens for glycemic and cardiovascular biomarkers. Examination of the impact of the study diets on these biomarkers is included in 4.3 Other Outcomes. After performing the laboratory tests, WashU will send JHU the laboratory results. Samples with adequate specimen remaining for additional studies will be returned to JHU for long-term storage or additional investigations. If

sufficient sample is not available for additional investigations, the remnant sample will be destroyed.

JHU will be responsible for analyses of the laboratory results in the context of the study data. The JHU team will prepare presentations and publications based on the analyses, and the WashU investigator(s) will be invited to be a co-author(s) on presentations or publications arising from this collaboration.

Transfer to University of Colorado Anschutz

Specimens (3 stool aliquots and 1 plasma aliquot per participant per visit) collected and stored during the trial will be sent to outside laboratories for fee-for-service analyses related to nutrition biomarkers (gut microbiome, metabolome, and short-chain fatty acid analyses) to address the aims of Dr. Noel Mueller's NIH-funded (R01HL166473) ancillary study to the DASH4D trial entitled "Effects of Dietary Patterns and Sodium Intake on the Gut Microbiome and Metabolome" in accordance with the terms of the JHU subcontract to site PI Dr. Lawrence Appel. These nutrition biomarkers were listed in section 4.3 Other Outcomes as contingent upon securing additional funding.

Following the external laboratory assays, any remnants will be shipped to University of Colorado Anschutz for long-term storage. Additionally, the remaining stool aliquot that was collected and stored at JHU during the trial will be shipped to University of Colorado. Specimens from participants who did not consent to specimen storage for future research will be destroyed, and the rest of these specimens will be put into long-term storage for future research as approved by the DASH4D Executive Committee.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas. In: Eighth ed.2017: https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/134-idf-diabetes-atlas-8th-edition.html.
2. Centers for Disease Control and Prevention. *National Diabetes Statistics Report, 2017.* Atlanta, GA: Centers for Disease Control and Prevention;2017.
3. Boehme AK, Esenwa C, Elkind MS. Stroke Risk Factors, Genetics, and Prevention. *Circ Res.* 2017;120(3):472-495. PMCID: PMC5321635
4. Banerjee C, Moon YP, Paik MC, et al. Duration of Diabetes and Risk of Ischemic Stroke. *Stroke.* 2012;43(5):1212-1217.
5. American Diabetes Association. 10. Cardiovascular Disease and Risk Management: Standards of Medical Care in Diabetes-2019. *Diabetes Care.* 2019;42(Suppl 1):S103-s123.
6. Centers for Disease Control and Prevention. *National Diabetes Statistics Report, 2014.* Atlanta, GA: Centers for Disease Control and Prevention;2014.
7. Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V, Patel A. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. *Jama.* 2015;313(6):603-615.
8. Brunström M, Carlberg B. Effect of antihypertensive treatment at different blood pressure levels in patients with diabetes mellitus: systematic review and meta-analyses. *Bmj.* 2016;352:i717. PMCID: PMC4770818
9. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. *Lancet.* 2008;371(9623):1513-1518.

10. Azadbakht L, Fard NR, Karimi M, et al. Effects of the Dietary Approaches to Stop Hypertension (DASH) eating plan on cardiovascular risks among type 2 diabetic patients: a randomized crossover clinical trial. *Diabetes Care.* 2011;34(1):55-57. PMCID: PMC3005461
11. Paula TP, Viana LV, Neto AT, Leitão CB, Gross JL, Azevedo MJ. Effects of the DASH Diet and Walking on Blood Pressure in Patients With Type 2 Diabetes and Uncontrolled Hypertension: A Randomized Controlled Trial. *J Clin Hypertens (Greenwich).* 2015;17(11):895-901.
12. Kwakernaak AJ, Krikken JA, Binnenmars SH, et al. Effects of sodium restriction and hydrochlorothiazide on RAAS blockade efficacy in diabetic nephropathy: a randomised clinical trial. *Lancet Diabetes Endocrinol.* 2014;2(5):385-395.
13. Parvanova A, Trillini M, Podestà MA, et al. Moderate salt restriction with or without paricalcitol in type 2 diabetes and losartan-resistant macroalbuminuria (PROCEED): a randomised, double-blind, placebo-controlled, crossover trial. *Lancet Diabetes Endocrinol.* 2018;6(1):27-40.
14. Dodson PM, Beevers M, Hallworth R, Webberley MJ, Fletcher RF, Taylor KG. Sodium restriction and blood pressure in hypertensive type II diabetics: randomised blind controlled and crossover studies of moderate sodium restriction and sodium supplementation. *Bmj.* 1989;298(6668):227-230. PMCID: PMC1835532
15. Houlihan CA, Akdeniz A, Tsalamandris C, Cooper ME, Jerums G, Gilbert RE. Urinary transforming growth factor-beta excretion in patients with hypertension, type 2 diabetes, and elevated albumin excretion rate: effects of angiotensin receptor blockade and sodium restriction. *Diabetes Care.* 2002;25(6):1072-1077.
16. Luik PT, Hoogenberg K, Van Der Kleij FG, et al. Short-term moderate sodium restriction induces relative hyperfiltration in normotensive normoalbuminuric Type I diabetes mellitus. *Diabetologia.* 2002;45(4):535-541.
17. Vedovato M, Lepore G, Coracina A, et al. Effect of sodium intake on blood pressure and albuminuria in Type 2 diabetic patients: the role of insulin resistance. *Diabetologia.* 2004;47(2):300-303.
18. Imanishi M, Yoshioka K, Okumura M, et al. Sodium sensitivity related to albuminuria appearing before hypertension in type 2 diabetic patients. *Diabetes Care.* 2001;24(1):111-116.
19. Yoshioka K, Imanishi M, Konishi Y, et al. Glomerular charge and size selectivity assessed by changes in salt intake in type 2 diabetic patients. *Diabetes Care.* 1998;21(4):482-486.
20. Trevisan R, Bruttomesso D, Vedovato M, et al. Enhanced responsiveness of blood pressure to sodium intake and to angiotensin II is associated with insulin resistance in IDDM patients with microalbuminuria. *Diabetes.* 1998;47(8):1347-1353.
21. Miller JA. Renal responses to sodium restriction in patients with early diabetes mellitus. *J Am Soc Nephrol.* 1997;8(5):749-755.
22. Mühlhauser I, Prange K, Sawicki PT, et al. Effects of dietary sodium on blood pressure in IDDM patients with nephropathy. *Diabetologia.* 1996;39(2):212-219.
23. Suckling RJ, He FJ, Markandu ND, MacGregor GA. Modest Salt Reduction Lowers Blood Pressure and Albumin Excretion in Impaired Glucose Tolerance and Type 2 Diabetes Mellitus: A Randomized Double-Blind Trial. *Hypertension.* 2016;67(6):1189-1195.
24. Evert AB, Dennison M, Gardner CD, et al. Nutrition Therapy for Adults With Diabetes or Prediabetes: A Consensus Report. *Diabetes Care.* 2019;42(5):731-754.
25. Suckling RJ, He FJ, Macgregor GA. Altered dietary salt intake for preventing and treating diabetic kidney disease. *Cochrane Database Syst Rev.* 2010(12):Cd006763.
26. Ekinci EI, Clarke S, Thomas MC, et al. Dietary salt intake and mortality in patients with type 2 diabetes. *Diabetes Care.* 2011;34(3):703-709. PMCID: PMC3041211
27. Thomas MC, Moran J, Forsblom C, et al. The association between dietary sodium intake, ESRD, and all-cause mortality in patients with type 1 diabetes. *Diabetes Care.* 2011;34(4):861-866. PMCID: PMC3064042
28. National Academies of Sciences, Engineering, and Medicine. *Dietary Reference Intakes for sodium and potassium.* Washington, DC: The National Academies Press;2019.

29. Evert AB, Boucher JL, Cypress M, et al. Nutrition therapy recommendations for the management of adults with diabetes. *Diabetes Care.* 2014;37 Suppl 1:S120-143.
30. Martin SS, Blaha MJ, Elshazly MB, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *Jama.* 2013;310(19):2061-2068. PMCID: PMC4226221
31. Appel LJ, Moore TJ, Obarzanek E, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med.* 1997;336(16):1117-1124.
32. Sacks FM, Svetkey LP, Vollmer WM, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med.* 2001;344(1):3-10.
33. Appel LJ, Sacks FM, Carey VJ, et al. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *Jama.* 2005;294(19):2455-2464.
34. Sacks FM, Carey VJ, Anderson CA, et al. Effects of high vs low glycemic index of dietary carbohydrate on cardiovascular disease risk factors and insulin sensitivity: the OmniCarb randomized clinical trial. *Jama.* 2014;312(23):2531-2541. PMCID: PMC4370345
35. Lloyd-Jones DM, Hong Y, Labarthe D, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation.* 2010;121(4):586-613.
36. U.S. Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Food Surveys Research Group (Beltsville, MD), and U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics (Hyattsville, MD). What We Eat in America, NHANES 2013-2014 Data: Dietary Interview - Total Nutrients Intakes -- First Day (DR1TOT_H). (2016, September). Available from: https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/DR1IFF_H.XPT.
37. U.S. Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Food Surveys Research Group (Beltsville, MD), and U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics (Hyattsville, MD). What We Eat in America, NHANES 2015-2016 Data: Dietary Interview - Total Nutrients Intakes -- First Day (DR1TOT_I). (2018, July). Available from: https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/DR1IFF_I.XPT.
38. McClure ST, Schlechter H, Oh S, et al. Dietary intake of adults with and without diabetes: results from NHANES 2013-2016. *BMJ Open Diabetes Res Care.* 2020;8(1). PMCID: PMC7590352
39. U.S. Department of Health and Human Services, U.S. Department of Agriculture. *2015-2020 Dietary Guidelines for Americans. 8th Edition.* December 2015.
40. American Diabetes Association. 5. Lifestyle Management: Standards of Medical Care in Diabetes-2019. *Diabetes Care.* 2019;42(Suppl 1):S46-s60.
41. Anderson CA, Cobb LK, Miller ER, 3rd, et al. Effects of a behavioral intervention that emphasizes spices and herbs on adherence to recommended sodium intake: results of the SPICE randomized clinical trial. *Am J Clin Nutr.* 2015;102(3):671-679. PMCID: PMC4548171
42. White WB, Anwar YA. Evaluation of the overall efficacy of the Omron office digital blood pressure HEM-907 monitor in adults. *Blood Press Monit.* 2001;6(2):107-110.
43. Ryan DH, Espeland MA, Foster GD, et al. Look AHEAD (Action for Health in Diabetes): design and methods for a clinical trial of weight loss for the prevention of cardiovascular disease in type 2 diabetes. *Control Clin Trials.* 2003;24(5):610-628.
44. American Diabetes Association. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2019. *Diabetes Care.* 2019;42(Suppl 1):S90-s102.
45. Musso C, Liakopoulos V, Stefanidis I, De Miguel R, Imperiali N, Algranati L. Correlation between creatinine clearance and transtubular potassium concentration gradient in old people and chronic renal disease patients. *Saudi J Kidney Dis Transpl.* 2007;18(4):551-555.

