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Inflammatory Biomarkers and Serum Fatty Acids in High-Risk Populations: A Research Protocol

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Abstract

Background: Advances in hypertension management have resulted in a growing number of survivors in the United States. Despite the success of treatments, patients face long-term challenges and changes in health, and with the likelihood of developing more age-related comorbid disorders, related to cardiovascular, renal, pulmonary conditions, and stroke due to uncontrolled blood pressure and a reduction in life expectancy. This study hypothesizes that the increased risk of morbidity and mortality may be due to increased fat intake, resulting in the acceleration of hypertension.

Methods: To address these questions, this study leverages an existing TLIP P-50 project (5P50MD01347-04) at Meharry Medical College in Nashville, TN. Participants will be examined at baseline and prospectively followed at 6, 12, 24, and 36 months to address three specific aims:

Results:1) to examine differences at baseline and in prospective rate of change of metabolic fatty acid assays as a function of blood Pressure progression; 2) to examine differences at baseline and in prospective rate of change of bio- markers assays as a function of Blood Pressure progression; 3) Inflammatory Bio markers and metabolic acid assay in relation to reduction of comorbidities associated with blood pressure.

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Conclusions: This study will determine whether monitoring inflammatory biomarkers and serum fatty acid assays over time can detect the progression of hypertension, with independent effects or through the interaction of behavioral factors in high-risk populations, and whether this approach can reduce the risk of age-related morbidities. Such information is necessary to define the risk population and to selectively target inflammatory biomarkers and fatty acid assays with the most effective efficacy and potential to reduce the risk of age-related morbidities.

Keywords: Hypertension; systolic blood pressure; diastolic blood pressure; Biomarkers; serum fatty acids.

1. Introduction

In line with the Clinical Research Initiative, this study identifies the fatty acids that contribute to overweight, obesity, and hypertension, which are interrelated. It is imperative to determine whether monitoring of inflammatory and fatty acid biomarkers independently has an influence and whether these biomarkers play a role in the progression of hypertension in high-risk populations. These findings have implications for monitoring progression of hypertension, predicting morbid outcomes, and for the development of interventions that can target vulnerable groups with precision (i.e., elevated levels of inflammation) and mitigate age-related morbidity associated with these biomarker risk profiles.

2. Project narrative

This proposal addresses a key clinical research priority in Hypertension: monitoring fatty acids, biomarkers, and interventions to address Hypertension and reduce the medical complications arising from uncontrolled Hypertension. The overarching goal of this proposal is to evaluate serum fatty acid levels and the progression of Hypertension, and to determine whether monitoring serum fatty acid assays helps monitor Hypertension and whether assessment-driven interventions can assist in better management of Hypertension. This proposal is essential because Hypertension is more prevalent in black Americans than in the general population. According to CDC data, 58% of the African American Population is hypertensive. This will be a longitudinal study design that shall involve repeated observation and measurement of dietary fatty acids (Initial assessment, 6, 12, 24, 36 months follow-up). The study will adhere to DASH diet scores measured at 6, 12, 24, and 36 months of follow-up. These scores will be computed from survey self-reports. The intake intervention, developed using Community Engagement Research principles [20], will be delivered in person, with selected education sessions. The intervention will be designed to provide knowledge, competence, and skills that empower participants to implement lifestyle modifications necessary to control Hypertension and prevent adverse complications.

A cross-sectional study from 18 countries [19] has revealed that dietary consumption of monounsaturated fatty acids (MUFAs) was inversely associated with Hypertension. Due to inconclusive results on the association between dietary fatty acid intake and the risk of HTN, and limited studies on this relationship, this study would assess the relationship between excess fatty acid consumption and progression/relation to HTN in a high-risk community population. No standard approach exists to identifying risk and implementing interventions to prevent adverse outcomes related to fatty acid consumption; analysis and follow-up in hypertensive clinics. The

overarching goal of this proposal is to evaluate whether providing cardiologists with lifestyle modification interventions —such as the DASH diet, lifestyle7 plus sleep modification, and follow-up with serum fatty acid analysis at 6, 12, 24, and 36 months —improves outcomes. About expected outcomes, this proposal will fill vital gaps in knowledge regarding serum fatty acid levels and progression of Hypertension in the high-risk population, and the mechanism of how monitoring serum fatty acid levels will help cardiologists and medical practitioners improve decision–making and improve quality of life in hypertensive patients, and help the high-risk population in self-monitoring their blood pressures.

