THE IMPACT OF

SODIUM AND FRUCTOSE

ON

BLOOD PRESSURE AND INFLAMMATION

by

Ronald K. McMillan

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences

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I'd like to begin by expressing thanks to my family and to God, it was because of their support and guidance that made all of this possible. Like countless others, my fascination with science began during my middle to late elementary school years, growing steadily alongside my experiences. As a first-generation African American college student, I encountered numerous hurdles in my early academic pursuits. Yet, through resilience and the invaluable guidance of key mentors, I overcame these obstacles, finally reaching my goal of achieving a doctoral degree. Along each step of this journey, I've encountered individuals whose guidance and support have been indispensable. To them, I owe a debt of immense gratitude. In this acknowledgment section, I wish to extend my heartfelt appreciation to a select few who have played pivotal roles in shaping my path.

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ABSTRACT

Increased dietary sodium is associated with hypertension. Yet, many young healthy adults have "sodium-resistant" blood pressure (BP), meaning that increased dietary sodium is not associated with increased BP[1]. This is because normal functioning kidneys can excrete excess dietary sodium, preventing a sodium-induced expansion of the extracellular fluid volume (ECV) and consequent increase in BP. However, rodent studies demonstrate that increased dietary fructose impairs renal sodium handling, which is associated with increased BP and hypertension risk[2,3]. Moreover, both dietary salt and fructose have been shown to be pro-inflammatory [4–6].

The immune mechanisms underlying the pro-inflammatory effects of sodium and fructose are poorly understood. The impact of the Western diet on the peripheral blood mononuclear cells (PBMCs) has garnered attention due to associations with vascular-related diseases [7–10]. There is evidence demonstrating that increased dietary sodium induces pathogenic subtypes of PBMCs, resulting in an exacerbated production of proinflammatory interleukins[11]. In rodents fed increased sodium, T-cell infiltration in the kidneys was increased in conjunction with higher levels of reactive oxygen species (ROS)[12]. Moreover, increased fructose consumption is linked to elevated *de novo* lipogenesis and plasma triglyceride levels, both contributing to increased oxidative stress [13–15].

The combined consumption of increased dietary sodium and fructose, prevalent in typical Western diets, may exacerbate hypertension and inflammation more than sodium loading alone. While numerous rodent studies support these associations, few have sought to validate them in humans [16–20]. This research gap is significant, given the prevalence of Western dietary patterns and their link to cardiovascular health. Here we hypothesize that 1) the combination of increased dietary fructose and sodium will increase BP in normotensive adults by preventing the kidneys from properly excreting excess sodium and 2) the combination of increased dietary fructose and sodium will induce increased ROS and inflammation resulting in an impairment in endothelium function when compared to an increased sodium alone. To investigate our hypotheses, we gathered data from 36 participants using a randomized double-blind clinical trial. Our study demonstrates that within a single week of consuming increased dietary fructose in conjunction with high sodium, healthy normotensive adults display a modest reduction in urinary sodium excretion and an increase in BP. Additionally, we find that the combination of increased fructose and sodium induces a greater pro-inflammatory (e.g., TNF alpha and IL-6) and oxidative stress (e.g., intracellular ROS) response when compared to sodium loading alone. Overall, these data demonstrate that increased sodium and fructose lead to alterations in renal function while promoting inflammation in young healthy adults.

Chapter 1

REVIEW OF LITERATURE

1.1 Introduction

Hypertension, sometimes referred to as the "silent killer," increases the risk of cardiovascular disease (CVD) by appearing without noticeable external symptoms. The Center for Disease Control and Prevention (CDC) estimates approximately 50% of adults have high blood pressure (BP) and further estimates that only 25% of that population have their blood pressure under control through medication and lifestyle improvements[21]. BP is the force applied by circulating blood against the major arteries, and it is typically measured in millimeters of mercury[22]. It is traditionally divided into systolic and diastolic measurements. Systolic blood pressure (SBP) denotes the highest blood pressure recorded during ventricular contraction, whereas diastolic blood pressure (DBP) represents the lowest pressure measured just before the next contraction [22].

A person is considered to have normotensive blood pressure when the SBP is below 120 millimeters of mercury (mmHg) and the DBP is below 80 mmHg[23]. Blood pressure assessments often categorize systolic values between 120-129 mmHg and diastolic values below 80 mmHg as elevated blood pressure. When SBP and/or DBP exceed 130 and 80 mmHg, respectively, hypertension stage 1 is diagnosed, which is associated with an increased risk of cardiovascular diseases[23]. Hypertension stage 2 is identified when blood pressure consistently measures above an SBP of 140 and/or a

DBP of 90 mmHg, requiring medical treatment and lifestyle interventions to lower BP levels. Blood pressure readings higher than an SBP of 180 and/or a DBP of 120 mmHg warrant immediate medical attention due to the significantly heightened risk of adverse cardiac events.

BP-related diseases, specifically stroke, coronary heart disease, heart failure, and kidney disease, are the leading causes of morbidity and mortality throughout the world[24]. Indeed, over one-third of Americans, or about 868,000 people, die due to heart disease or stroke each year[25]. The financial burden of heart disease in the United States, encompassing healthcare expenses, medication costs, and lost work productivity, amounted to approximately $240 billion from 2018 to 2019 [26]. Several factors, including genetics, obesity, stress levels, and lifestyle choices, contribute to BP related diseases [27]. This review will focus on the consumption of dietary sodium and fructose, which are characteristic of the Western diet.

Excess dietary sodium intake on a typical Western diet has continued to exceed the AHA's recommendations [28]. The sodium intake guidelines suggest a daily limit of approximately a teaspoon of salt or 2300 mg of sodium per day, yet the average American consumes over 3400 mg of sodium per day [29,30]. Excess sodium consumption has been linked to hypertension while also affecting vascular structure, inflammatory signaling, and the central nervous system [31]. In addition to increased sodium intake, the western diet is also associated with high fructose consumption[32]. There are no specific guidelines specifically to fructose intake, however, to avoid deleterious effects it is advised to limit added sugars to less than 40g per day[33]. Added

sugars typically describe sucrose and high fructose corn syrup (HFCS). Sucrose is a disaccharide of glucose and fructose thus limiting your total fructose intake to 20g per day is recommended[33]. The average American is estimated to consume 54g of fructose daily [34]. Chronic fructose consumption can cause insulin resistance, non-alcoholic fatty liver disease, and metabolic syndrome, all of which are associated with hypertension[35–37].

Sodium sensitive BP (SSBP) describes a phenotype where BP is increased after dietary sodium is increased. SSBP is associated with an increased risk of mortality and developing CVD[38]. There are several factors implicated in the generation of SSBP such as an augmented RAAS, impaired renal function, endothelial dysfunction, and an overly stimulated immune system[39–42]. Although previous research has linked overconsumption of sodium and fructose independently to BP-related diseases, the mechanisms have yet to be fully understood[6,16,43]. Furthermore, the interaction that occurs when both are consumed in excess at the same time has not been thoroughly investigated in humans. Our review provides a summary of information to provide support for how dietary sodium and fructose contribute to the phenotype of the previously described SSBP.

1.2 Impact of Salt on Blood Pressure

1.2.1 RAAS

High salt intake suppresses the renin angiotensin aldosterone system (RAAS) whereas periods of sodium depletion have a stimulatory effect[44]. The RAAS is also

stimulated in response to a decline in blood volume, a decline in BP, and decreased plasma sodium levels [45]. In response to a decline in BP, the kidneys will secrete renin from juxtaglomerular cells into the circulatory system. When renin encounters circulating angiotensin, it cleaves the N-terminal region of the peptide generating the peptide known as angiotensin I (AngI). Ang I's C-terminal region is metabolized further by Angiotensin-converting enzyme (ACE), leading to the generation of angiotensin II (Ang II), the active enzyme. ACE is expressed in many tissues, but it is especially abundant in vascular organs such as the retina and the lung.

Ang II stimulates vasoconstriction and influences sodium and fluid reabsorption within regions of the nephrons[46–49]. In the kidney, Ang II regulates the glomerular filtration rate and sodium reabsorption by binding to angiotensin II receptor types 1 (AT1) and 2 (AT2) in the proximal and distal tubules[50]. Crajoinas et al. investigated the role of AT1 receptors in sodium transport by creating proximal tubule AT1 knockout mice [51]. Their study showed that AT1 knockout mice significantly reduced BP and NHE3 activity in response to Ang II, impairing sodium transport [51]. Furthermore, Nickening and colleagues demonstrate that AT1 is overexpressed in vascular smooth muscle cells collected from Sprague Dawley rats fed a high salt diet[52]. This not only provides evidence that the AT1 receptor plays a direct role in the reabsorption of sodium in the nephron, but a high salt diet can enhance its activity.

A high sodium balance in the body is widely acknowledged to inhibit the RAAS[44]. This is due to the stimulation of RAAS which aims to maintain the body's

salt content, leading to a negative feedback loop between sodium intake/body sodium content and renin levels. During times of sodium deprivation, the adrenal gland secretes aldosterone, which stimulates sodium reabsorption by acting on mineralocorticoid receptors located on the distal tubule of the nephron [53]. Studies in rodent models investigating the role of aldosterone in hypertension have shown that increasing aldosterone levels can have negative effects, including structural remodeling of the vasculature[54]. This effect can be attributed to aldosterone directly interacting with the endothelian-1(ET-1) receptor resulting in increased oxidase activity and inhibitory eNOS actions[55]. In treating cases of hypertension, the combination of a low-sodium diet in combination with aldosterone receptor blockers has shown poor effectiveness. Thus, research on aldosterone inhibitors has expanded from the use of mineralocorticoid receptor blockers to novel synthase inhibitors, which would increase the effectiveness of inhibiting sodium and water reabsorption and lowering blood pressure[56].