46. He FJ, MacGregor GA. Fortnightly review: Beneficial effects of potassium. *BMJ.* 2001;323(7311):497-501. PMCID: PMC1121081
47. Bourgoignie J, Oster JR, Perez GO, Green DF. Disorders of Potassium Metabolism. In: Suki W, Massry S, eds. *Therapy of Renal Diseases and Related Disorders.* Second ed.: Springer; 1991.
48. Komers R, Meyer T, Anderson S. Pathophysiology and Nephron Adaptation in Chronic Renal Failure. In: Schrier R, ed. *Diseases of the Kidney and Urinary Tract.* Vol III. Lippincott, Williams, and Wilkins; 2001:2695.
49. Kelleher C, Linas S. The Patient with Hypokalemia or Hyperkalemia. In: Schrier R, ed. *Manual of Nephrology.* Lippincott, Williams, and Wilkins; 2004:38.
50. Ben Salem C, Badreddine A, Fathallah N, Slim R, Hmouda H. Drug-induced hyperkalemia. *Drug Saf.* 2014;37(9):677-692.
51. Bakris GL, Siomos M, Richardson D, et al. ACE inhibition or angiotensin receptor blockade: impact on potassium in renal failure. VAL-K Study Group. *Kidney Int.* 2000;58(5):2084-2092.
52. Mangrum AJ, Bakris GL. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in chronic renal disease: safety issues. *Semin Nephrol.* 2004;24(2):168-175.
53. Maschio G, Alberti D, Janin G, et al. Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. *N Engl J Med.* 1996;334(15):939-945.
54. Hou FF, Zhang X, Zhang GH, et al. Efficacy and safety of benazepril for advanced chronic renal insufficiency. *N Engl J Med.* 2006;354(2):131-140.
55. Ruilope LM, Aldigier JC, Ponticelli C, Oddou-Stock P, Botteri F, Mann JF. Safety of the combination of valsartan and benazepril in patients with chronic renal disease. European Group for the Investigation of Valsartan in Chronic Renal Disease. *J Hypertens.* 2000;18(1):89-95.
56. American Diabetes Association. 5. Facilitating Positive Health Behaviors and Well-being to Improve Health Outcomes: Standards of Care in Diabetes-2024. *Diabetes Care.* 2024;47(Suppl 1):S77-s110. PMCID: PMC10725816
57. Patel SM, Cobb P, Saydah S, Zhang X, de Jesus JM, Cogswell ME. Dietary sodium reduction does not affect circulating glucose concentrations in fasting children or adults: findings from a systematic review and meta-analysis. *The Journal of nutrition.* 2015;145(3):505-513.
58. American Diabetes Association. 6. Glycemic goals and hypoglycemia: Standards of Care in Diabetes—2024. *Diabetes Care.* 2024;47(Supplement_1):S111-S125.
59. Battelino T, Alexander CM, Amiel SA, et al. Continuous glucose monitoring and metrics for clinical trials: an international consensus statement. *Lancet Diabetes Endocrinol.* 2023.
60. Wadwa RP, Reed ZW, Buckingham BA, et al. Trial of Hybrid Closed-Loop Control in Young Children with Type 1 Diabetes. *N Engl J Med.* 2023;388(11):991-1001. PMCID: PMC10082994
61. Brown SA, Kovatchev BP, Raghinaru D, et al. Six-Month Randomized, Multicenter Trial of Closed-Loop Control in Type 1 Diabetes. *N Engl J Med.* 2019;381(18):1707-1717. PMCID: PMC7076915
62. Ware J, Allen JM, Boughton CK, et al. Randomized Trial of Closed-Loop Control in Very Young Children with Type 1 Diabetes. *N Engl J Med.* 2022;386(3):209-219.
63. Bionic Pancreas Research Group. Multicenter, Randomized Trial of a Bionic Pancreas in Type 1 Diabetes. *New England Journal of Medicine.* 2022;387(13):1161-1172.

Appendix A: Literature Review of Randomized Controlled Trials on Blood Pressure in Persons with Diabetes

A-1. Trials on the Effects of the DASH Diet on Blood Pressure in Persons with Type 2 Diabetes

Study	Design	Type of intervention	Comparison	N	Age (yrs)	Males (%)	Race	Population	Study Dur (wks)	BP Δ in intervention group	BP Δ in control group	Between-group Difference in BP Δ	Contrast in urine sodium
Paula, 2015	Parallel study	Behavioral	DASH vs. control	40	61.8-62.5	45	White: 80-90%	Type 2 diabetes, uncontrolled BP, BMI≤40	4	All ABPM reductions were sig Office systolic BP Δ: -17.6 (-44.0 to 14.0), p<0.001 Office diastolic BP Δ: -3.9 (-14.0 to 7.0), p=0.045	Only 24-hr diastolic and nighttime, systolic and diastolic, ABPM reductions were sig Office systolic BP Δ: -6.3 (-54.0 to 24.0), p=0.246 Office diastolic BP Δ: -0.9 (-21.0 to 11.0), p=0.589	ABPM reduction greater in intervention vs. control group in all periods, except for diastolic ABPM at nighttime (no diff between groups) Office systolic BP Δ: P=0.053 Office diastolic BP Δ: P=0.06	Sig reduction in urinary sodium in intervention group but not control group. Between group difference in 24h urine Na: P=0.17
Azadbakht, 2011	Crossover study	Behavioral	DASH vs. control	31	NR	42	NR	Type 2 diabetes	8	Systolic BP Δ: -13.6±3.8	Systolic BP Δ: -3.1±2.7	p=0.02	NR

A-2. Trials on the Effects of Sodium Reduction on Blood Pressure in Persons with Diabetes

Study	Design	Comparison	N	Age (yrs)	Males (%)	Race	Population	Study Dur (wks)	BP Δ in intervention group	BP Δ in control group	Between-group Difference in BP Δ	Contrast in urine sodium
Dodson, 1989	Parallel study	Moderate Na restriction vs. control	34	61	71/65	NR	Type 2 diabetes, mild hypertension, no proteinuria, no past or current use of insulin	12	Systolic mean % change -12.0, p<0.001 Diastolic mean % change -3.1, p<0.05	Systolic mean % change 4.2, NS Diastolic mean% change 5.1, NS	P<0.03 for systolic BP NS for diastolic BP	Na restriction group had sig greater reduction in UNa (p<0.001) vs. control, between group diff (p<0.05)
Houlihan, 2002	Parallel study	Placebo vs. Losartan→ washout→ Low (50-70mmol) vs. normal Na diet	21	60.5-63.1	95	NR	Type 2 diabetes, with hypertension, elevated urinary AER	2	NR	NR	Losartan treatment associated w/ similar decreases in MAP during regular-and low Na phases, and in placebo group, Δ in MAP not sig diff between regular- and low-Na phases. Changes in MAP in losartan group were sig greater vs. placebo group for both regular- and low-Na phases	Equivalent levels of Na restriction were achieved with urinary Na excretion of 80±22 and 91±14 mmol/ day in placebo and losartan groups, respectively (both P<0.001 vs. regular-sodium diet).
Luik, 2002	Crossover study	Low (50mmol) vs. high (200mmol) Na intake; control vs. diabetes	24	29	63	NR	Type 1 diabetes, normotensive, normoalbuminuric	1	NR	NR	MAP similar in both groups during liberal Na intake. The decreases in MAP during low Na diet were not significant in either group	NS difference in UNaV between control vs. diabetes on liberal sodium diet or on low Na diet
Kwakernaak, 2014	Crossover study	Low (50mmol) vs. normal Na diet Plus HCTZ vs placebo	45	65	84	White: 100%	Type 2 diabetes, albuminuria	24			% reduction in SBP sig larger for HCTZ vs. Na restriction (diff in relative response 4·3% [95% CI 1·0–7·5], p=0·012) and even larger w/ combination than w/ either Na restriction alone (diff in relative response 8·0% [4·9–10·9], p<0·0001) or HCTZ alone (diff in relative response 3·5% [0·8–6·1], p=0·012).	Na excretion sig decreased from 224 mmol (SD 73)/day during regular Na diet to 148 mmol (SD 65)/day during Na restriction when combined with placebo and 164mmol (SD 73)/day with HCTZ (both p<0·0001 vs regular Na intake). In control, urinary Na excretion was 189 mmol (SD 80)/day. In age-matched and sex-matched reference population, mean urinary Na excretion was 207 mmol (SD 79)/day—ie, similar to trial population (p=0·20) during regular Na intake.
Parvanova, 2018	Crossover study	Low (100mEq) vs. high (200 mEq) Na diet Paricalcitol to placebo vs placebo to paricalcitol	115	64	89	NR	Type 2 diabetes, macroalbuminuria, RAS inhibitor therapy	12			Systolic and diastolic BP did not change appreciably in high-sodium diet group, whereas they slightly but significantly decreased in the low Na diet group.	At month 1, month 2, and month 3 after randomization, natriuresis in high-Na diet group was about 200 mEq/day vs. 170 mEq/day in low Na group Between group diff: 35·2 mEq (SD 61·8)/day at month 1 (p=0·0003), 27·4 mEq (56·2)/day at month 2 (p=0·01), and 35·3 mEq (58·6)/day at month 3 (p=0·0002), even after adjustment for baseline sodium excretion.