3. Specific aims and project Summary

Four fatty acids were associated with blood pressure, independent of differences in dietary fat intake. Stearic acid (18:0), palmitoleic acid (16:1), and eicosatrienoic acid (20:3) are nonessential fatty acids. Dihomogammalinolenic acid (20.3), an essential fatty acid, is derived primarily from linoleic acid (18.2); the differences in the serum levels of these fatty acids reflect fatty acid metabolism as well as dietary intake and therefore may be modifiable. [1, 2, 3, 4]

Cholesterol ester stearic acid (18.0), a long-chain saturated fatty acid, was associated with lower blood pressure levels, and cholesterol ester palmitoleic acid (16:1), a monounsaturated fatty acid, and phospholipid eicosatrienoic acid (20:3), and ω9 polyunsaturated fatty acid, were associated with higher blood pressure levels. Cholesterol esters, dihomogammalinolenic acid (20.3), ω9 polyunsaturated fatty acid, and ω6 fatty acid were also associated with higher blood pressure.

The observational studies that have examined the relation of fatty acid concentrations in their blood or adipose tissue to blood pressure have not reported consistent results [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]

The possibility that differences in blood pressure influence serum fatty acid composition of serum lipoproteins, affecting blood pressure, cannot be excluded. Current dietary guidelines for US adults recommend total fat intake between 20% and 35% of calories, saturated FAs intake less than 10% of calories, and trans FAs consumption as low as possible [11]. Epidemiological evidence on the relevance of such dietary recommendations to the prevention of Hypertension is surprisingly limited. With total fat intake as a percentage of calories falling over recent decades, more specific recommendations on the optimal amount and type of FAs intake for Hypertension and cardiovascular disease prevention are needed. An adverse diet profile, along with an unhealthy lifestyle, may increase the risk of Hypertension through promoting obesity [12]. In recent years it has been shown that Omega-3 fatty acids have blood pressure-lowering, antithrombotic, inflammatory status- improving, and endothelial -protective properties, which in turn suggests that Omega-3 fatty acids have a better efficacy in enhancing both the composition and stability of coronary atherosclerotic plaques [13], thus further demonstrating that Omega-3 fatty acids are associated with the additional benefit of lowering blood pressure [14].

Hypertension is extensively associated with most chronic diseases, such as cardiovascular disease (CVD), chronic kidney disease (CKD), end-stage renal disease (ESRD), and mortality [15]. The American Heart Association expected that the direct and indirect costs of HTN in the US population would rise to 240 billion USD by 2030 [16]. Based on evidence, lifestyle factors like dietary habits, alcohol consumption, and physical inactivity are the main contributors to the high

prevalence of this disease [17]. Preventing Hypertension through dietary modification is the best solution for public health to promote the population's well-being [14]. Although the effects of dietary sodium, potassium, fruit, vegetables, and obesity on blood pressure control are well established, the roles of fatty acids remain ambiguous [17, 18]. The macronutrient dietary fat plays a crucial role in providing energy for physical activity, as well as aiding in the transport of fat-soluble vitamins and in the construction of cell membranes [14]. However, overconsumption of fat can lead to weight gain and the risk of developing various diseases [14]. A cross-sectional study across 18 countries [19] found that dietary consumption of monounsaturated fatty acids (MUFAs) was inversely associated with Hypertension. Due to inconclusive results on the association between dietary fatty acid intake and the risk of HTN and limited studies on this relationship, this study would assess the relationship between excess fatty acid consumption and progression/relation to HTN in the high-risk community population.

4. Research strategies and methods

To examine these questions, this study leverages an existing TLIP P-50 project (5P50MD01347-04) at Meharry Medical College [21]. Participants from Nashville general hospital (NGH) clinics/ Metropolitan development housing authority (MDHA) housing projects/ Mathew Walker clinics and participants from blood pressure screening at various community centers and churches in Davidson county, Nashville, TN diagnosed with hypertension in the last 3 years identified from the clinic records, and new patients with hypertension will be invited to participate by their physician to examine the relation between serum fatty acids and blood pressure. (see table 1). Flyers will be distributed in the clinics, and a poster will be displayed in the waiting area.