As one would expect, the RAAS must be tightly regulated on a molecular and physiological level to avoid chronic increases in BP. Abnormal activation of the RAAS is a risk factor for conditions such as stroke, atherosclerosis, and renal failure. RAAS dysfunction was linked to several diet-related factors in human clinical trials[57]. Elevated plasma renin activity (PRA) contributes to RAAS dysfunction and can serve as an indicator for kidney damage in hypertensive patients [58]. Mengyue and colleagues found a significant association between PRA and the incidence rate of chronic kidney disease based on the elevations in proteinuria within hypertensive subjects [59].

Under normal physiological conditions, renin inhibition can be triggered by increased BP or elevations in circulating AngII resulting in a decrease of intracellular calcium within juxtaglomerular cells[44]. However, patients suffering from hypertension demonstrate an altered response within this negative feedback loop resulting in renin secretion levels remaining elevated, leading to increased cleavage of angiotensinogen [60]. Furthermore, there have been preclinical studies that show the differential expression of angiotensin receptors is suggested to be one of many BP control factors that can contribute to the development of hypertension[61].

The RAAS plays a pivotal role in the regulation of BP and interacts with other physiological systems[44]. ANG II exerts specific effects on the sympathetic nervous system and baroreceptor reflexes[62]. There is evidence to support a dysregulated RAAS impairs the autonomic control of blood pressure. The dysregulation of the RAAS can be exacerbated by intrarenal factors, such as inadequate suppression of renin [39,63,64]. This can ultimately contribute to the development of SSBP. The SSBP phenotype is linked to impaired renal function, although the underlying mechanisms remain incompletely understood.[40,65,66].

1.2.2 Renal Function

Excess dietary sodium can impair kidney function and is associated with the onset of kidney disease[67–69]. The kidney functions to selectively filter waste products from the blood while also facilitating metabolite secretion through the production of urine. Prolonged excessive intake of dietary sodium can disturb molecular interactions in the three primary anatomical regions of the kidney: the renal cortex, renal medulla,

and renal pelvis [70–72]. Each kidney contains approximately 1 million nephrons, which are the individual filtering subunits of the kidney and reside primarily in the renal cortex[73]. Transitioning to the structure and components of the nephron, blood travels from the afferent arteriole to the efferent side, passing through the glomerulus—a pivotal network of small blood vessels encased by the Bowman's capsule. The glomerulus, characterized by a double layer of epithelial cells, represents a critical site for renal function and filtration [74].

The evaluation of renal function often involves assessing biomarkers in urine, including neutrophil gelatinase-associated lipocalin (NGAL), serum creatinine, kidney injury molecule-1 (KIM-1), Galectin-3, and urinary vanin-1, all indicative of kidney injury [75]. The mechanisms underlying the generation of NGAL, KIM-1, and vanin-1 are believed to involve heightened neutrophil infiltration and increased oxidative stress, processes that may be exacerbated by elevated sodium intake [76–79]. Barnett et al. provided evidence that consuming a high-sodium diet can significantly increase NGAL while also impairing creatinine clearance, an important measure of kidney function, within 10 days in normotensive adults [80]. Creatinine, a byproduct of creatine phosphate metabolism in muscle, is excreted through glomerular filtration at a constant rate mirroring its plasma concentration[81]. Although the investigation conducted by Barnett et al., did not identify an effect of sodium on KIM-1, elevated KIM-1 levels have been observed during cases of advanced stages of kidney damage [82]. Washino et al. demonstrated that vanin-1 can detect early-stage kidney injury in normotensive rats exposed to salt loading [83]. Changes in urinary NGAL, vanin-1, and creatinine clearance seem to be early indicators of kidney damage, detectable before

the presence of albumin in the urine [80,83]. Elevated levels of KIM-1 suggest a more advanced stage of chronic kidney disease [82]. These findings highlight the importance of monitoring urinary biomarkers and creatinine clearance in individuals with high salt intake, providing insights into the early detection and management of renal dysfunction associated with excessive sodium consumption.

Excess dietary salt consumption significantly impacts renal sodium transport, especially within the proximal convoluted tubule (PCT), where the bulk of sodium reabsorption takes place [84]. This process involves the renal system adjusting to effectively handle the increased sodium load by changing the activity and expression of sodium transporters and exchangers within the PCT to maintain sodium balance despite increased intake [85]. In normally functioning kidney, high salt intake acutely triggers adaptive responses within PCT cells, leading to a downregulation of sodium transport proteins such as sodium glucose transporter 2 (SGLT2), sodium phosphate cotransporter type 2 (NPT2), sodium hydrogen exchanger isoform 3 (NHE3), sodium bicarbonate transporter (NBC1), sodium-potassium-adenosine triphosphatase (Na/K ATPase), as well as sodium amino acid transporters, resulting in reduced sodium reabsorption (Figure 1)[86].

Chronic exposure to elevated salt levels can overwhelm renal compensatory mechanisms, leading to maladaptive responses and detrimental effects on renal function [87]. Changes in the expression and function of renal sodium transporters, such as the NHE3, have been linked to the pathogenesis of hypertension, fluid retention, and cardiovascular complications [88]. NHE3 plays a crucial role in mediating bulk

sodium transport within the proximal convoluted tubule (PCT), being one of nine isoforms expressed primarily at the apical membrane in the S1 and S2 segments [89]. Its function is tightly regulated at various levels, including transcriptional regulation, protein abundance, trafficking, and interactions with other proteins [90,91]. Expression of NHE3 is modulated in a protein kinase C (PKC)-dependent manner, with increased levels of Angiotensin II (Ang II) and dietary salt intake enhancing intrarenal PKC activity, thereby impairing the pressure natriuretic response[92]. Elevated PKC activity leads to the upregulation of NHE3, thereby impeding the pressure natriuretic response [92].The impact of increased salt intake on renal sodium handling provides insight on the pathophysiology of SSBP. Considering the crucial function of NHE3 in sodium transport in the PCT, even slight alterations in its activity can have notable effects on the balance of electrolytes and fluids in the body. This demonstrates the significance of ongoing investigations into the modulation of renal sodium transport by diverse factors, to identify therapeutic targets to optimize the management of CVD, hypertension, and SSBP.

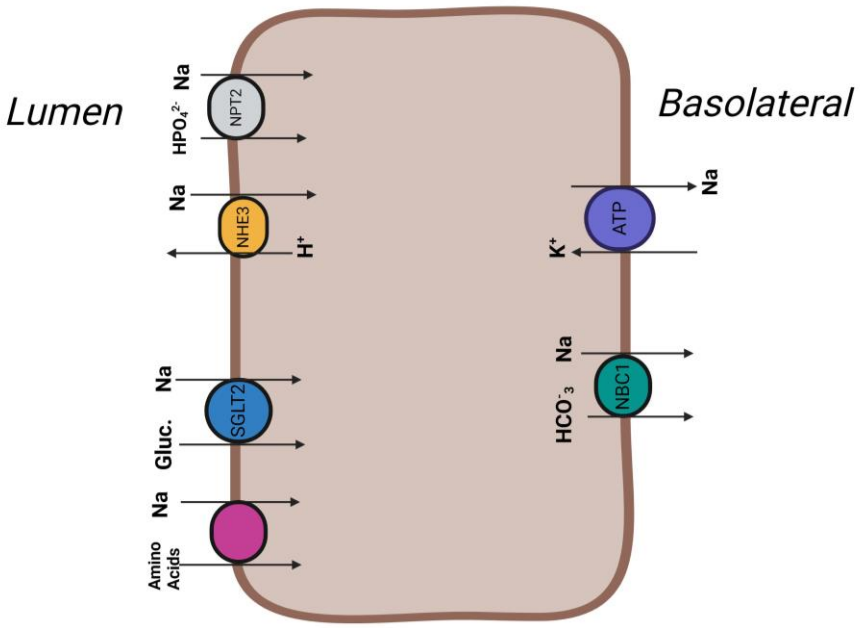

Figure 1 Sodium Transporters in Early Proximal Tubule. Created in Biorender.

1.2.3 Endothelial function

Dietary sodium has been shown to impact endothelial function, by altering factors that contribute to regulating vascular tone and blood flow [93]. Numerous studies have demonstrated the impact of sodium consumption on endothelial function, highlighting its role in cardiovascular health [93–95]. For instance, Dupont et al. found that higher sodium intake was associated with impaired vascular function, as assessed by flow-mediated dilation (FMD), in salt-resistant adults after increasing sodium intake for 7 days[96]. Another study by Dickinson et al. showed that acute salt loading significantly impaired endothelial function in healthy individuals, as evidenced by reduced FMD and increased arterial stiffness[94]. These studies provide a link between excess dietary salt intake and the endothelium.

The endothelium, which is a monolayer of endothelial cells lining blood vessels, regulates vasodilation in response to various stimuli, including changes in BP, mechanical stimuli, oxygen levels, nutrient availability, and temperature [97,98]. Structurally, blood vessels are organized into layers, including the tunica externa, tunica media, and tunica intima [99]. The tunica intima, primarily composed of the endothelial layer, subendothelial stellate cells, and elastic fibers like the glycocalyx layer, serves as a protective barrier against mechanical stressors and contributes to vascular homeostasis [100,101]. Within this endothelial layer, the synthesis of vasodilators such as nitric oxide (NO) is vital for maintaining vascular relaxation and blood flow regulation [102].

The enzyme endothelial nitric oxide synthase (eNOS) produces NO, a soluble gas generated from the amino acid L-arginine, as shown in Figure 2[102]. eNOS is a critical enzyme that produces NO in endothelial cells and plays a pivitol role in vascular homeostasis and BP regulation[103]. The activity of eNOS is tightly regulated by various signaling pathways and cellular mechanisms to ensure precise control over NO production[103]. eNOS can be activated using either a calcium-dependent or calcium-independent pathway, with phosphorylation occurring at specific serine, threonine, and tyrosine residues critical for its enzymatic activity [104]. Mechanical forces and hormonal signaling pathways, such as the protein kinase C (PKC) and phosphoinositide 3-OH kinase (PI3K)/Akt pathways, play important roles in regulating eNOS activity [105–109]. When activated, eNOS catalyzes the conversion of the amino acid L-arginine to NO and citrulline, triggering a series of events that leads to vascular smooth muscle relaxation and vasodilation[102].