A-3. Trials on the Effects of Sodium Reduction Feeding Intervention on Blood Pressure in Persons with Diabetes

Study	Design	Comparison	N	Age (yrs)	Males (%)	Race	Population	Study Dur (wks)	BP Δ in intervention group	BP Δ in control group	Between-group Difference in BP Δ	Contrast in urine sodium
Miller, 1997	Crossover study	Low (20mmol Na) vs. High (200mmol Na) diet; control vs diabetic	12	23	100	NR	Type 1, insulin-dependent, within 5 years of diagnosis, normotensive, no proteinuria	1	NR	NR	MAP did not change in response to diet in either group	UNa excretion (mmol/day) for diabetics: Sodium replete, 254±34 Sodium restricted, 124±33 P≤0.05 comparing groups
Trevisan, 1998	Parallel study	Low (25mmol) to high Na (250mmol) diet; no vs. micro-albuminuria	9	42	67	NR	Type 1, insulin-dependent, BMI<27kg/m², persistent microalbuminuria, untreated seated BP<140/90	1	With microalbuminuria: Increase in MAP 9.6%, p<0.001	Without microalbuminuria: no change in MAP, 3.5%, NS	NR	Mean 24-hr urinary Na excretion was sig higher in high Na vs. low Na diet within groups (p<0.0001) but not between groups
Yoshioka, 1998	Crossover study	Low (85mEq) vs. high salt (225mEq) diet; normo vs micro vs. advanced albuminuria	19	60	58	NR	Type 2 diabetes, in patients, excluded those on prior or current BP treatment or elevated BP	1	NR	NR	With either diet, MAP was similar in all 3 groups. In all groups, MAP not significantly higher during high-salt diet vs. low-salt diet	NR
Imanishi, 2001	Crossover study	Low (80mmol) vs. normal (200 mmol) diet	32	59-62	59	NR	Type 2 diabetes, inpatients, simple diabetic retinopathy, excluded those on BP meds	1	NR	NR	SBP higher in normal vs low Na diet: p<0.001 No change in DBP	NR
Vedovato, 2004	Crossover study	Low (20mmol) vs. High (250mmol) Na diet; control (diabetes w/o micro-albuminuria) vs. diabetes w/ micro-albuminuria	20	57	75	NR	Type 2 diabetes, microalbuminuria, normotensive	1	On switching from low to high Na diet, 24-h MAP increased from 95±2 mmHg to 103±2 (p<0.0001), percentage increase of 7.1±0.9%.	No significant change in MAP from switching low to high Na diet (from 94±1 to 95±1 mmHg;1.3±0.8%).	No diff in BP between microalbuminuric and normoalbuminuric patients after low Na. BP higher in microalbuminuric patients after high-Na diet period.	NR

A-4. Trials on the Effects of Sodium Supplementation on Blood Pressure in Persons with Diabetes

Study	Design	Comparison	N	Age (yrs)	Males (%)	Race	Population	Study Dur (wks)	BP Δ in intervention group	BP Δ in control group	Between-group Difference in BP Δ	Contrast in urine sodium
Mulhauser, 1996	Parallel study	Sodium supplement vs. placebo	14	31	75	NR	Type 1 diabetes, on insulin, increased proteinuria, high normal or mildly hypertensive BP	4	Systolic mean diff, 3.2 (-2.6 to 9) Diastolic mean diff, 2.2 (-1.8 to 6.2)	Systolic mean diff, -1.7 (-8.6 to 5.3) Diastolic mean diff, -3.1 (-5.9 to -0.4)	Systolic: 4.9 (-3.3 to 13.1) NS Diastolic: 5.3 (1 to 9.7) p=0.02	Urinary Na excretion (mmol/day): Placebo, 92±33 Supplement, 199±52 p=0.0002 compared to placebo
Suckling, 2016	Crossover study	Reduced salt (90mmol) vs. placebo	26	56	54	White: 65 Black: 23 Asian: 12	Diet-controlled type 2 diabetes or IGT, untreated normal or high normal BP	12			BP fell from 135.5±2.0/81.3±1.1 mmHg with salt to 131.2±1.9/79.7±1.2 mm Hg with placebo, that is, a fall of 4.2 mmHg in SBP (P<0.01) and fall in DBP of 1.7 mmHg (p=0.055)	During randomized crossover phase, mean 24-hour UNa was 165.1±9.0 mmol/24 hours (9.7g salt) on salt and 116.6±9.5 mmol/24 hours (6.8g salt) on placebo. The reduction in salt intake was, therefore, 48.6±9.3 mmol (2.9g salt) from salt to placebo. UNa excretion was lower in placebo vs. salt group (p<0.001)

Appendix B: Continuous Glucose Monitoring (CGM) Ancillary Study

1. Ancillary Study Details

Official Project Title: Effects of the DASH diet on glucose patterns in adults with type 2 diabetes

Principal Investigator: Elizabeth Selvin, PhD, MPH
Johns Hopkins Bloomberg School of Public Health

Funded By: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Grant #: R01DK128900

2. Rationale

The overarching goal is to evaluate how the DASH4D dietary pattern modifies glucose patterns in adults with type 2 diabetes. This ancillary study to the DASH4D protocol will add continuous glucose monitoring (CGM) combined with symptom reporting in all participants in the DASH4D trial (N=~100) during screening (between SV2 and the start of the run-in) and during the two weeks at the end of each of four feeding periods. This ancillary study also includes laboratory testing for diabetes and cardiac biomarkers of biospecimens collected as part of the main DASH4D trial. This study will answer the question: Does the DASH4D diet (a DASH-style diet modified for people with diabetes) improve glucose control and reduce glycemic variability in adults with type 2 diabetes?

3. Background

Dietary guidelines for persons with diabetes are largely directed at reducing hyperglycemia. The DASH diet[31] is rich in fruits, vegetables, and low-fat dairy products with reduced saturated fat and cholesterol. The DASH eating plan is recommended by the ADA for adults with diabetes based on evidence that it helps control blood pressure and lowers cardiovascular risk in the general population.[40,56] The direct effects of the DASH diet on glucose control and glycemic excursions are uncharacterized. The DASH4D trial provides an opportunity to evaluate how diet may affect hyperglycemia and glucose patterns using CGM. Glucose patterns in adults with type 2 diabetes are relatively uncharacterized, and no prior study has examined the effect of diet on CGM parameters in a controlled feeding study. The addition of the symptom reporting survey during CGM wear will allow us to understand how certain symptoms relate to changes in glucose levels.

4. Study design

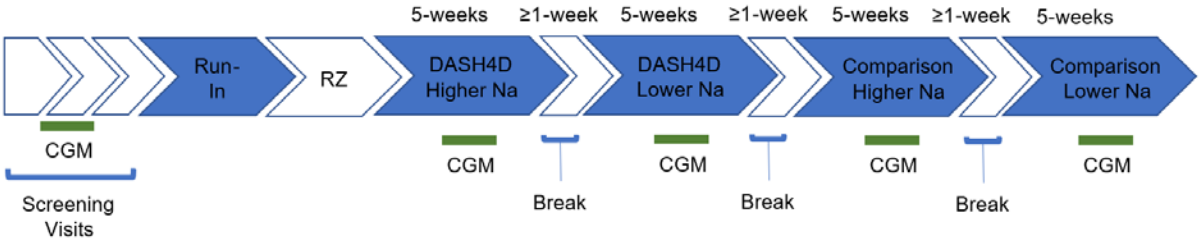
We propose to add 14-day CGM assessment in participants in the DASH4D trial (N=~100) during screening (up to 14 days, or the start of Run-In, whichever comes first) and each of the four feeding periods, for a total of five 14-day CGM periods (see green lines in **Figure CGM-1**).

As part of the main trial, each participant will complete screening, a run-in period, and four feeding periods lasting 5 weeks each, separated by at least 1 week of a break (eating own food).

During the screening phase and at randomization, DASH4D screenees will be invited to participate in, and consent for, this ancillary study, which is optional. CGM sensors will be placed during week 3 of each feeding period (typically on Thursday, which is the last day of in-person feeding every week). Sensors will be worn up to 14 days. Thus, CGM data collection will typically begin during week 3 and end during week 5 for every feeding period.

During each period of CGM device wear, we will also ask participants to complete a symptom reporting survey at least daily to indicate whether they experienced any symptoms that may relate to changes in their glucose levels, along with reporting the time of symptom onset.

Figure CGM-1. Design of the DASH4D CGM Study.



Abbreviation: RZ = randomization
Note: Figure shows an example of randomization to 1 of 24 possible orders of diet administration.
Total of 7-9 months per participant including 20 (=4 periods * 5) weeks of controlled diet

5. Exclusions

Participants will be excluded from participating in the CGM ancillary study if they have a history of allergic skin reaction to adhesive or an implantable medical device, such as a pacemaker. Participants who do not wish to participate in the CGM ancillary study will still be allowed to participate in the DASH4D trial.