Table 1: Enrollment

Visit 1: Enrollment; 153	**Visit 2: Bio-Sample collection; 53**
Public Housing (MDHA): 76	Public Housing communities: 30
Nashville General; 39	Nashville General: 14
Mathew Walker; 25	Mathew walker; 8
Health fair; 10	Health fair: 1
Non- Public Housing; 2	

Participants will be examined at baseline and prospectively followed at 6, 12, 24, and 36 months to address three specific aims as outlined in the research proposal (see Table 2).

Table 2: Study/Project outcomes

Primary Outcome Measures	Secondary Outcome Measures	Other Pre-Specified Outcomes
Systolic and Diastolic pressure changes At enrollment Follow up at 6,12, 24, 36 months.	LS7 Score and DASH score Each item on LS7/DASH will be scored at enrollment Follow up at 6,12, 24, 36 months	Selected Biomarkers and metabolic fatty acid assays for assessing nutritional status and progression of hypertension Follow up at 6, 12, 24, 36 months

4.1 Research Tools

Demographics & Medical History; -BP and Physical Measurements; -Life's Simple 7 Assessment Survey; DASH Eating Plan Assessment Survey; -Bio samples: blood collection protocols.

4.2 Human Subject Safety and Protection materials

Consent forms, HIPAA forms, Recruitment flyers, Posters.

4.3 Bio sample collection & handling

Biomarkers of body mass index (BMI), which include C-Reactive Protein (CRP), Interleukin (IL)-10, and Adiponectin.73 Biomarkers for obesity that can be measured in saliva include IL-10, Leptin, CRP, Insulin, and Vascular Endothelial Growth Factor (VEGF). These are all associated with cardiovascular disease and pulmonary arterial hypertension [22]. In addition to these biomarkers Plasminogen activator inhibitor-1 (PAI-1) B-type are currently recommended as risk factors in PAH, inclusion of CRP, Plasminogen activator inhibitor-1 (PAI-1) [23]. In addition, Galectin-3 (Gal-3) and Tissue inhibitor of metalloproteinase 4 (TIMP-4) have been suggested as essential biomarkers for PAH and progression of hypertension [22, 23, 24].

This study is an opportunity to discover biomarkers and metabolic fatty acids using the U-PLEX Metabolic Group 1, which evaluates 87 analytes in human plasma or serum (Cat # K151ACM). Standard biosafety protocols will collect 5.0 ml of serum/plasma. (collected at the time of participation, 6, 12, 24, and 36 months, follow-up). Samples will be stored as 250 µL aliquots at -80 °C to avoid freeze-thaw cycles. Biomarkers and metabolic fatty acids will be assayed using the human metabolic panel kits (Meso Scale Discovery, Rockville, MD, USA) and read on the MESO Quick Plex SQ 120MM (Meso Scale Discovery) located at the Molecular Biology Core Facility at Meharry Medical College.