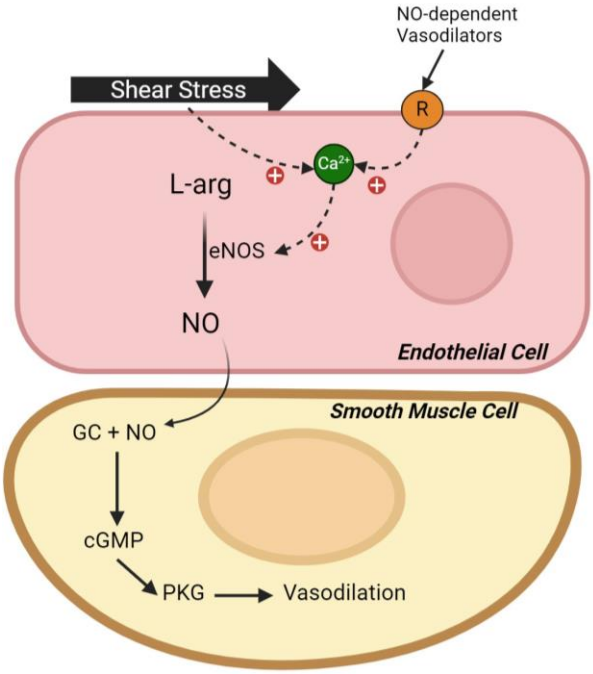

Figure 2 Nitric oxide-mediated vasodilation. Created in Biorender

Evidence indicates a dynamic interaction between dietary salt intake and endothelial function, with chronic exposure to elevated salt levels inducing endothelial damage [110,111]. Increased sodium contributes to endothelial dysfunction by disrupting the glycocalyx layer, increasing oxidative stress, and impairing NO-mediated vasodilation[70,112,113]. This damage leads to alterations in vascular homeostasis and contributes to the development of cardiovascular pathologies such as hypertension and atherosclerosis [114].

Oberleithner et al., conducted an in vitro experiment using tissue from a human umbilical cord to investigate the effects of sodium loading on endothelial cell stiffness [112]. The study found that when extracellular sodium concentrations were elevated for 5

days, the endothelial glycocalyx decreased by approximately 50%[112]. Additionally, Dickinson et al., found that reducing daily sodium intake by 3g resulted in a significant improvement in FMD [95]. Further mechanistic insights were provided by Ramick et al. demonstrated that a diet high in salt can increase oxidative stress in endothelial cells, leading to a decline in endothelial function [41]. This establishes additional links between excessive salt intake and endothelial function, while using FMD as an assessment method.

Comprehending the mechanisms that contribute to the negative effects of high salt intake on endothelial function provides clinically relevant information since reducing dietary salt can effectively improve endothelial function and decrease the risk of CVD[95]. In brief, excess salt intake induces eNOS uncoupling, alters vascular structure, and elevates ROS concentrations, consequently augmenting oxidative stress and impairing vascular function [115]. Hence, reducing sodium intake should be accompanied by strategies to decrease oxidative stress, forming a comprehensive approach to reduce CVD effectively.

1.2.4 Impact of Salt on PBMCs

Salt promotes infiltration of immune cells into the vasculature, impacting immune function and systemic health [116–118] . Peripheral blood mononuclear cells (PBMCs), comprised of lymphocytes and monocytes, are sensitive to sodium fluctuations [119,120]. In vitro studies show that PBMCs grown in the presence of high sodium stimulate the differentiation of pathogenic T helper 17 (Th17) cells [121]. Th17 cells produce interleukin 17 (IL-17), which is a proinflammatory cytokine associated

with hypertension [122]. IL-17 causes eNOS to be phosphorylated at Thr495, which is facilitated by RhoA-Rho-kinase, resulting in decreased NO production and impaired NO-dependent vasodilation [123]. Figure 3 shows the population and ratio of PBMCs within the immune system. T cells and B cells are central to adaptive immunity and regulate immune responses against pathogens [124]. $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells activate immune responses, whereas B cells produce antibodies that bind to pathogens or foreign substances, such as toxins, to neutralize them [124].

In a study conducted by Chan et al., using B cell-deficient mice, they observed a significant decrease in BP in the rodents given a hypertensive stimulus when compared to the control group [125]. Guzik et al., similar to Chan, depleted mice of T cells and detected a decrease in BP along with an increase in tumor necrosis factor (TNF) alpha [126]. Providing evidence that T and B cells are involved in hypertension and secrete proinflammatory cytokines like IL-17 and TNF alpha, influencing immune function and cardiovascular health.

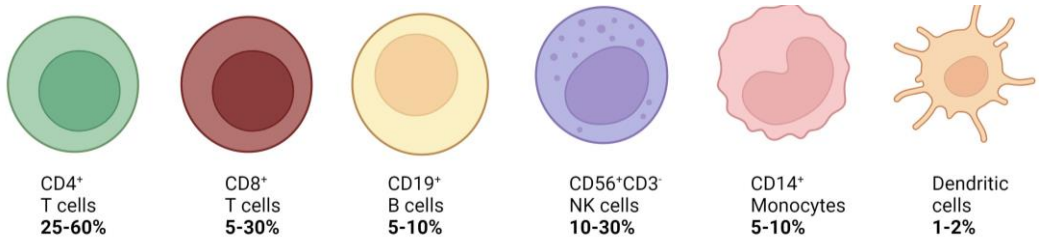

Figure 3 PBMC population. Created in Biorender

Macrophages carry out a variety of functions, including pathogen and debris phagocytosis, antigen presentation, and cytokine production[124]. Wendstedt et al. provide evidence that 2 weeks of increased dietary salt feeding in humans stimulates monocyte differentiation into macrophages, while increasing proinflammatory cytokines IL-6 and TNF alpha [127]. Increased sodium levels can also enhance macrophage phagocytic activity, facilitating the clearance of pathogens and cellular debris, which is critical for reducing microbial infections and promoting tissue repair[119]. Moreover, while investigating the mechanism by which salt stimulates macrophage activation in mice, Zhang et al. identified sodium-sensing macrophages, which can detect elevated sodium levels in the extracellular milieu [113]. When exposed to high sodium concentrations, sodium-sensing macrophages exhibit increased antimicrobial activity and production of proinflammatory cytokines, which contribute to immune activation and inflammatory responses[120,128].

As the immune system is stimulated, B and T lymphocytes secrete interleukins to carry out various signaling mechanisms [124]. Factors that stimulate growth, differentiation, and inflammation all induce interleukin signaling [124]. Interleukins 17a (IL-17a) is associated with hypertension and decreases of circulating IL-17a are associated with a reduction in BP [129]. Orejudo et al. observed improvements in renal function by measuring a decrease in NGAL during a study in which mice were infused with Ang II for two weeks and given an IL-17a neutralizing antibody[130]. Saleh et al., like Orejudo et al., found evidence of reduced renal inflammation as well as a reduction in BP when using an IL-17a antagonist[131]. In vitro studies using proximal and distal convoluted tubule cell lines demonstrated that IL-17A increased NHE3

expression and sodium chloride co-transporter (NCC) activity in each cell type [132]. These findings suggest that IL-17A may play a role in promoting sodium reabsorption within the nephron.

IL6 is strongly linked to the promotion of atherosclerotic factors that elevate blood pressure [133,134]. As with IL17a, decreasing levels of IL6 have led to decreased BP in the hypertensive population [135]. Brands et al. demonstrated this in their study of IL-6 knockout mice[133]. They observed a significant decrease in BP in the experimental Ang II infusion rodents, demonstrating that targeting IL6 completely mediates Ang II-induced hypertension[133]. IL-6 enhances the production and functionality of the epithelial sodium channel (ENaC) [136]. Notably, ENaC mutations are linked to increased sodium reabsorption, cystic fibrosis, and Liddle's syndrome [137]. IL-6 can stimulate the intrarenal renin-angiotensin system (RAS), leading to an indirect promotion of sodium reabsorption[138]. The intrarenal RAS is a localized autocrine and paracrine system in the kidney that performs both angiotensin-dependent and independent functions [63].

The interaction between dietary salt and immune cells extends beyond antimicrobial defense to encompass broader implications for systemic health. Salt intake influences immune function and associated inflammatory responses, which are linked to heightened risks of hypertension and CVD. Of particular interest is investigating how salt-induced immune alterations intersect with the generation of ROS, which are potent mediators of inflammation and oxidative stress. Understanding the crosstalk between salt-induced immune responses and ROS secretion is essential

for unraveling the complex interplay between dietary salt, immune function, and systemic health.

TNF alpha is elevated in cases of hypertension and is a known proinflammatory cytokine[139–141] . According to a study conducted by Shahid et al., the infusion of TNF alpha into mice resulted in renal vasoconstriction and a decrease in glomerular filtration rate [142]. This was likely caused by the increased levels of superoxide that were measured in the kidney. Huang et al. conducted a study that showed enhanced localization of TNF alpha in the renal medulla in a salt-sensitive rodent model[141]. Additionally, they showed that the rodent models treated with a TNF alpha-neutralizing antibody exhibited a diminished mean arterial pressure (MAP) response following the administration of a hypertensive stimulus [141]. These studies establish a direct relationship between immune system stimulation and hypertension.