6. Procedures

We will use the FreeStyle Libre Pro CGM device (Abbott; https://provider.myfreestyle.com/freestyle-libre-pro-product.html) to measure glucose in up to five 14-day periods (during screening, CGM wear may be <14 days) in all participants who consent to this protocol (**Figure CGM-2**). The version of the device used in this ancillary study does not reveal the glucose readings to participants, which will be provided to participants after the data collection phase of the trial ends (see section 9 Reporting below).

Figure CGM-2. FreeStyle Libre Pro CGM System.

The FreeStyle Libre Pro system is FDA approved for use in continuous monitoring of glucose in persons with diabetes who are aged 18 or older. The system is designed for professional use to reveal glucose trends and patterns, which are downloaded from the sensor using a reader following up to 14 days of wear. The sensor is held in place with an adhesive and has two main parts: 1) a transmitter patch, which is about the diameter of a quarter and thickness of two quarters that collects and stores data on glucose, and 2) a flexible very thin filament (<4mm) sensor inserted just under the skin to measure interstitial glucose. The application of the sensor is painless and most patients report that they cannot feel the sensor when it is being worn.

After obtaining informed consent as part of the main screening and trial consents, trained study staff will place the sensor on the back of the upper arm using a disposable applicator at SV1 and during the third week of each of the four feeding periods. The sensor will be covered with an adhesive bandage designed for use with the Libre sensor to prevent accidental dislodgement. At least 2 minutes after placement, staff will check the status of each sensor to ensure it is working properly.

The sensor is worn on the arm for 14 days (may be shorter during screening) and is factory calibrated (no fingerstick is required). The Libre Pro system records interstitial glucose every 15 minutes and stores the 14 days of data. DASH4D participants will be provided with instructions for wear of the device. The device stays on for each 14-day period and can be worn while showering, swimming, or exercising. Staff will be available (in-person and by phone) to answer any questions and will encourage participants to wear the device for the full 14-day period. After day 14 of each wear period, staff will remove the device during the next pre-scheduled trial visits. The Libre Pro sensors require a hand-held reader to download information on glucose values. After removal, sensors will be labeled, and the data will be downloaded by staff using the Libre Pro Reader (**Figure CGM-3**).

Figure CGM-3. Libre Pro Reader.

7. Symptom Reporting Survey

During each period of CGM device wear, participants will be asked to complete a symptom reporting survey to report each time they experience one of the listed symptoms, along with the time of symptom onset, or to report that none of the symptoms of interest occurred that day. Participants will ideally complete the symptom reporting via REDCap survey links that will be sent via text message, so that they can better report symptoms in real-time or near real-time. However, participants may elect to complete the symptom reporting as a paper-based diary if they do not wish to receive text messages or complete a mobile survey.

8. Safety/Risks

There is minimal risk or discomfort associated with use of the CGM sensor. The main risk is skin irritation from the adhesive. There is a very small risk of developing a local skin infection at the site of the sensor filament placement. The risk will be mitigated by using sterile technique during placement. The Libre Pro system does not have alarms and does not provide any real-time information to the participant. Throughout the trial, participants will continue routine glucose monitoring as recommended by their doctor. Participants will be counseled not to ignore symptoms that may be due to low or high blood glucose, or dehydration. The CGM device is contraindicated for wear during MRI, CT scan, X-ray, or diathermy treatment. During any medical treatment, participants should inform their health care provider that they are wearing a CGM sensor. The sensor should be removed before any MRI, CT scan, X-ray, or diathermy treatment. Confidentiality will be maintained by labeling the used sensors with a coded number and following data security procedures already in place as part of the DASH4D trial.

9. Reporting

Participants will not be aware of their glucose measures collected during the 14-day wear periods. A report with a summary of the 14-day glucose data from the screening phase (mean glucose, standard deviation, and a graph of glucose values with information on the normal range)

will be provided back to participants after they complete the feeding phase of the trial or are closed out of screening (i.e., not randomized). CGM technology is often used in the care of adults with type 1 diabetes. It is not standard of care for adults with type 2 diabetes, and it is not commonly used in this population. Even if subsequent analysis of the CGM data reveals clinically relevant glucose patterns, this information is not directly clinically actionable at a later date, especially in the absence of insulin therapy.

10.Study Outcomes and Objectives

Based on prior studies, we hypothesized that salt would have no effect on glucose.[57] Therefore, we plan to combine data from the lower and higher sodium feeding periods for each diet to increase statistical power (i.e., combine data collected during the DASH4D lower sodium diet with data collected during the DASH4D higher sodium diet). Our primary aim is to examine the effects of the DASH4D diet (vs. comparison diet) on glucose control and glycemic variability in adults with type 2 diabetes.

Primary, secondary, and exploratory CGM outcomes were selected based on clinical guidelines and recommendations from a consensus statement on CGM endpoints for clinical trials.[58,59] CGM outcomes will be based on CGM data captured by sensors worn from week 3 to week 5 (up to 14 days) of each 5-week feeding period in participants included in the DASH4D-CGM ancillary study. Per current analytic recommendations,[59] CGM outcomes will be generated using all available CGM data for each feeding period.

10.1 Primary CGM outcomes

10.1.1 **CGM mean glucose (mg/dl):** The mean of glucose measurements collected during the 14-day wear period will be used as a primary outcome of the corresponding feeding period.

10.1.2 **Percentage of time between 70 and 180 mg/dL:** The percentage of time glucose was between 70 and 180 mg/dL (time-in-range) during the 14-day wear period will be used as a primary outcome of the corresponding feeding period.

10.1.3 **Coefficient of variation (%):** The coefficient of variation (standard deviation divided by mean glucose, multiplied by 100) of glucose measurements collected during the 14-day wear period will be used as a primary outcome of the corresponding feeding period.

10.2 Secondary CGM outcomes

10.2.1 **Glucose standard deviation (mg/dL):** The standard deviation of glucose measurements will be used as a secondary outcome of the corresponding feeding period.

10.2.2 **Percentage of time above 180 mg/dL:** The percentage of time glucose was above 180 mg/dL will be used as a secondary outcome of the corresponding feeding period.

10.2.3 **Percentage of time above 250 mg/dL:** The percentage of time glucose was above 250 mg/dL will be used as a secondary outcome of the corresponding feeding period.

10.2.4 **Percentage of time below 70 mg/dL:** The percentage of time glucose was below 70 mg/dL will be used as a secondary outcome of the corresponding feeding period.

10.2.5 **Percentage of time below 54 mg/dL:** The percentage of time glucose was below 54 mg/dL will be used as a secondary outcome of the corresponding feeding period.

10.3 Exploratory CGM outcomes

10.3.1 **Percentage of time between 70 and 140 mg/dL:** The percentage of time glucose was between 70 and 140 mg/dL will be used as an exploratory outcome of the corresponding feeding period.

10.3.2 **Percentage of time above140 mg/dL:** The percentage of time glucose was above 140 mg/dL will be used as an exploratory outcome of the corresponding feeding period.

10.3.3 **Proportion of participants with >70% time between 70 to 180 mg/dL:** The proportion of participants that spent >70% of time with glucose between 70 and 180 mg/dL will be used as an exploratory outcome of the corresponding feeding period.

10.3.4 **Proportion of participants with coefficient of variation <36%:** The proportion of participants with coefficient of variation <36% will be used as an exploratory outcome of the corresponding feeding period.

10.3.5 **Proportion of participants with <25% time above 180 mg/dL:** The proportion of participants that spent <25% of time with glucose above 180 mg/dL will be used as an exploratory outcome of the corresponding feeding period.

10.3.6 **Proportion of participants with <5% time above 250 mg/dL:** The proportion of participants that spent <5% of time with glucose above 250 mg/dL will be used as an exploratory outcome of the corresponding feeding period.

10.3.7 **Proportion of participants with <4% time below 70 mg/dL:** The proportion of participants that spent <4% of time with glucose below 70 mg/dL will be used as an exploratory outcome of the corresponding feeding period.

10.3.8 **Proportion of participants with <1% time below 54 mg/dL:** The proportion of participants that spent <1% of time with glucose below 54 mg/dL will be used as an exploratory outcome of the corresponding feeding period.

10.4 Primary aim

Examine the mean differences in mean glucose, time-in-range, and glucose coefficient of variation on the DASH4D diet compared to the comparison diet, in a combined analysis across both levels of sodium.

- We hypothesize that the DASH4D diet will result in improved glucose control (lower mean glucose, higher time-in-range) and glycemic variability (lower glucose coefficient of variation) compared to the comparison diet.

10.5 Secondary aims

10.5.1 Examine the mean differences in glucose standard deviation and time spent with hyperglycemia and hypoglycemia on the DASH4D diet compared to the comparison diet, in combined analyses across both levels of sodium.

10.5.2 Examine the mean differences in exploratory outcomes (described in 10.3) on the DASH4D diet compared to the comparison diet, in combined analyses across both levels of sodium.

11. Statistical Analysis

11.1 Analytic population

Among all randomized participants, those who completed CGM during one or more feeding period will be included in the analyses.

11.2 Statistical analyses

All primary analyses are summarized in **Table CGM-1**. We will conduct all primary analyses using an intention-to-treat approach. We will use linear (for continuous outcomes) or logistic (for binary outcomes) mixed effect models and compare differences in CGM outcomes while consuming the DASH4D (versus comparison) diet.

Our primary contrast will be between the DASH4D and comparison diet (combining across both levels of sodium) on mean glucose, time-in-range, and coefficient of variation (**Primary Aim – 10.4**). Secondary contrasts will assess the effects of the DASH4D (versus comparison) diet on glucose standard deviation, time spent with hyperglycemia, and time spent with hypoglycemia (**Secondary Aim – 10.5.1**) and on exploratory outcomes (**Secondary Aim – 10.5.2**). Because CGM outcomes are not independent, we will not make adjustments for multiple comparisons.