CONCEPTUAL FRAMEWORK OF THE PROJECT/STUDY

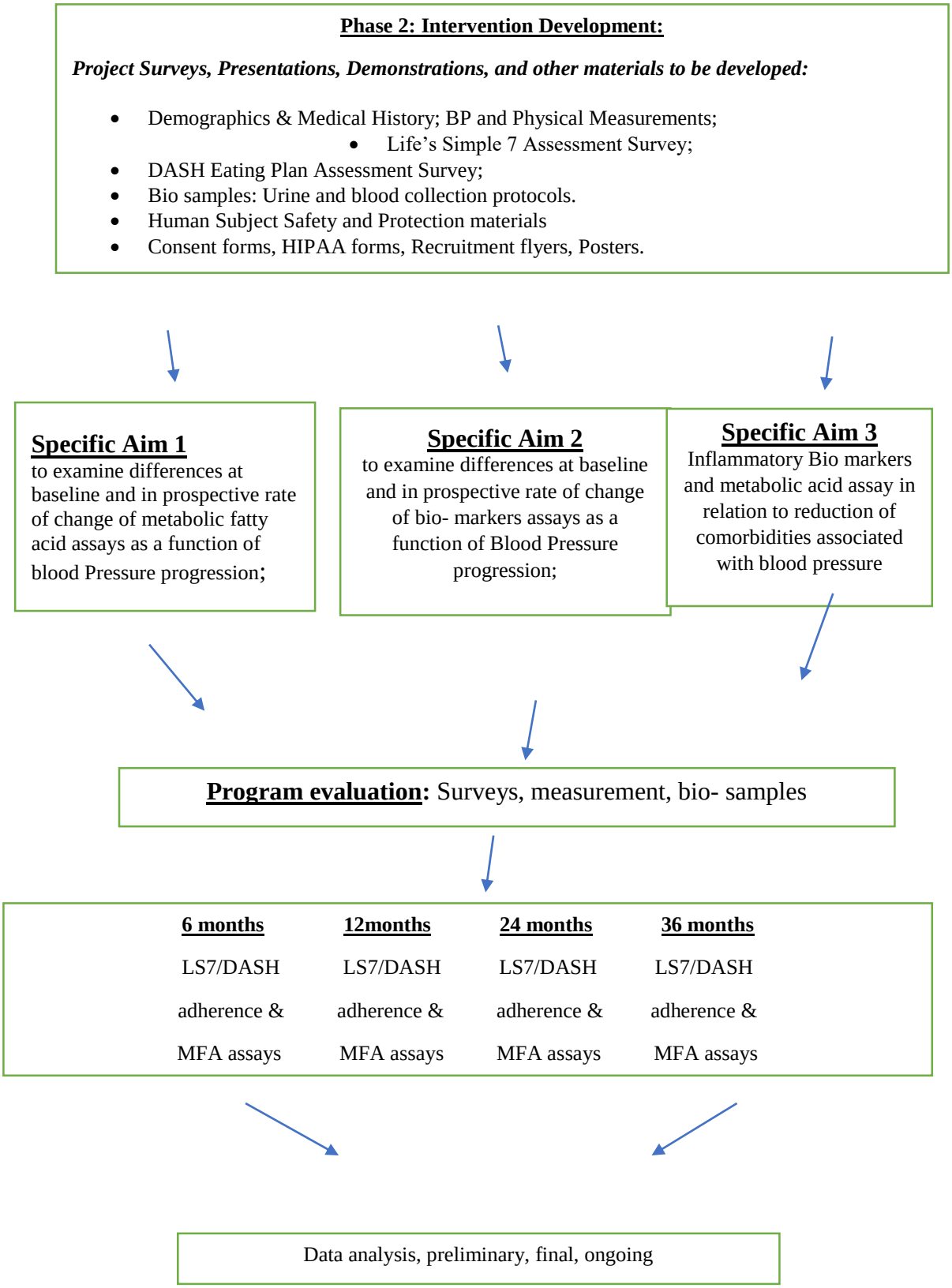

Figure 1: Conceptual framework of the project/study

5.Data analysis plan

The primary study outcomes are monitoring metabolic fatty acid assays as a function of blood pressure progression at 6, 12, 24, and 36 months. The secondary outcomes are LS7 and DASH adherence scores at 6, 12, 24, and 36 months (see figure 1). Descriptive demographic statistics will be tabulated by study arm and study site. Continuous variables such as SBP, DBP, BMI, Waist, and Biomarker measures will be assessed for normality and log10 transformed as needed. All analyses will be performed on an intention-to-treat basis, and statistical tests will be two-sided with a 5% significance level. Percent adherence (Low, Medium, High) for both LS7 and DASH, and patient healthy lifestyle knowledge will be based on the difference from baseline to follow-up, using matched t-tests and chi-square tests, which are appropriate. Changes in LS7 and DASH scores will be measured using repeated measures ANOVA followed by Tukey's post-hoc test. If the data are heteroscedastic, the nonparametric Friedman's test will be used in conjunction with the Games-Howell post hoc test. The model will include terms for education, age, hypertension status, marital status, and employment, and secondary outcomes and biomarker measures will be used as an unstructured covariance matrix. All analyses will be performed in R/RStudio, SPSS, and SAS software (version 9.4; SAS Institute, Cary, NC).

6.Declarations

6.1. Ethics approval and consent to participate

The study is conducted in-accordance with the declaration of Helsinki and was reviewed and approved by the Meharry Medical College IRB (IRB@mmc.edu). All study procedures were approved by Meharry medical College committee for medical and health research Ethics. (IRB Project Number 25-02-1555).

7.Competing interests

The authors declare no competing interests.

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