1.2.5 Salt on PBMC-derived ROS

An elevation in extracellular sodium levels triggers the production of ROS, a process with significant implications for vascular health [143,144]. ROS, such as hydrogen peroxide and superoxide anion as depicted in Figure 4, are naturally produced during cellular metabolism. However, they can also be generated from external sources influenced by environmental factors such as diet [144]. During the immune response to microbial pathogens, phagocytes undergo rapid intracellular accumulation of ROS, followed by ROS release to serve as a signaling mechanism to facilitate pathogen destruction [145]. Recent studies have highlighted the role of excessive salt intake, particularly characteristic of Western diets, in promoting ROS production[146,147]. For

instance, high salt consumption has been shown to induce oxidative stress in endothelial cells, contributing to endothelial dysfunction and vascular damage [148].

The mechanistic link between dietary salt and ROS is intricate and multifaceted, involving multiple pathways. Increased sodium can alter mitochondrial function, particularly in endothelial cells, leading to enhanced ROS production as a byproduct of oxidative phosphorylation[149]. Moreover, evidence suggests that elevated sodium intake can modulate metabolic processes in the liver, potentially upregulating genes involved in peroxisome proliferation[150]. The peroxisome is an organelle that produces ROS in response to a variety of stimuli including fatty acid β-oxidation, photorespiration, nucleic acid, polyamine catabolism, and ureide metabolism. Another implicated pathway involves the RAAS, particularly in the context of salt-induced kidney disease [151]. Li et al. demonstrated that high salt intake in mice with chronic kidney disease amplifies ROS production, enhancing the contractility of afferent arterioles through Ang II-mediated mechanisms[151]. Activation of the RAAS promotes ANG II synthesis, which has been linked to stimulating ROS generation via the activation of Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[152].

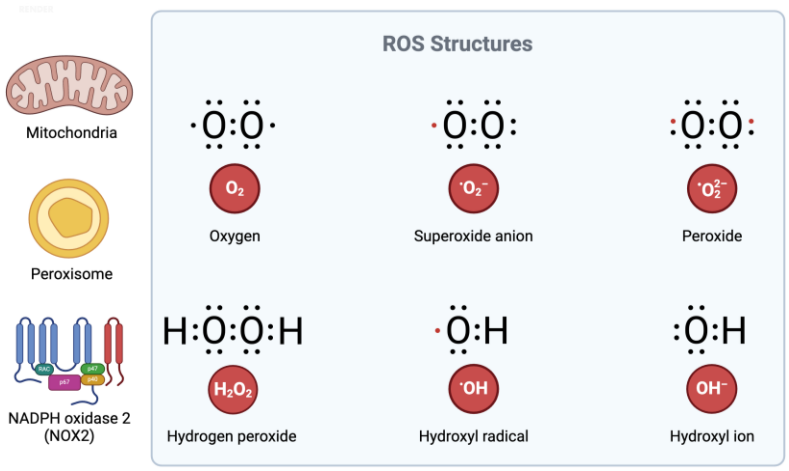

Figure 4 Summary of ROS structures and sources. Created in Biorender

NADPH oxidases are protein complexes responsible for generating ROS [153]. Extensively studied for their signaling roles across various tissues, including the endothelium and the kidney, the precise involvement of ROS generated by resident or infiltrating immune cells in renal physiology remains unclear [153,154]. One such NADPH oxidase, NOX2, also known as the phagocytic NADPH oxidase, is prominently expressed in macrophages/monocytes and granulocytes[153,154]. Upon activation by specific stimuli, NOX2 recruits cytosolic cofactors (p47, p40, p67, and the small GTPase Rac) to the membrane-bound complex, leading to enzymatic activity and the production of superoxide[143,153].

Excessive ROS production resulting from high salt intake contributes to oxidative stress, a state characterized by an imbalance between ROS generation and antioxidant defense mechanisms [155]. Oxidative stress triggers inflammation and damages cellular components, including lipids, proteins, and DNA, which can further exacerbate vascular dysfunction [77,115,156]. Moreover, ROS-induced oxidative stress has

been implicated in the development and progression of hypertension [157]. Animal studies have shown that elevated levels of ROS levels contribute to endothelial dysfunction, vascular remodeling, and reduced availability of nitric oxide, which are all characteristics of hypertension [115,147,158].

To summarize, increased dietary sodium contributes to the development of physiological diseases and disorders, independent of its impact on BP. This aligns with the notion of BP-related conditions being dubbed the "silent killer". As discussed in our review, increased sodium consumption is linked to immune cell differentiation towards more pathogenic phenotypes, elevated secretion of inflammatory cytokines, increased tissue infiltration affecting renal function, and augmented production of ROS leading to vascular damage. Despite compelling preclinical evidence, further investigations are necessary to understand how dietary patterns and oxidative stress interplay in BP regulation[146,147,159]. Continued research into the interactions of common components in Western diets, such as salt, shows promise for lowering the risk of CVD. Moreover, investigating the effects of other prevalent dietary elements, like fructose, on vascular function will offer additional insights into the complex relationship between diet and BP regulation, particularly in the context of SSBP.

1.2.6 Salt-sensitive BP

Dietary salt increases the risk for hypertension, yet many young to middle-aged adults display "salt resistance", with their BP remaining stable despite increased salt intake [160,161]. Alternatively, some normotensive adults exhibit heightened sensitivity to dietary salt, experiencing a significant BP increase of 10% or more[1].

Identifying individuals demonstrating characteristics of the salt-sensitive phenotype can serve as a predictive marker for hypertension among healthy adults [39,40]. However, there is a substantial knowledge gap regarding the factors and mechanisms underlying SSBP. Research using rodent models has provided valuable insights into salt-sensitvity[18]. Through preclinical research studies, factors such as genetic predisposition, renal dysfunction, endothelial dysfunction, inflammation, and disturbances in autonomic control have all been established as contributing factors to the salt-sensitive phenotype [41,143,162,163]. There is evidence indicating that sex plays a role in contributing to SSBP [164]. Recent findings indicate that females exhibit heightened salt sensitivity relative to males [165]. Interestingly, it has been noted that female sex steroid hormones, help to reduce this increased sensitivity. Furthermore, studies suggest that women have a lower ability to suppress the RAAS compared to men, resulting in sex-specific imbalances that favor increased activation of the RAAS when consuming higher amounts of sodium [166,167]. When comparing males, research shows that males have a higher level of immune system activation, leading to SSBP, while females display a stronger anti-inflammatory response[168,169]. These mechanisms specific to sex offer an understanding of the interaction between biological factors and SSBP.

1.2.7 Genetic factors contributing to SSBP

In normotensive adults, there are genetic regions that distinguish between salt-sensitive and salt-resistant populations. While age, racial disparities and sex differences may influence the prevalence of SSBP, our review will exclude those factors when discussing underlying mechanisms[170,171]. For example, alterations in the

expression of hormones that affect proteins in the RAAS are crucial factors to consider because of their influence on the regulation of BP [172]. The GenSalt study is the most comprehensive investigation that has been conducted to identify genetic variations that contribute to SSBP[162]. The single polymorphic analysis (SNP) analysis conducted by Dongfeng et al. confirmed previously identified genes associated with the RAAS, but also expanded the database to include angiotensin II receptor type 1 (AGTR1), Hydroxysteroid 11-Beta Dehydrogenase 2 (HSD11B2), and Renin Binding Protein (RENBP) as genes related to SSBP[162].

In the context of immune system proteins, P-selectin-glycoprotein-1 (PSGL-1) has emerged as a genetic marker of interest [10]. PSGL-1, expressed on circulating leukocytes, modulates T-cell differentiation and cytokine secretion [173]. Studies with PSGL-1 deficient mice reveal its role in salt-sensitive hypertension, exacerbating inflammatory responses in the vasculature and promoting vascular injury [10]. Additionally, genes that stimulate immune cells expressing NOX2 contribute to elevated BP in salt-sensitive rodent models [143]. Genetic factors influencing sympathetic nervous activity also impact sodium transport and SSBP[174]. Variations in tyrosine hydroxylase (TH) expression, pivotal for neurotransmitter production, directly influence norepinephrine and epinephrine levels [175,176]. Based on these insights, there is a need to further investigate genetic variations contributing to SSBP.

1.3 Dietary fructose as contributing factor to SSBP

The association between excessive fructose intake and the increased risk of CVD, fatty liver disease, and metabolic syndrome is well-documented [177]. In the

context of SSBP, the Western diet is a significant factor. The consumption of fructose is high in the US population. Reports indicate that 25% of Americans consume 200 daily calories from sugar, while 5% consume 600 or more calories. Preclinical research performed in vitro and in vivo provides a compelling link between dietary fructose consumption and SSBP. While excess sodium intake is recognized as a risk factor for hypertension, studies suggest that excess fructose consumption contributes to the salt-sensitive phenotype in salt-resistant rodent models [17,18,178]. Given that fructose metabolism dysfunction has been linked to several diseases, understanding fructose transport and metabolism is critical for investigating its role in hypertension development, particularly in relation to SSBP [179].

The metabolic pathways of fructose play a significant role in the onset of several health conditions such as insulin resistance, metabolic syndrome, and fatty liver disease[14,36,37]. Unlike glucose, which is metabolically prioritized by various tissues throughout the body, fructose is primarily metabolized within hepatocytes in the liver [180]. Its uptake into enterocytes is facilitated by glucose transporter 5 (GLUT5), which includes the liver exhibits high expression levels in the small intestine and lower expression levels in tissues such as skeletal muscle and the kidneys [181]. Intriguingly, fructose absorption in the small intestine stimulates sodium transport in the jejunum, influencing sodium reabsorption processes[178,181].

The transport of fructose into enterocytes via GLUT5 is facilitated by the upregulation of luminal transporters, such as the proton-coupled transporter (PAT1) and NHE3 [174]. Additionally, glucose transporter 2 (GLUT2), the principal glucose

transporter, albeit at a lower affinity can also transport fructose [182]. GLUT2 is widely expressed in tissues, such as the liver, pancreas, hypothalamus, small intestine, and kidney[183]. Fructolysis, in contrast to glycolysis, lacks several regulatory checkpoints found in the glycolytic pathway [184]. As a result, it leads to increased (Adenosine Triphosphate) ATP consumption and the production of inflammatory metabolic byproducts.