We will conduct several sensitivity analyses. First, to test our assumption that sodium does not affect glucose, we will assess the effects of the higher (versus lower) sodium diet on all primary CGM outcomes, stratified by different diets (**Sensitivity analysis 1a**). We will also examine whether the effects of the DASH4D diet on all CGM outcomes are modified by sodium levels by exploring diet-sodium interactions (**Sensitivity analysis 1b**). Second, we will re-estimate all analyses using a per-protocol approach **(Sensitivity analysis 2)**. For these analyses, participants without perfect adherence on >90% of days for a given diet will be excluded. Perfect adherence

was defined as eating all provided study foods and not eating any non-allowed foods or beverages.
For each feeding period, we will only include data from participants who wore CGM sensors for 10 or more days (**Sensitivity analysis 3**). Current guidelines suggest that sensors may need to be worn ≥70% of the time (~10 out of 14 days for the Abbott Libre Pro) to accurately represent glycemic control.[59]

11.3 Exploratory analyses

We will conduct two sets of exploratory analyses. First, we will estimate the linear mixed models to examine the effect the DASH4D (versus comparison) diet on the main CGM outcomes, stratified by age (<65, 65+), sex (male, female), baseline HbA1c (<7%, 7-7.9%, 8-8.9%), number of glucose-lowering medications used (0, 1, 2+), and type of diabetes medications used (no medication used, non-insulin or sulfonylureas, insulin or sulfonylurea). The DASH4D-CGM study is not powered to detect differences across any specific subgroup. Therefore, these analyses are exploratory and will not be adjusted for multiple comparisons.

Second, we will visually compare average hourly and daily mean glucose, time-in-range, and coefficient of variation across the DASH4D (versus comparison) diet. These analyses are exploratory and designed to examine how long participants may need to consume the DASH4D diet before an effect is evident and whether diet effects vary across daytime versus nighttime. Daily averages will be calculated using each 24-hour of CGM data. These analyses are similar to results reported in other major CGM trials.[60-63]

Table CGM-1: Summary of main comparisons for the CGM ancillary study

Aim	Outcomes	Contrast	Timing
Primary - 10.4	Mean glucose, TIR, CV	DASH4D vs Comparison	FP week 3-5
Secondary - 10.5.1	SD, TA180, TA250, TB70, TB54	DASH4D vs Comparison	FP week 3-5
Secondary - 10.5.2	See 10.3 for full list	DASH4D vs Comparison	FP week 3-5
Sensitivity analysis 1a	Mean glucose, TIR, CV	DH vs DL; CH vs CL	FP week 3-5
Sensitivity analyses 1b	Mean glucose, TIR, CV	(DH-DL) vs (CH-CL)*	FP week 3-5
Sensitivity analysis 2	Mean glucose, TIR, CV	DASH4D vs Comparison (Per-protocol)	FP week 3-5
Sensitivity analysis 3	Mean glucose, TIR, CV	DASH4D vs Comparison (CGM wear time >70%)	FP week 3-5

Note: All analyses will be conducted using an intention-to-treat approach unless otherwise stated.

Abbreviations: TIR = time-in-range; CV = coefficient of variation; SD = standard deviation; TA180 = time above 180 mg/dL; TA250 = time above 250 mg/dL; TB70 = time below 70 mg/dL; TB54 = time below 54 mg/dL; CH = Comparison diet with higher sodium; CL = Comparison diet with lower sodium; DH = DASH4D diet with higher sodium; DL = DASH4D diet with lower sodium; FP = feeding period

*Examination of interaction (difference of differences)

Appendix C: Statistical Analysis Plan

DASH4D Statistical Analysis Plan
Version 1.1
July 26, 2024

HISTORY OF STATISTICAL ANALYSIS PLAN CHANGES

The following table summarizes substantive changes between Statistical Analysis Plan (SAP) versions. Minor changes, such as minor corrections and clarifications, page numbering, and formatting are not described in the table but can be seen in the track changes version of the document.

SAP version	Affected section(s)	Brief description of change	Brief rationale for change
v1.0 (10-27-2024)	Initial complete version of the DASH4D Statistical Analysis Plan.		
v1.1 (07-26-2024)	1 Introduction; 2.1.1 Primary outcome; 2.1.2 Secondary outcome; 4.2 Primary outcome and primary testing contrast	Clarified that the primary and secondary outcome blood pressures are the average of the daily averages, rather than the average of all available measurements.	SAP was not previously clear, and the DSMB recommended that we clarify how the averages would be calculated in the event of missing data points.
	1.2.8; 4.7 Subgroup analysis	Added baseline number of medications used to treat the outcome as a pre-specified subgroup analysis.	Number of medications may be an important factor in examining the effects of the study diets on the outcomes. At the time of addition of this subgroup analysis, no analyses related to baseline medication use have been performed.
	4.1.1 Analytic population	Updated the analytic population to reflect that participants who do not have at least 1 end-of-period BP measurement will be excluded from the analysis population.	Inclusion of participants lacking any end-of-period BP data points would require changing the modelling approach, which relies on having at least 1 end-of-period BP data point.

1 INTRODUCTION

The objective of the DASH4D trial is to determine the effects, alone and combined, of (a) the DASH4D diet (a DASH-style diet modified for people with diabetes) vs. comparison diet that is typical of what many Americans with diabetes eat and (b) lower sodium intake vs. higher sodium intake on blood pressure (BP). The study population includes approximately 100 adults who have Type 2 diabetes, a systolic BP of 120-159 mmHg, and diastolic BP <100 mmHg (see DASH4D protocol section 6.2 for the full eligibility criteria). The core design is a single-site, four-period, crossover feeding study with 5-week feeding periods. Participants are fed each of four iso-caloric diets, presented in random order. The four diets are:

- the DASH4D diet with lower sodium
- the DASH4D diet with higher sodium
- the comparison diet with lower sodium
- the comparison diet with higher sodium (reference diet).

Outcomes (unless otherwise specified) are measured at the end of each feeding period, with end-of-period assessments captured during the final 2 weeks of each 5-week feeding period. In rare instances when extenuating circumstances require it, feeding and outcome assessments may be extended briefly into a 6th week to complete the end-of-period measures.

The primary outcome is end-of-period, office-based systolic BP, where seated BP is measured in triplicate on five separate days during the last 2 weeks of each 5-week feeding period, and the mean of these daily systolic BP measurements is used as the primary outcome of the corresponding feeding period.

1.1 Primary aim

To determine the mean difference between end-of-period systolic BP on the DASH4D diet with lower sodium and the end-of-period systolic BP on the comparison diet with higher sodium ("primary testing contrast").

Hypothesis: Mean end-of-period systolic BP will be significantly lower on the DASH4D diet with lower sodium than mean end-of-period systolic BP on the comparison diet with higher sodium.

1.2 Secondary aims

1.2.1 To determine the mean difference between end-of-period systolic BP for the lower and higher sodium intake separately in (a) the comparison diet and (b) the DASH4D diet.

1.2.2 To determine the mean difference between end-of-period systolic BP for the DASH4D diet and comparison diet separately at (a) higher sodium intake and (b) lower sodium intake.

1.2.3 To examine whether there is an interaction between the effects of diet and sodium intake on systolic BP.

1.2.4 To determine the time course over 5 weeks of lower and higher sodium intakes on systolic BP in the DASH4D and comparison diets.

1.2.5 To determine the time course over 5 weeks of the DASH4D and comparison diets on systolic BP at lower and higher sodium intakes.

1.2.6 To conduct corresponding analyses (repeat above aims) with diastolic BP as the outcome.

1.2.7 To determine the effects, alone and combined, of the DASH4D diet (vs. comparison diet) and lower sodium intake (vs. higher sodium intake) on the other end-of-period outcomes, each from a single assessment during the final 2 weeks of each 5-week feeding period, including:

a. Glycemia measures, including fasting glucose (mg/dL), glycated albumin (%), fructosamine (µmol/L), and HbA1c (%). Of these, our principal measures of glycemia are fructosamine and HbA1c, both of which are used clinically as integrated measures of glycemia. Fructosamine represents the average level of glycemia over 2 to 3 weeks prior to the test date, while HbA1c represents average level of blood glucose level over the preceding 3 months.
b. Lipid levels (mg/dL), including total cholesterol, low-density lipoproteins [LDL] cholesterol (Martin-Hopkins equation), high-density lipoproteins [HDL] cholesterol, and triglycerides
c. Estimated CVD risk (%): current 10-year atherosclerotic cardiovascular disease risk, using the ACC/AHA ASCVD risk equation
d. Orthostatic hypotension outcomes, including presence of orthostatic hypotension (yes, no), postural change in systolic BP (mmHg), and postural change in diastolic BP (mmHg)
e. 24-hour urinary albumin excretion (mg/24hr), which is our principal measure of urinary protein excretion; the albumin-creatinine ratio, used clinically, will also be reported
f. Patient-reported symptoms (any; any moderate or severe)
g. Diet acceptability, as rated by participants on two questions with a 9-point Likert scale for how much the participant liked the diet (1=disliked extremely, 9=liked extremely) and how hungry the participant felt while eating the diet (1=not at all hungry, 9=extremely hungry)

Additionally, safety outcomes including hypoglycemia, hypokalemia, hyperkalemia, elevated BP, low BP, serious adverse events [SAEs], and adverse medical events, which are assessed throughout each of the intervention periods, will also be documented for and compared across the study diets.

1.2.8 To conduct subgroup analyses (by gender, race, age, baseline level of the outcome variable [i.e., BP for the primary and secondary outcomes], baseline number of medications used to treat the outcome, and chronic kidney disease [CKD] status), corresponding to the above aims.