Fructose metabolism in hepatocytes results in increased production of acetyl coenzyme A (acetyl CoA), which is subsequently converted into fatty acids by fatty acid synthases[185]. The fatty acids generated combine with glycerol 3-phosphate and contribute to the formation of triglycerides, thereby playing a significant role in the development of nonalcoholic fatty liver disease (NAFLD)[36]. Consequently, this metabolic process leads to elevated levels of serum triglycerides, LDL, VLDL, and uric acid, all established as contributors to CVD [4,186]. Fructolysis stimulates the production of increased uric acid by converting fructose to fructose-1-phosphate through the action of fructokinase. This is an uncontrolled ATP-dependent process that leads to a reduction in ATP levels[185].

The ATP depletion pathway, stimulated by fructolysis, is a complex metabolic process involving the deamination of ATP and its conversion into inosine monophosphate (IMP) by the enzyme adenosine deaminase. IMP undergoes additional metabolic processes and is converted into xanthine and subsequently uric acid by the enzyme xanthine oxidase. This pathway results in the further depletion of ATP and the concurrent production of uric acid, which has been implicated in various pathological

conditions, including hyperuricemia and gout. Clinical studies have provided substantial evidence linking hyperlipidemia and hyperuricemia to fructose metabolism[187,188]. Furthermore, a longitudinal study conducted by Wang et al. observed a positive correlation between hyperuricemia and dyslipidemia, highlighting the interplay between these metabolic abnormalities in the progression of cardiovascular disorders [189]. Additionally, research performed by Nakagawa et al. revealed that fructose-induced hyperuricemia exacerbates endothelial dysfunction and oxidative stress, predisposing individuals to hypertension, atherosclerosis, and coronary artery disease [190].

1.3.1 Fructose and Endothelial Function

Excess fructose consumption increases oxidative stress, inflammation, and dyslipidemia, each of which are associated with endothelial dysfunction[4,185]. The impact of fructolysis on endothelial function is crucial, given the implications for the risk of CVD. Katakam et al. fed rodents a 66% fructose-enriched diet and detected a significant decline in vascular function by the end of the 14-day diet [191]. This was not observed during an acute fructose feeding study performed by Muhammed et al. [192]. This implies that the effect on the endothelium might not be observable in an immediate reaction, necessitating further investigation. The interaction between fructose and endothelial function is complex and poorly understood. Our review discusses various factors that have been established as possible contributors to fructose-induced endothelial dysfunction.

Fructolysis results in the generation of metabolic intermediates that elevate the risk of cardiovascular disorders by inducing dysfunction in endothelial cells[185]. In addition to glucose, high levels of fructose can lead to adverse modifications of reactive proteins, such as peptide glycation[193]. Emerging evidence suggests a link between fructose metabolites and circulating insulin levels in the pathogenesis of endothelial dysfunction[16,194]. Moreover, the correlation between fructolysis byproducts and endothelial dysfunction is associated with decreased NO bioavailability and increased intracellular adhesion molecule 1 (ICAM-1) expression, both of which are implicated in hypertension and insulin resistance, respectively[195]. Jin et al. conducted experiments on rodents and showed that fructose influences eNOS by activating protein phosphatase 2 A (PP2A) [196]. This leads to a decrease in eNOS phosphorylation at Ser1777, which impairs the release of NO and reduces the vasodilation response[196].

ICAM-1, a cell surface glycoprotein involved in mediating immune responses, modulates leukocyte recruitment from the circulation, thus changing mechanical shear stress and promoting leukocyte adhesion to the vascular endothelium [197]. The dysregulation of ICAM-1 expression by fructose metabolism provides evidence of its role in promoting vascular inflammation, further contributing to endothelial dysfunction and cardiovascular pathology. Gaining a better understanding of these mechanisms may reduce the detrimental cardiovascular consequences resulting from excessive fructose intake and inflammation-induced dysfunction of the endothelium.

1.3.2 Fructose, ROS, and Inflammation

High fructose consumption is associated with various metabolic abnormalities, that stimulate immunostimulatory such as elevated triglycerides, total cholesterol, and uric acid levels, all of which are associated with metabolic syndrome[187,188,198]. Additionally, chronic intake of excess dietary fructose imposes a metabolic burden on hepatocytes and the generation of ROS[199]. The resultant oxidative stress has been implicated in the development of insulin resistance, which, along with ROS production, is independently associated with impaired BP regulation. This provides a link between fructose metabolism, oxidative stress, and cardiovascular function[43,194].

Chronic overconsumption of dietary fructose induces systemic inflammation through its metabolic effects[200–203]. Increased fructose intake stimulates de novo lipogenesis, leading to elevated plasma triglycerides and hepatic lipids, which can adversely affect the gut microbiota [204]. The resulting damage to gut microbes releases lipopolysaccharides (LPS), potent endotoxins that trigger immune responses via toll-like receptor 4 (TLR4) ligands on immune cell membranes [205]. Upon binding to TLR4, the ligands undergo a conformational change, dimerize, and initiate a signaling cascade involving the classical mitogen-activated protein kinase (MAP-K) cascade. This cascade activation can subsequently induce nuclear factor kappa B (NF-kB) activation via the MyD88-dependent pathway, prompting the transcription of inflammatory cytokines. The activation of NF-kB signaling pathway and subsequent cytokine secretion have been associated with increased ROS production, exacerbating oxidative stress-mediated cellular and tissue damage [206]. Moreover, elevated plasma fatty acids and triglycerides resulting from excess fructose consumption further contribute to PBMC activation and systemic inflammation [207,208]. Understanding the

mechanisms underlying fructose-induced inflammation and oxidative stress is crucial for reducing a reduction in endothelial function and reducing the risk for developing CVD.

Furthermore, emerging evidence suggests an interplay between oxidative stress and the development of SSBP. Compelling evidence from rodent models demonstrates the critical involvement of NOX2 in SSBP. Rodent models have provided compelling evidence of the crucial involvement of NOX2 in SSBP, demonstrating that inhibiting ROS derived from NOX2 significantly alleviates the hypertensive response compared to control groups with normal ROS levels[143]. Further investigations in rodent models, particularly those utilizing NOX2-expressing T cells, indicate that SSBP is exacerbated by T cell-mediated mechanisms, characterized by heightened T cell infiltration, renal damage, and an amplified hypertensive response. The probability of NOX2 oxidase activation due to underlying inflammation is substantial in cases of hypertension.

There is a critical knowledge gap regarding whether T cells trigger hypertension or if elevated blood pressure triggers increased cytokine production, resulting in NOX2-mediated ROS secretion. Furthermore, while these findings shed light on the role of NOX2 and T cell-mediated mechanisms in SSBP, there is still a knowledge gap about what stimulates the immune system in the context of SSBP. Based on recent research conducted on rodents, dietary factors have an impact on SSBP, particularly diets that are high in fructose and salt. Hence, it is important to perform additional research to investigate this interaction. Investigating the potential

synergy between excess dietary fructose and salt intake in the progression of SSBP could yield further understanding.

1.3.3 Fructose and Salt SSBP Preclinical Studies

SSBP is recognized as a predictive marker for hypertension and is closely associated with increased mortality and CVD risk. Despite continuous research endeavors, the exact mechanisms and factors that contribute to SSBP are still not well understood, which has led to an increasing interest in studying the effects of different dietary factors. Recent studies have suggested a possible link between increased consumption of fructose, along with higher intake of salt, and the development of SSBP[17–19]. Although this area of research is still in its early stages, most of the studies conducted so far have used different rodent models. Our review will primarily examine rodent studies that have investigated the effects of diets containing both salt and fructose to induce a salt-sensitive response, as there is a lack of human studies on this topic.

Studies using salt-resistant rodents indicate that diets containing moderate to high amounts of fructose, along with elevated sodium levels, are linked to the generation of a salt-sensitive phenotype. In a 2009 study led by Vasdev and his team, a 6-week feeding study using male Wistar Kyoto (WKY) rats revealed that moderately high salt diets (4%) combined with a 4% fructose diet led to a significant increase in blood pressure after just 3 weeks[209]. According to the study's conclusion, the rats exhibited signs of insulin resistance and elevated markers of oxidative stress. Similarly, a 2014 study by Cabral and colleagues conducted a 2-week study using salt-

resistant male Sprague Dawley (SD) rats, corroborated these findings[17]. Figure 5 portrays the effects of fructose consumption in a normotensive rodent model displaying SSBP, displaying a significant BP increase in the salt-resistant rats fed a high-salt diet (8%) when combined with moderate fructose intake (20%). Additionally, Gordish and colleagues conducted a similar study involving a high-salt and moderate fructose diet, yielding results consistent with previous findings, attributing the blood pressure elevation to heightened sodium retention and impaired renal NO[18]. Furthermore, in 2022, Brostek et al. conducted a study using both male and female SD rats, determining that sex had no discernible impact on the BP response to the diet [210]. Collectively, the studies conducted by Vasdev, Cabral, Gordish, and Brostek demonstrate a synergistic effect of fructose and salt intake in the development of salt-sensitive hypertension.