1.2.9 To establish a repository of biospecimens (plasma, serum, urine, stool, PAXgene).

2 OUTCOMES AND DATA SOURCES

2.1 Outcomes

2.1.1 Primary outcome

- **End-of-period, office-based systolic BP (mmHg):** seated blood pressure will be measured in triplicate on five separate days during the last 2 weeks of each 5-week feeding period. Daily mean systolic BP (SBP) will be averaged based on the triplicate measures, and the mean of these daily SBP measurements will be used as the primary outcome of the corresponding feeding period.

2.1.2 Secondary outcome

- **End-of-period diastolic BP (mmHg):** seated blood pressure will be measured in triplicate on five separate days during the last 2 weeks of each 5-week feeding period. Daily mean diastolic BP (DBP) will be averaged based on the triplicate measures, and the mean of these daily DBP measurements will be used as the secondary outcome of the corresponding feeding period.

2.1.3 Other outcomes

- Other end-of-period outcomes, each from a single assessment during the final 2 weeks of each 5-week feeding period, include:
    - Glycemia measures, including fasting glucose (mg/dL), glycated albumin (%), fructosamine (µmol/L), and HbA1c (%). Of these, our principal measures of glycemia are fructosamine and HbA1c, both of which are used clinically as integrated measures of glycemia. Fructosamine represents the average level of glycemia over 2 to 3 weeks prior to the test date, while HbA1c represents average level of blood glucose level over the preceding 3 months.
    - Lipid levels (mg/dL), including total cholesterol, LDL cholesterol (Martin-Hopkins calculation), HDL cholesterol, and triglycerides
    - Estimated CVD risk (%): current 10-year atherosclerotic cardiovascular disease risk, using the ACC/AHA ASCVD risk equations
    - Orthostatic hypotension outcomes, including presence of orthostatic hypotension (yes, no), postural change in systolic BP (mmHg), and postural change in diastolic BP (mmHg)
    - 24-hour urinary albumin excretion (mg/24hr), which is our principal measure of urinary protein excretion; the albumin-creatinine ration, used clinically, will also be reported
    - Patient-reported symptoms (any; any moderate or severe)
    - Diet acceptability, as rated by participant on two questions with a 9-point Likert scale for how much the participant liked the diet (1=disliked extremely, 9=liked extremely) and how hungry the participant felt while eating the diet (1=not at all hungry, 9=extremely hungry)
- Other outcomes assessed throughout the intervention include:
    - Safety outcomes, including hypoglycemia, hypokalemia, hyperkalemia, elevated BP, low BP, SAEs, and adverse medical events

2.2 Data Sources

Data will include physical measurements (e.g., BP and weight), questionnaire responses (e.g., daily diaries, self-reported symptoms, diet acceptability), and laboratory tests (e.g., urinary electrolyte

excretion). Data will primarily be entered by clinic staff into a web-based data system (REDCap). Data will also be automatically transferred from central laboratories. The Data Management and Analysis Unit of the trial will oversee data storage. Backup files of the database will be generated automatically at the server level and stored at regular intervals in a secure, off-site location, to permit regeneration of the database in the event that it is destroyed. Freeze dates for data sets created for interim and publication analyses will be documented with datasets used in analyses archived in secured network attached storage (NAS) folders managed by institutional IT.

3 POWER AND SAMPLE SIZE

The trial aims to enroll 100 eligible participants. This sample size goal is selected based on resources and logistic considerations, after confirmation of sufficient statistical power to detect meaningful minimal detectable difference (MDD) for the primary testing contrast (see 1.1) under plausible and yet conservative parameter scenarios, as detailed below.

Minimal Detectable Difference (MDD) for systolic blood pressure (SBP) reduction between DASH4D diet with lower sodium (DL) and the comparison diet with higher sodium (CH), with 2-sided type 1 error of 0.05 and 90% power. The observed effect from the DASH-Sodium trial was 8.9 mmHg.

Sample Size (N)	Standard Deviation (SD)	Within-person Correlation (r)	Minimal Detectable Difference (MDD), mmHg
100	14	0.5	4.71
100	14	0.75	3.34
90	14	0.5	4.99
90	14	0.75	3.53
80	14	0.5	5.28
80	14	0.75	3.84
100	12	0.5	4.04
100	12	0.75	2.86
90	12	0.5	4.28
90	12	0.75	3.03
80	12	0.5	4.53
80	12	0.75	3.21

Data from DASH-Sodium trial showed that cross-sectional SD for SBP at baseline and after intervention ranged from 9.4 to 11.9 mmHg across intervention arms. We conservatively assumed a cross-sectional SD of 14 mmHg and within-person correlation between SBP measures from different end-of-feeding periods of 0.75, which would result in a SD for SBP difference between feeding periods of 9.9 mmHg, a value that is conservative compared to previous trials with similar designs for different patient populations (e.g., 6.1 mmHg from OmniHeart, and between 7.4 and 9.2 mmHg across arms of DASH-Sodium).

4 STATISTICAL ANALYSIS FRAMEWORK

4.1 Overview

This trial is a randomized, four-period crossover study that tests the effects of 4 diets (specified by the combinations of 2 diet factors – DASH4D vs Comparison and 2 sodium levels – Higher vs Lower) on a specified set of outcomes. The 4 diets are denoted DH (DASH4D-Higher sodium), CH (Comparison-Higher sodium), DL (DASH4D-Lower sodium), and CL (Comparison-Lower sodium). Each participant receives all 4 diets. Each diet is eaten for 5 consecutive weeks. The order in which participants eat these diets is random. A break of at least 1 week (typically 2 weeks), where participants are eating their own food, will separate each consecutive set of feeding periods.

		DIETARY PATTERN	
		***C**omparison*	***D**ASH4D*
SODIUM LEVEL	***H**igher (3700mg)*	***CH (ref*)***	***DH***
	***L**ower (1500 mg)*	***CL***	***DL***

**reference group for the primary contrast*

The primary outcome of the trial is end-of-period systolic BP (SBP), with the primary contrast testing the mean SBP difference between the DL and the CH diets (Primary Aim). The secondary contrasts focus on the effects of sodium on SBP stratified by diet types (DH vs DL and CH vs CL, Secondary Aim **1.2.1**), and the effect of diet on SBP stratified by sodium levels (DH vs CH and DL vs CL, Secondary Aim **1.2.2**). The trial will examine whether the diet and sodium effects are additive through exploring for diet-sodium interaction on SBP (Secondary Aim **1.2.3**), and assess the time course of the sodium effects on SBP by diet types (Secondary Aim **1.2.4**) and the diet effects on the time course of SBP by sodium levels (Secondary Aim **1.2.5**) over the 5-week feeding period. We will also assess the effects of these diet contrasts on other protocol-specified outcomes, namely, diastolic BP (DBP; Secondary Aim **1.2.6**), fasting glucose, fructosamine, plasma lipids, CVD risk scores, patient-reported outcomes, and specified safety outcomes, SAEs and adverse medical events (Secondary Aim **1.2.7**). We will assess the differences of the aforementioned outcomes under the specified diet contrasts by pre-specified subgroups (Secondary Aim **1.2.8**).

Aim	Outcome	Timing	Contrast
Primary **1.1**	SBP	End of Period	CH vs DL
Secondary **1.2.1**	SBP	End of Period	DH vs DL; CH vs CL
Secondary **1.2.2**	SBP	End of Period	DH vs CH; DL vs CL
Secondary **1.2.3**	SBP	End of Period	(DH-DL) vs (CH-CL)*
Secondary **1.2.4**	SBP	Period Trajectory	DH vs DL; CH vs CL
Secondary **1.2.5**	SBP	Period Trajectory	DH vs CH; DL vs CL
Secondary **1.2.6**	DBP	Same as all of above for SBP	
Secondary **1.2.7**	fasting glucose; glycated albumin; fructosamine; HbA1c; plasma lipids; 24-hour urinary albumin excretion; CVD risk score; patient-reported outcomes	End of Period	CH vs DL; DH vs DL; CH vs CL; DH vs CH; DL vs CL; (DH-DL) vs (CH-CL)*
	safety data (incl. hypoglycemia, hyperkalemia, elevated BP, low BP, SAEs & adverse medical events)	Cumulative over the entire period	CH vs DL; DH vs DL; CH vs CL; DH vs CH; DL vs CL; (DH-DL) vs (CH-CL)*
Secondary **1.2.8**	All of above, by pre-specified subgroups		

*examination of interaction (difference of differences)

4.1.1 Analytic population

All randomized participants who completed at least 1 day of intervention feeding and at least 1 end-of-period BP assessment will be included in the analyses, unless otherwise noted below. For practical reasons related to food preparation, there is an interval between date of randomization and initial day of feeding, and participants who were randomized but withdrew from the trial prior to any intervention feeding will not be included in analyses. Additionally, participants who did not complete at least 1 end-of-period BP assessment during the first feeding period (i.e., dropped out or withdrew within the first 3 weeks of the study) will be excluded from the analytic population, as the modelling approach depends on having at least 1 end-of-period BP data point.

4.1.2 Missing data

Prevention is far superior to the statistical treatments for missing data, as there is no statistical cure, only treatments, for missing data. The primary solution is improved retention. Every effort will be made to reduce participant burden and facilitate continuing participation. We will talk to participants to identify reasons for dropout and will use findings to direct retention efforts.

We will anchor our main analyses on the assumption of missing at random (MAR), and conduct sensitivity analyses according to sensible not missing at random (NMAR) scenarios to evaluate the robustness of results under MAR. We will analyze available data, including incomplete series of outcome data up to the dropout to assess possible relationships between dropout tendency and study outcomes to identify observed factors that may be associated with dropouts for the MAR-based analyses.

For continuous outcomes with repeated measures over time, the MAR based analyses will be carried out using repeated-measures mixed-effects regression modeling approach. For binary outcomes and count data where the analyses will employ nonlinear link functions, multiple imputation for the missing data

under MAR will be carried out before conducting generalized estimating equations (GEE) analyses to produce population-average inferences.

Unfortunately, MAR is an assumption that cannot be empirically tested using observed data without auxiliary information on the missing values that are not observed. We will conduct multiple imputation using the MAR-based models, preserving the estimated variance-covariance structure, while modifying the predicted means for multiple imputation under expert guided non-MAR, or NMAR, scenarios, and analyze such imputed data through standard approach as sensitivity analyses under NMAR to evaluate the robustness of the results under MAR assumption. These model-based sensitivity analyses according to sensible NMAR scenarios will be presented to foster clear interpretation of study results (Daniels and Hogan, 2000).

4.1.3 Interim analysis

There is no planned interim analysis for the outcomes. An independent Data and Safety Monitoring Board will periodically review data from the trial to ensure participant safety.

4.1.4 Multiple comparisons

The primary analysis evaluates a single primary testing contrast on the primary outcome of SBP (Primary Aim **1.1**). No multiple comparisons adjustment on type 1 error is necessary.

For all other aims, multiple comparison adjustments are not adopted. Estimated effects will be reported with corresponding 95% confidence intervals. A crucial feature of our approach to study interpretation is the requirement of biological coherence of statistically significant findings. Estimates that do not fit biologically with the bulk of our findings, even those that are large relative to estimated standard errors, will be reported with explicit warnings about coherence and the risk of chance errors. Unexpected significant findings will be reported as requiring independent confirmation. Findings that do not arise from pre-specified comparisons noted in the study protocol or statistical analysis plan will be explicitly identified as such.

4.2 Primary outcome and primary testing contrast

End-of-period, office-based systolic and diastolic BP will be measured by the Omron HEM 907XL device, which records BP using an oscillometric technique. The Omron device has been validated. BP is obtained by trained and certified data collectors according to a standard protocol, adapted from that used in the OmniHeart, SPRINT, and other trials. Three consecutive measurements (each separated by 30 seconds) are obtained on the right arm of participants after they rest quietly in the seated position for at least 5 minutes. Heart rate and average BP will also be reported by the device.

The primary outcome is systolic BP (SBP) and is constructed as follows. The SBP measure associated with a given diet and sodium level is the mean of the five sets of three SBP measures (total of 15 BPs) obtained in the final two weeks of the feeding period for that diet. Daily mean systolic BP (SBP) will be averaged based on the triplicate measures, and the mean of these daily SBP measurements will be used as the primary outcome of the corresponding feeding period. This calculation is straight forward in the absence of missing data. We will use repeated-measures mixed-effects regression modeling to address missing data under the assumption of MAR as the primary analysis. The primary testing contrast tests the mean difference of SBP between the DASH4D diet with lower sodium (DL) and the comparison diet

with higher sodium (CH), which will be conducted using the mixed-effects modelling approach as described below.

4.2.1 Primary analysis: Intention-to-treat analysis

The primary analysis will be conducted according to the intention-to-treat (ITT) principle. Without missing outcome data, the primary analysis can be carried out using the following repeated-measures mixed-effects regression model, where the feeding period-specific study outcomes can be viewed as a high-dimensional vector of repeated measures on individual participants, with diet assignment indicators, feeding period indicators, and additional relevant variables either as time-invariant or time-dependent (e.g., period-specific) covariates. This provides a very general framework for the ITT analysis and can be used to explore potential carryover effects by incorporating the diet-by-period cross-product interaction terms into the model. Sub-vectors of the entire outcome vector may be appropriately analyzed using the mixed effects model:

$$Y_{ij} = \beta_0 + \alpha_j \, I(\text{Period} = j) + \beta_k \, I(\text{Diet} = k) + f(W_{ij}; \theta) + e_{ij} \qquad \text{(Model 1)}$$

where i = 1, …, n indexes individuals and j = 1 to 4 indexes repeated measures on outcome Y over each of the 4 feeding periods, respectively, with $\alpha_1 = 0$ and α_j , j = 2 to 4, indicating the incremental increase in period effect over the effect of period 1, $\beta_1 = 0$ and β_k , k = 2 to 4, indicating the incremental increase in diet effect of CL, DH, and DL diets over the effect of the reference diet CH, respectively. With this parametrization, β_4 estimates the intervention effect under the primary testing contrast for testing the mean difference of SBP between the DASH4D diet with lower sodium (DL) and the comparison diet with higher sodium (CH), which will be tested with a 2-sided significance level of 0.05, with the corresponding 95% confidence interval for the estimated effect difference reported.

Note that the outcome Y here will be the average of the 15 end-of-period SBP measurements in the absence of missing data, and I(•) denotes the indicator function which returns the value 1 if the argument is true and 0 otherwise. The error term e_{ij} is assumed as independently Gaussian distributed vector for i = 1, …, n, with mean vector zero and a 4x4 unstructured variance-covariance matrix to allow flexible outcome variances at different feeding periods (j = 1 to 4) and covariances between feeding periods. The model component $f(W_{ij}; \theta)$ is some function of covariates W_{ij} which may include baseline characteristics and/or period-specific covariates. Given the crossover trial design, $f(W_{ij}; \theta)$ will only be included when necessary, as part of sensitivity analyses to augment the primary analysis in the absence of missing data.

When any of the individual end-of-period SBP measures is missing while multiple SBP assessments during the diet period are available, we will expand Model 1 and put repeated SBP measure as $Y_{ij\ell}$, where ℓ indexes the 5 end-of-period measures within period, and structure the model as an observed-data likelihood-based longitudinal model using the repeated-measures mixed-effects modelling approach, that is,

$$Y_{ij\ell} = \beta_0 + \alpha_j \, I(\text{Period} = j) + \beta_k \, I(\text{Diet} = k) + f(W_{ij}; \theta) + e_{ij\ell} \qquad \text{(Model 2)}$$

with appropriate covariance structure of the repeated measurements of SBP within (e.g., compound symmetric) and across (e.g., unstructured) feeding periods, while incorporating relevant covariates in the mean model to implicitly impute the missing SBP within the end-of-period, and derive the desired change of BP contrast between specific periods under the MAR assumption. All data from the entire ITT sample will be structured accordingly and used in this analysis, with missing values indicated using the

software-designated missing indictor. Model 2 may be considered if there is a sizable number of individual end-of-period SBP measures missing, while very few periods are without end-of-period measures completely.

To perform ITT analysis with participants who are lost-to-follow-up prior to outcome measure assessment, especially when a sizable number of periods are without end-of-period measures completely, we will construct an observed-data likelihood-based regression model under the MAR assumption using Model 1, incorporating covariates relevant to the MAR mechanism in the mean model and an appropriate variance-covariance structure, analyzing the entire ITT sample with software-designated missing indicator to indicate a missing value. Additionally, we could also utilize the weekly SBP assessments prior to the end-of-period as auxiliary information on SBP, if available, and incorporate them to better address the missing end-of-period SBP data, for example through a time-course based model incorporating these additional SBP measurements for multiple imputation under MAR. See Model 4 in **4.4** for additional model descriptions.

Validity of assumptions for statistical approaches and models will be evaluated, and proper outcome transformation will be carried out if necessary. Equivalently, the aforementioned, model-based approaches can also be done through multiple imputation of the missing data using the observed data likelihood model as the imputation model. Sensitivity analyses based on sensible NMAR scenarios will be carried out through multiple imputation.

A parallel approach will be used for Secondary Aims **1.2.1**, **1.2.2**, and **1.2.6**, and the continuous outcomes in Aim **1.2.7** (see **4.6**).

4.2.2 Exploring for carryover effects

Carryover effects are not expected, given the interval between outcome ascertainment in consecutive periods (at least 6 weeks apart, given the duration of each period [5 weeks] and the interval between periods [at least 1 week]) and the observed time course of BP and plasma lipids in prior feeding studies. Nonetheless, we will explore for potential carryover effects by expanding Model 1 and including additional covariates for carryover effects.

$$Y_{ij} = \beta_0 + \alpha_j\, I(\text{Period} = j) + \beta_k\, I(\text{Diet} = k) + \gamma_q\, I(\text{Diet} = q \text{ in Period } j\text{-}1) + f(W_{ij}; \theta) + e_{ij} \quad \text{(Model 3)}$$

Where $\gamma_1 = 0$ and γ_q, q = 2 to 4 and q ≠ k, indicating the incremental increase in carryover diet effect of CL, DH, and DL diets, respectively, from the previous feeding period over the similar carryover effect of the reference diet CH. Small estimates of γ_q for q = 2 to 4 support the notion of no carryover effects from diets. We will report finding of significant or large (in the context of hypothesized intervention effect) estimates of carryover effect. In additional sensitivity analyses, we will repeat our aims using only data from the first period.

4.2.3 Sensitivity analysis addressing BP medication changes during the trial

Data on BP medication at baseline and any changes in BP medication during the trial will be tracked. An investigator masked to intervention assignment will code the change into categories: increased BP medication (above dose and/or frequency of baseline medication or addition of new medication), decreased BP medication (below dose and/or frequency of baseline medication or stopped medication), or swapped BP medication, against the reference group of no change in BP medication from baseline.

We will describe the frequency distribution of this variable by intervention diets. As the distribution of this variable would allow, we will include this variable as a time-dependent categorical covariate for each feeding period in Model 1 (or Model 2 if used as the basis of the primary analysis) as a sensitivity analysis to evaluate the robustness of the intervention effects established in the primary analysis. BP medication changes after baseline is a post-randomization variable which has to be dealt with carefully and the related results interpreted cautiously.

We will also exclude participants who experienced BP medication change during the trial in an additional sensitivity analysis using the primary analysis approach with data from only the remaining participants who did not have any BP medication alteration during the trial.