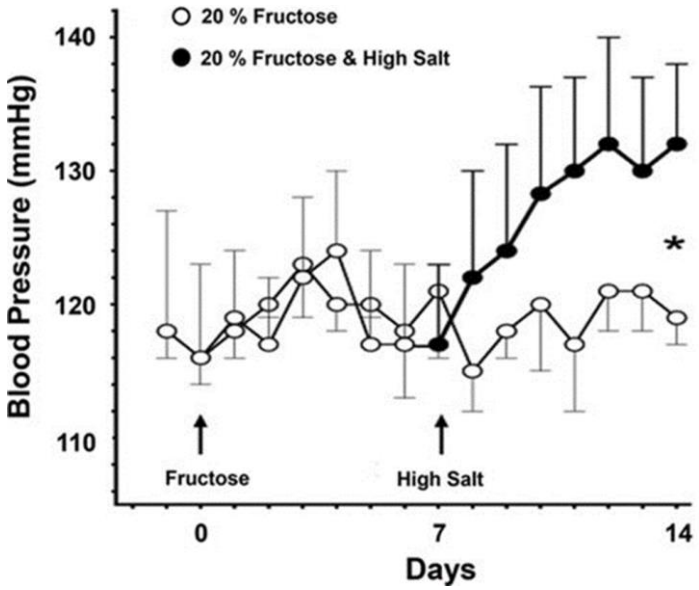

Figure 5 Fructose-Induced Salt-Sensitive BP in Salt Resistant rodents. Cabral 2014

Research efforts have been performed to investigate the impact of fructose on renal sodium transport, with a particular focus on its role in modulating NHE3 activity. In a study by Gonzelez-Vicente et al., proximal tubules of rodents subjected to a fructose-enriched diet exhibited impaired sodium excretion, attributed to heightened Ang II activity and sodium transport stimulation [19]. This observation was reinforced by the enhanced proximal tubule sodium reabsorption capacity in cells derived from fructose-fed rodents, even at lower Ang II concentrations. Similarly, Yang et al. demonstrated that fructose can directly enhance the activity of renal sodium transporters, thereby augmenting sodium reabsorption [92]. This mechanism potentially culminates in an expansion of extracellular fluid volume and subsequent elevation of BP.

There are limited studies investing the direct impact of fructose and salt on endothelial function however, Dornas et al., during a 10-week rodent feeding study found that the rodents fed high salt combined with high fructose exhibited a decrease in serum Paraoxonase 1 (PON1) [211]. PON1 is an important anti-atherosclerotic component of high-density lipoprotein (HDL), which highlights how this diet promotes hyperlipidemia. Within this study, they also detected an increase in oxidative stress based on the expression of the immune cells within the rodents. Furthermore, in study conducted by Nishimoto et al., using an insulin resistant model rat, they found that the group fed a high fructose diet in conjunction with a high sodium diet detected reduction in eNOS within the renal medulla [212].

Immune system dysfunction is considered a contributing factor to mechanisms underlying increased sodium retention in the kidney and SSBP[142,213,214]. A growing body of preclinical studies continues to investigate the association between an overstimulated or hypersensitive immune system in the context of SSBP. For instance, Zenner et al., in line with Gordish et al., further explored the relationship between immune cell activity and SSBP, expanding on previous findings related to fructose-salt-induced SSBP[198]. Their study using SD rats revealed elevated levels of 8-isoprostane, a marker of renal ROS, in urine samples. The observed effect was reversed upon administration of a free radical scavenger. These findings suggest that the increase in BP may be attributed, at least in part, to renal impairment resulting from heightened ROS levels.

1.4 Closing remark

Several mechanisms are currently under consideration to elucidate the relationship between fructose, salt, and the SSBP phenotype. My review presents theoretical frameworks proposing that an up-regulation of sodium and chloride transporters within the kidney may contribute to SSBP by inducing a state of salt overload, thereby increasing blood pressure through enhanced retention of dietary sodium. Additionally, evidence suggests a potential association between insulin resistance and hypertension in the context of fructose consumption. Furthermore, my review highlights the role of dietary interactions affecting the central nervous system in modulating blood pressure. Moreover, it presents evidence indicating that the combined dietary intervention may lead to an overstimulated immune system,

consequently leading to elevated blood pressure. The exploration of these proposed mechanisms using rodent models has been instrumental in advancing the field. However, there remains a noticeable gap in the literature regarding studies conducted in human subjects, with only a limited number of research groups having pursued this avenue of inquiry.

Chapter 2

THE IMPACT OF DIETARY SODIUM AND FRUCTOSE IN BP NORMOTENSIVE ADULTS

2.1 Introduction

The Western diet contributes to the development of cardiovascular disease (CVD), largely due to the increased consumption of sodium and fructose[32]. Chronic consumption of these nutrients has been linked to the development of hypertension[3,16,215]. While there has been extensive research on the effects of sodium alone and fructose alone on blood pressure (BP) regulation, their synergistic effects are underexplored in the scientific literature[16,85,86,215]. Many normotensive adults demonstrate "sodium resistant" BP, meaning they maintain a stable BP despite increases in sodium intake contrasting sharply with the pronounced BP elevation observed in SS individuals[1]. However, preclinical research suggests increased fructose consumption contributes to sodium-sensitive blood pressure (SSBP); a condition characterized by an augmented BP response to increased sodium intake [3,16,18].

The development of SSBP is influenced by multiple physiological mechanisms, with the disruption of renal sodium handling by fructose playing a possible role[3,20,212]. Preclinical studies in rodents suggest that a diet high in fructose content could impair the kidneys' ability to eliminate excess sodium, potentially contributing to elevated BP levels[3,20]. For example, after an 8-week high-fructose diet with fructose comprising 66% of total nutrition, C57 sodium-resistant mice exhibited a

notable elevation in BP[216]. Sprague Dawley rats demonstrated similar results during an intervention of 14 days when fed a combination of high-salt (8% NaCl) chow and 20% fructose[17]. These findings align with additional investigations using sodium-resistant rats placed on a diet high in both sodium (4% NaCl) and fructose (20%) over 14 days[18]. In addition to observing an increase in BP a decline in renal function was also observed, as indicated by a decrease in sodium excretion [18].

One underlying mechanism implicated in the development of the SSBP phenotype in rodents when exposed to a combined diet of fructose and sodium is the marked elevation in sodium hydrogen exchanger 3 (NHE3) expression[217]. NHE3 is an important mediator of sodium transport in the proximal tubules [217]. This increased expression leads to an enhanced reabsorption of sodium[218]. Dietary fructose has been shown to increase NHE3, thereby potentiating sodium transport mechanisms[178]. Evidence suggests that the increase in BP is mediated by protein kinase C within the proximal tubules—a pivotal regulator of NHE3[92]. Furthermore, there is evidence indicating that excess dietary fructose can counteract the inhibitory effect of sodium on the renin-angiotensin-aldosterone system (RAAS)[17,19,92]. This is corroborated by studies demonstrating that higher consumption of fructose leads to increased sensitivity of the proximal tubule to lower concentrations of Angiotensin II (AngII), resulting in greater stimulation of the RAAS[17].

Although fructose consumption is associated with the development of sodium-sensitive hypertension in rodent models, it is important to note that these findings have not yet been corroborated in human subjects[3,17,18]. Therefore, the objective of this

study was to examine the impact of a high dietary intake of fructose and sodium over a period of 7 days on renal function and BP in young healthy adults. We hypothesized that adding fructose to a high-sodium diet would impair the kidneys' ability to excrete excess sodium and increase BP in otherwise healthy sodium-resistant adults.

2.2 Methods

2.2.1 Study design

I designed a randomized double-blind crossover study for this dissertation. The study design was comprised of three 7-day interventions involving the consumption of a daily low-fructose beverage or a high-fructose beverage, along with either sodium or placebo pills. The Institutional Review Board at the University of Delaware (IRB #1617405-2) approved the informed consent and all procedures by the Declaration of Helsinki. I recruited participants locally in the vicinity of Newark, DE through the distribution of campus flyers and online advertisements. Before enrolling in the study, I or a member of our team formally obtained informed consent from the participant. The study is registered on clinicaltrials.gov with the identifier NCT04994418.

2.2.2 Participant Screening

This study included healthy adults with normal blood pressure, aged between 18 and 45 years. Women and minorities were selected to ensure a proportional representation of the overall population. Following the acquisition of both written and verbal consent, I provided participants with questionnaires to gather information on their habitual physical activity readiness and their medical history. Subsequently, I

evaluated and collected data on each participant's height, weight, and BMI. Brachial blood pressure measurements were taken three times while participants were seated after they had rested quietly for five minutes. The measurements were performed using the Dash 2000 device from GE Medical Systems in Milwaukee, WI. The inclusion criteria for this study were individuals aged between 18 and 45 years, with a resting systolic blood pressure below 140 mmHg, a resting diastolic blood pressure below 90 mmHg, and a body mass index below 30 kg/m2 at the screening stage. Additional inclusion criteria encompassed individuals who were non-smokers, devoid of any documented cardiovascular ailment, and exhibited no indications of metabolic, neurological, renal, or pulmonary disorders. The exclusion criteria encompassed the following factors: pregnancy; body mass index (BMI) exceeding 30 kg/m^2; previous occurrences of cardiovascular disease; kidney disease; diabetes; thyroid disease; asthma; heart disease; high blood pressure; cancer; utilization of antibiotics; and consumption of tobacco products. The confirmation of COVID-19 vaccination was obtained during the enrollment process.

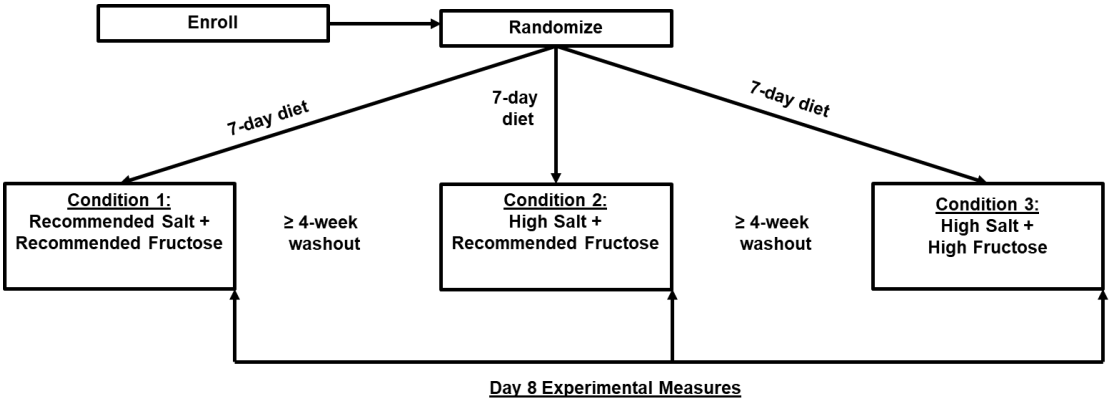

Figure 6 Overall Study Design

2.2.3 Habitual Diet and Dietary Intervention Protocol

The dietary intervention conditions for each subject were accomplished by having a registered dietitian (RD) individually meet with and make recommendations to each participant. Participants were advised by the RD to limit consumption of products containing added sugars outside of what is provided and maintain a diet that does not exceed the daily recommended dietary sodium intake (2300mg) during the respective dietary conditions. Participants were asked to maintain a similar diet during the 3 study trials and provided a diet intake log of their food and water intake for the duration of each of the 7-day interventions. Each participant was randomly assigned to a cross-over dietary intervention of a daily recommended or high fructose drink in combination with a placebo or salt pills. The randomization was accomplished using research randomizer software (Version 4.0). This program has been extensively utilized and referenced in the industry as a technique for randomization in clinical trial investigations [219].

2.2.4 Dietary intervention, sodium, and fructose supplementation

This study consisted of three 7-day interventions separated by a washout period of at least 4 weeks. Participants were instructed to resume their habitual diet throughout the washout period. During the 7-day intervention, the participants consumed a daily recommended fructose drink containing 20g of fructose (RF) or a high fructose (HF) drink containing 200g of fructose in combination with an additional 3900 mg sodium (HS) in the form of pills or an equal number of placebo (RS) pills containing dextrose. The amount of sodium and fructose intake was established to stimulate a perturbation effect and to align with the upper quartile

consumption rates in the population[220,221]. The quantity of capsules (9) and volume of the fructose drink (32oz) provided were equivalent during each intervention. The capsules utilized were empty and delayed-release/acid-resistant capsules (PureCaps USA (Size 00)). The clear plastic juice bottles obtained from ULINE were filled with purified drinking water to a total volume of 32 fluid ounces and sealed with the provided tamper-evident lid before being provided to the participants. The drinks were flavored using a calorie-free lemon flavoring packet (True Lemon). To mitigate the chances of experiencing symptoms associated with the excessive consumption of salt and sugar, participants were given a document including explicit and comprehensive instructions on how to consume the intervention supplements. Participants were instructed to ingest the 3 capsules with whole foods at 3 different times a day, ensuring a minimum interval of 6 hours between each dose. Participants were instructed to consume the fructose drink in moderation throughout the day, with a 2-hour interval between each 2.3 oz serving. The participants were given a document tracker to record any further water consumption and any residual supplements not consumed. The fructose drink, sodium, and dextrose capsules were made in a metabolic kitchen under the supervision of the registered dietician. On the last day of each condition, I requested that the participants refrained from consuming caffeine, alcohol, and exercise 24 hrs. before the lab testing.

2.2.5 Physical activity monitoring

To obtain physical activity data I asked the participants to wear an accelerometer (ActiGraph wGT3X-BT, Pensacola, FL) on their dominant side hip during each seven-day dietary intervention. I requested them to exclude wearing the

accelerometer while sleeping and during activities involving water such as swimming or showers. Participants logged the periods during which the accelerometer was utilized and not utilized to enhance adherence and the accuracy of the data. Data was considered valid if there were a minimum of four days with a cumulative wear time of at least 23 hours. The accelerometer data was evaluated using ActiLife software.

2.2.6 24-hour Ambulatory Blood Pressure and Urine Collection

On the last day of each diet, the participants were asked to wear a BP monitor (SpaceLabs, Inc.) on their non-dominant upper arm for 24 hours. This BP cuff was set to automatically take the participants' BP every 20 minutes during awake hours and every 30 minutes during sleeping hours. The monitor records and saves each BP measurement automatically. The participants documented their sleep and wake times on a given datasheet. The BP data collected during periods of sleep and wakefulness were used to examine fluctuations in blood pressure levels such as nocturnal decline (dipping) and morning surge[41,222–224]. Observations were omitted if fewer than 20 readings were recorded during the daytime and 7 readings were recorded at night[225]. During the same 24 hours that the participant wore the BP monitor on their arm, participants also collected 24 hours' worth of urine into a plastic container. The urine sample was analyzed for sodium concentration to assess urinary sodium excretion, osmolality, urine specific gravity, and creatinine clearance.

2.2.7 24-hour Urine Collection and Measurements

Each participant received training on how to independently collect 24-hour urine samples using a provided container for urine collection. The first void of the day in the morning was discarded, and all subsequent urine produced within the next 24 hours was collected. Participants were given a sheet to indicate the time at which they disposed of their first urine sample and the time at which they collected their final urine sample into the container. After receiving the urine container for analysis, the participant's urine was thoroughly mixed. The overall volume of the collection was measured. Testing was conducted to measure the levels of sodium (Na^+), potassium (K^+), and chloride (Cl^-) in the urine. Additional calculations included free water clearance, osmolar clearance, and creatinine clearance using standard formulas. A coded sample was stored in a freezer at a temperature of -80 °C for further testing purposes for future analysis of fluid regulatory hormones. A sample of the urine was provided to LabCorp for the measurement of creatine clearance.

2.2.8 Experimental Visit

Upon arrival to the laboratory, each participant provided a spot urine sample. Body weight was recorded at the beginning of each study visit (Tanita Body Composition Analyzer, Model TBF-300A; Arlington Heights, IL), and the participants were asked a series of questions including any adverse symptoms that may have occurred during the intervention. Afterward, the subjects were positioned in a supine posture for a duration of 15 minutes before a venous blood sample was obtained from the antecubital vein. Participants were subsequently equipped with devices to measure beat-to-beat blood pressure (BP), heart rate (HR), and respiratory excursions (using a

strain-gauge pneumograph). Data was then collected during a 10-minute baseline period as participants rested quietly in a dimly lit, temperature-controlled room (22-24°C).

2.2.9 Blood and Urine Analysis

The serum and urine electrolyte concentrations were analyzed using the EasyElectrolyte Analyzer by Medica, located in Beford, MA, USA. Additionally, the plasma and urine osmolality were measured using the 3D3 Osmometer by Advanced Instruments, located in Norwood, MA. The Hb (Hb 201+; Hemocue, Lake Forest, CA, USA) and Hct (Pre-calibrated Clay Adams, Readacrit Centrifuge; Becton Dickinson, Sparks, MD, USA) levels were measured in venous blood samples. In addition, the urine-specific gravity was measured for both the spot urine sample and the 24-hour urine sample. Pregnancy status in female participants was confirmed using spot urine samples (hCG cassettes, Moore Medical).

2.2.10 Estimation of 24-hour Sodium Excretion

The calculation of 24-hour urinary sodium excretion was based on the measurement of sodium levels in a 24-hour urine sample. Equation provided below:

24hr urinary sodium excretion= Na concentration (mmol/L) x volume (L) x molecular mass of Na

2.2.11 Statistical Analysis

The primary outcome measure of this aim is the effect of the HF+HS when compared with RF+HS and RF+RS on BP responses and sodium excretion. I conducted an a priori power analysis based on an effect size of 0.25 using previously collected data to determine the appropriate sample size (GPower 3.1)[41]. Based on a moderate effect size, it was calculated that a sample size of 35 participants would be sufficient to achieve 90% statistical power, with a significance level (α) of 0.05, to detect a 5-mmHg difference in MAP between the three dietary conditions. An increase of a similar magnitude in BP change of 5mmHg has been associated with an increased risk for CV diseases[226]. Repeated measured one-way ANOVA with the Newman-keuls method of multiple comparisons was used to analyze characteristics that contribute to differences in BP and sodium excretion of the participants who completed all 3 conditions. For characteristics that demonstrated a significant correlation ($p<0.05$) among the experimental measures, we performed post hoc comparisons with a Bonferroni correction. The differences in systolic BP, mean BP, and diastolic BP following the diet were examined using a one-way repeated-measures ANOVA. I conducted a one-way repeated-measures ANOVA to evaluate the alterations in serum Na+ and pOsm. The data is presented as the mean value plus or minus the standard deviation, with the predetermined α level set at 0.05. I conducted the statistical analysis for my data set using GraphPad Prism 8 software (GraphPad Software Inc., La Jolla, California, USA).

2.3 RESULTS

2.3.1 Baseline Subject Characteristics

Thirty-six participants took part in this study. Participant screening characteristics are presented in **Table 1**. Participants were young (18-45yr), normotensive, and non-obese. **Table 2** describes the habitual dietary intake of the cohort. Participants consumed an average of 2,055±698 calories in their habitual diet before starting the dietary intervention. Prior to the intervention participants consumed an average of 15±14g of fructose and 3309±1025mg of sodium each day. During each of the three diets, the participants were counseled by a registered dietician to limit their daily intake of sodium to 2300 mg and eliminate excess added sugars outside of the supplements provided by the study.

N, men/women	36 (17/19)
Race, White/ Black/ Asian	27/ 2/ 7
Age, yr**.**	30±8
Weight, kg	70.5±13.3
Height, cm	173.3±10.3
BMI, kg/cm^2	23.1±3.7
Systolic BP, mmHg	113±12
Diastolic BP, mmHg	67±8
MAP, mmHg	80±16

Table 1 Screening Characteristics. Data are expressed as means ±SD. *N*, number of participants, BMI: body mass index; BP blood pressure; MAP: mean arterial pressure.