A similar approach will be used for changed lipid lowering medications for analysis of fasting lipid outcome variables and changed glucose medication for analysis of glycemia outcomes (Secondary Aim **1.2.7**).

4.2.4 Sensitivity analysis evaluating the robustness of MAR assumption

Sensitivity analyses through multiple imputation based on sensible missing not at random (NMAR) scenarios will be conducted to further evaluate the robustness of the ITT analysis results under MAR. We will conduct multiple imputation using the MAR-based models, preserving the estimated variance-covariance structure, while modifying the predicted means for multiple imputation under expert guided NMAR scenarios, and analyze such imputed data through standard approaches.

4.2.5 Per protocol analyses

The main analyses will be supplemented by per protocol (PP) analyses, excluding information on study dropouts whenever incompleteness interferes with a given comparison. A PP analysis evaluates the intervention effects that could occur under optimal conditions and, in a crossover design, is less prone to confounding bias due to imbalance of personal characteristics between intervention arms induced by missing outcome data. PP analysis was used in the OmniHeart trial as the primary analytic approach.

Participants who do not have perfect adherence on >90% of days for a given diet will be excluded from any PP analysis that includes that diet. As such, participants who are lost to follow-up, drop out of the trial, or are withdrawn will be excluded for the feeding periods they do not complete. Perfect adherence on a given day reflects that the participant reported eating all provided study foods (none left over) and reported not eating or drinking any non-allowed foods or beverages. Separate analyses will be conducted for the paired comparisons of two study diets using data from participants who adhere (as defined above) to both diets. For example, if a participant missed one week of eating the study food only while in their CH feeding period (e.g., due to illness), or if the participant dropped out or was withdrawn from the study prior to their CH feeding period, the participant would not be included in the PP analysis for the primary contrast or any of the secondary contrasts that include the CH diet, but would be included in secondary contrasts that do not include the CH diet (e.g., DH vs DL) if they adhere to both diets being compared in the testing contrast).

4.3 Secondary contrasts for the primary outcome (Aims 1.2.1 and 1.2.2)

The secondary testing contrasts of the primary outcome include testing the difference of mean end-of-period SBP between lower and higher sodium level within DASH4D and comparison diet (**Secondary Aim 1.2.1)**, and the difference of mean SBP between DASH4D and comparison diet at each sodium level (**Secondary Aim 1.2.2)**. These analyses will be carried out using an observed-data likelihood approach implemented through mixed-effects regression models, or multiple imputation based on equivalent imputation models, under MAR assumption in the presence of missing data, similar to the approach described in the primary analysis (see **4.2**). Specifically, for **Aim 1.2.1**, the effect of lower sodium over higher sodium level in the DASH4D diet on SBP can be carried out by estimating $\beta_4 - \beta_3$ using Model 1 (or Model 2 if selected based on missing data pattern), while the effect of lower sodium over higher sodium level in the comparison diet on SBP can be carried out by estimating β_2 using the same mixed-effects regression model. For **Aim 1.2.2**, the effect of the DASH4D diet over the comparison diet on SBP in lower sodium level can be carried out by estimating $\beta_4 - \beta_2$, while the effect of the DASH4D diet over the comparison diet on SBP in higher sodium level can be carried out by estimating β_3 using the same model. These analyses will not be adjusted for multiple comparisons.

Sensitivity analysis under NMAR scenarios using multiple imputation for the primary aim will provide imputed data to examine the robustness of the finding under MAR for these two secondary aims (see **4.2.4**). Sensitivity analysis for addressing BP medication changes and per protocol analyses will also be carried out using similar approaches as described in **4.2**.**3** and **4.2.5**.

4.4 Additional contrasts for the primary outcome (Aims 1.2.3, 1.2.4 and 1.2.5)

We will evaluate whether the effects of diet and sodium intake on SBP are additive (Aim **1.2.3**) through examining the magnitude of estimate for $\beta_4 - \beta_3 - \beta_2$, where a small estimated value (close to 0) would be consistent with additive effects from diet and sodium level, while a large value in either direction away

from 0 would be suggestive of an interaction between diet and sodium effects. Uncertainty of this estimate will be quantified using the 95% CI corresponding to the estimate. This is an exploratory analysis, and the trial is not specifically powered to evaluate such a non-additive effect (i.e., statistical interaction).

To investigate dynamics of SBP change with increasing duration of diet exposure (Aims **1.2.4 and 1.2.5**), we expand Model 1 described in **4.2.1** to have up to 8 SBP measurements (i.e., $\ell = 1, \ldots, 8$) for participant *i* over time during the 5-weeks of feeding period *j*, i.e.,

$$Y_{ij\ell} = \beta_0 + \alpha_j \, I(\text{Period} = j) + \beta_k \, I(\text{Diet} = k) + f_k(W_{ij\ell}; \theta) + e_{ij\ell} \qquad \text{(Model 4)}$$

where the covariate $W_{ij\ell}$ now includes the time when SBP $Y_{ij\ell}$ is measured within the feeding period, and the function f_k is allowed to be different across diets to reflect the potentially different SBP patterns over the diet-specific 5-week period (e.g., straight lines with diet-dependent slopes), which may take the form of a quadratic, or segmentally linear, or simply a smooth nonparametric function of time elapsed on diet. We will explore the mean pattern of the SBP over time using nonparametric loess plot to guide the specification of appropriate mean model for SBP over time for the mixed-effects regression analysis, mimicking the observed mean time course of SBP for each feeding period. Actual day within each feeding period when SBP is measured will be used as the time variable in the analysis. Between- and within-subject variability in repeated outcome measures can be assessed by incorporating random effects δ_i and evaluating components of variance due to random effects and residual error. We will address these extended modeling activities using the Non-Linear Mixed-Effects modeling procedures in SAS or in R (Pinheiro and Bates, 2000). Model-based 95% confidence intervals (band) for the mean SBP level estimates over time will be derived.

4.5 Contrasts for the secondary outcomes

The contrasts to be evaluated for the secondary outcome include estimating the difference of mean end-of-period DBP between DL and CH diets, between lower and higher sodium level within the DASH4D and comparison diets, and between the DASH4D and comparison diet at each sodium level.

The DBP measure will be constructed analogously to the SBP measure. The analyses for DBP will be carried out similarly to analysis for the primary outcome of SBP using an observed data likelihood approach implemented through a mixed-effects regression models (e.g., Model 1), anchored on the MAR assumption (see **4.2.1, 4.3** and **4.4**). Similarly, sensitivity analysis under NMAR scenarios using multiple imputation will be carried out to examine the robustness of the finding under MAR (see **4.2.4**). Sensitivity analysis for addressing BP medication changes and per protocol analyses will also be carried out using similar approaches as described in **4.2.3** and **4.2.5**.

We will also evaluate whether the effects of diet and sodium intake on DBP are additive, as well as explore for the dynamics of DBP change with increasing duration of diet exposure for each diet. We will follow the same analytic approaches for SBP as described in **4.4**.

4.6 Evaluations for other outcomes

The contrasts of other outcomes include evaluating the mean difference of end-of-period fasting glucose, fructosamine, and plasma lipid levels; estimated CVD risk derived from the ACC/AHA ASCVD risk

equation; postural changes in systolic and diastolic BP; and diet acceptability patient rating scales between DL and CH diets, between lower and higher sodium level within the DASH4D and comparison diets, and between the DASH4D and comparison diet at each sodium level.

The analyses for the plasma lipid measures will be carried out similarly to analysis for the primary outcome of SBP using an observed data likelihood approach implemented through mixed-effects regression models (e.g., Model 1), anchored on the MAR assumption (see **4.2.1, 4.3** and **4.4**). Appropriate transformation of the outcome will be carried out when necessary to satisfy relevant model assumptions. Similarly, sensitivity analysis under NMAR scenarios using multiple imputation will be carried out to examine the robustness of the finding under MAR (see **4.2.4**), as well as sensitivity analysis to address relevant medication changes (see **4.2**.**3)**.

The safety outcomes are counts of binary outcomes collected throughout the feeding period, and SAEs and adverse medical events are similar count data (Poisson outcomes) that will be collected throughout the feeding period per trial protocol. We will evaluate the differences of the safety outcomes between DL and CH diets, between lower and higher sodium levels within the DASH4D and comparison diets, and between the DASH4D and comparison diets at each sodium level. The total number of events and the number of persons experiencing 1 or more events will be reported.

The safety outcomes of count data (or binary variables) will be analyzed using generalized estimating equations (GEE) approach, to derive population-average inferences while accounting for the correlation of outcome data from different feeding periods within the same participant. The mean model will be constructed similarly to the mean models used in the mixed-effects regression analysis previously described (e.g., same parametrization of predictors). Log link will be used for count outcomes and logit link will be used for binary outcomes. Unstructured working correlation will be used, and robust standard errors will be derived for statistical inferences. The potential for overdispersion in the count data will be explored, and negative binomial models will be used when appropriate.

Similar analytic strategies will be used for exploration of additional relevant outcomes that are not specified in the Aims.

4.7 Subgroup analysis

Protocol pre-specified subgroup analyses will be conducted using mixed-effects linear models or the GEE approach, consistent with the analyses proposed for each outcome as described previously. Pre-specified subgroups of interest are those defined by gender (women, men), race (Black, non-Black), baseline level of the outcome of interest, baseline number of medications used to treat the outcome, and chronic kidney disease status. The mean models for the analysis will be augmented with binary subgroup indicator(s) and appropriate cross-product interaction terms of subgroup indicator(s) by diet indicators. The differential intervention effects between subgroups will be evaluated through estimating the regression beta coefficient of the interaction terms and the corresponding 95% CIs. Subgroups with less than 10 participants will not be reported. The trial is not powered based on any specific subgroup analyses so these analyses are exploratory and will not be adjusted for multiple comparisons.