Energy intake, kcal	2055.6±697.7
Total Carbohydrate, g	232.1±81.1
Total Protein, g	88.8±41.7
Total Fat, g	85.4±38.3
Fructose, g	14.9±13.9
Glucose g/day	15.1±12.9
Sucrose, g	35.7±25.1
Total Fiber, g	22.5±13.3
Cholesterol, mg	311.6±162.3
Saturated fat, g	26.3±12.8
Sodium, mg	3308.7±1024.6
Potassium, mg	2594.3±1195.1
Magnesium, mg	334.2±183.4
Vitamin C, mg	68.4±45.2
Iron, mg	14.8±6.8
Zinc, mg	10.8±5.0
Alcohol, g	5.8±14.8

Table 2 Habitual daily diet intake. Data are expressed as means ± SD, *N*= 36

2.3.2 Intervention Adherence Measures

Participants self-reported water intake, capsules remaining, and beverage remaining at the end of each diet. There were no statistical differences in water intake (RF + RS: 51±28, RF + HS: 58 ± 29, HF+ HS: 58 ± 34 fl. oz/day, P = 0.31), capsules remaining (RF + RS: 0.11 ±0.4, RF + HS: 0.11 ±0.4, HF+ HS: 0.11 ±0.4, P > 0.99), or beverage remaining after each diet (RF + RS: 0.91± 2.5, RF + HS: 1.4 5± 2.6, HF+ HS: 2.31 ± 3.2 fl. oz/day, P = 0.09). Standard lab measures were performed at the conclusion of each diet. Physical activity was not different between any of the three diets in (Table 3, P = 0.92**)**. Lab and urinary measures are presented in **Table 4**. Body

weight was not different between the diets (Table 4, P = 0.40). Blood hemoglobin, hematocrit, serum electrolytes and plasma osmolality were all in normal ranges with no significant differences in response to the diet. We measured 24hr urine specific gravity (USG) to determine if the diet induced any changes in hydration status. We observed no differences in USG due to the dietary intervention (Table 4, P = 0.30). The HS + HF condition increased serum VLDL (Table 4, P = 0.01) and triglycerides (Table 4, P = 0.02). No significant differences were observed when assessing changes in glucose, HDL, or LDL. There is a trending decrease in serum creatinine in both RF + HS and HF + HS conditions compared to RF + RS (Table 3, P = 0.07). Serum uric acid decreased during both RF + HS and HF +HS conditions compared to RF + RS. (Table 3, P < 0.01).

	RF + RS	**RF + HS**	**HF + HS**	**P**
Sedentary, min/day	1067.9±85.7	1034±103.8	1065.85±103.3	0.36
Light PA, min/day	779.3±119.6	789.4±164.3	756.3±116.0	0.80
Moderate PA, min/day	62.4±19.8	59.1±16.9	60.1±18.3	0.98
Vigorous PA, min/day	7.7±7.7	6.3±7.3	6.25±7.2	0.78
Very vigorous, min/day	1.6±2.1	2.11±4.4	2.4±4.6	0.45
Steps / day	6951.2±2688.9	6724.1±2650.5	6741.4±2591.3	0.92

Table 3 Physical Activity Monitoring after each intervention. Recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 27. Data are expressed as means ± SD.

	RF + RS	**RF + HS**	**HF + HS**	**P**	***N***
Heart Rate, (bpm)	73±10	72±11	71±10	0.71	33
Weight, (Kg)	68±13	66±13	68±13	0.40	36
Serum Sodium, (mmol/L)	138.2±1.5	138.2±1.9	139.2±2.8	0.23	32
Serum Potassium, (mmol/L)	4.02±0.3	3.95±0.3	4.64±4.1	0.58	32
Serum Chloride, (mmol/L)	105.3±2.0*	105.7±1.8*	107.0±7.3	**0.03**	32
Plasma Osmolality, (mOsm/kg/H^20)	287.0±6.6	288.3±5.4	288.0±3.4	0.37	28
Hemoglobin (g/dl)	13±2	13±2	13±2	0.17	32
Hematocrit, (%)	42±4	42±3	42±4	0.13	32
Glucose (mg/dL)	88.5±8.8	87.9±6.3	90.6±7.1	0.11	26
Total Cholesterol (mg/dL)	166±30	165±27	168±34	0.43	26
HDL, Cholesterol, (mg/dL)	53.8±13.6	53.0±14.6	50.7±13.1	0.10	26
VLDL, Cholesterol, (mg/dL)	14.5±4.5	16.5±8.0	21.7±15.8*	**0.02**	26
LDL. Cholesterol, (mg/dL)	97.7±25.8	95.0±21.5	96.0±24.2	0.65	26
Triglycerides, (mg/dL)	75.1±28.9	88.4±50.0	117.2±88.5*	**0.01**	26
Uric Acid, (mg/dL)	4.7±1.2	4.2±1.2*	4.2±1.2*	**<0.01**	26

Serum Creatinine	0.87±0.2	0.82±0.2	0.83±0.2	0.07	21
Urinary Sodium excretion, mmol/24hr	121.5±42.2	277.9±121.2*	235.1±85.0*♦	**<0.01**	34
24-hr Urine Osmolality, mOsm/kg	421.4±219.3	539.8±253.4*	517.1±243.0*	**0.01**	34
Urine flow rate	1.6±0.86	1.75±1.4	1.54±1.0	0.10	36
24-hr Urine specific gravity	1.01±0.01	1.01±0.01	1.01±0.01	0.30	36
Free water clearance (ml/min)	-0.33±0.8	-0.7±0.8*	-0.54±0.9	**<0.01**	28
Osmolar clearance (ml/min)	1.79±0.7	2.36±0.9*	2.08±0.7*♦	**<0.01**	28
Creatinine clearance (ml/min)	109.5±25.2	118.4±32.1	110.4±29.1	0.15	19

Table 4 Laboratory and urinary measures after each intervention. Recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). Data are expressed as means ± SD. *N* = number of participants. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison. * Denotes $P < 0.05$ respective condition vs RF + RS, ♦ denotes $P < 0.05$ respective condition vs RF + HS. Boldface indicates significant values.

	RF + RS	RF + HS	HF + HS	P	*N*
Overall SBP, mmHg	113±10	115±10	116±10*	**0.04**	33
Overall DBP, mmHg	65±6	66±6	67±6*	**0.05**	33
Overall MAP, mmHg	81±7	83±7	84±7*	**0.03**	33
Awake SBP, mmHg	118±11	120±12	121±11	0.11	33
Awake DBP, mmHg	70±7	71±7	71±7	0.29	33
Awake MAP, mmHg	81±7	83±7	84±7*	**0.03**	33
Asleep SBP, mmHg	102±9	104±9	105±10	0.09	32
Asleep DBP, mmHg	56±5	57±7	58±6*	**0.04**	32
Asleep MAP, mmHg	65±12	65±12	64±11	0.49	32
Overall cSBP, mmHg	103±8	104±8	105±8*	**0.01**	30
Overall cDBP, mmHg	67±6	68±6	68±6	0.06	30
Awake cSBP, mmHg	107±7	108±10	109±9	0.13	30
Awake cDBP, mmHg	71±7	72±9	72±6	0.45	30
Asleep cSBP, mmHg	94±7	96±8	96±10	0.11	29
Asleep cDBP, mmHg	57±5	59±6	56±6	0.07	29

Table 5 Ambulatory blood pressure measures after each intervention. Recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). Data are expressed as means ± SD. *N* = number of participants. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison. * P <0.05 respective condition vs RF + RS. Boldface indicates significant values.

Name	Abbreviation
Sodium Hydrogen Exchanger 3	NHE3
Endothelial Nitric Oxide Synthase	eNOS
Angiotensin II	AngII
Sodium	Na
Potassium	K
Chloride	Cl
Urine Specific Gravity	USG
Free water Clearance	FWC
Systolic Blood Pressure	SBP
Diastolic Blood Pressure	DBP
Mean Arterial Pressure	MAP
Heart Rate	HR
Millimeter of mercury	mmHg
Blood Pressure	BP
Recommend Fructose	RF
Recommend Salt	RS
High Salt	HS
High Fructose	HF
Sodium Sensitive	SS
Sodium Resistant	SR
Sodium Sensitive Blood Pressure	SSBP
Flow mediated dilation	FMD
Area Under Curve	AUC
Electron Paramagnetic Resonance	EPR
Peripheral Blood Mononuclear Cells	PBMC
Reactive Oxygen Species	ROS
Cardiovascular Disease	CVD
Tumor Necrose Factor Alpha	TNFα
Interleukin 6	IL6
Cyclic hydroxylamine	CMH
Superoxide Dismutase	SOD
Relative fluorescent Units	RFU
Nicotinamide adenine dinucleotide phosphate	NADPH
Very low-density lipoprotein	VLDL
Low-density lipoprotein	LDL
High-density lipoprotein	HDL

Table 6 List of Abbreviations

2.3.3 Renal perturbation measures

Participants were provided 24 hours of urine after each condition. Participant data was excluded from total N due to inadequate blood collection or poor quality of measure. There were no significant differences in hydration status. Urine osmolality increased in both RF + HS and HF + HS conditions when compared to RF + RS (Table 3, P = 0.01). As depicted in F**igure 7**, sodium excretion was significantly increased in both sodium loading conditions, (Figure 7, P <0.001) The addition of high fructose slightly decreased the amount of sodium excreted (277.9 ± 121.2, mmol/24hr vs 235.1 ± 85.0, mmol/24hr, p = 0.05). Osmolar clearance provides information of the total amount of osmotically active solutes excreted from the urine. We hypothesized that the osmolar clearance rates would follow a similar trend to the sodium excretion data. Osmolar clearance increased in both RF+HS and HF+HS when compared the RF + RS condition (1.79±0.7 vs 2.36±0.9 ml/min, 2.08±0.7 ml/min, P <0.001). Urine flow rate remained unchanged in response to increased sodium or the combination of sodium and fructose (Table 4, P = 0.10). Free water clearance provides data on the amount of solute free water being excreted within the urine. We hypothesized that the combination of fructose would decrease the amount of solute free water excreted from in the urine. Free water clearance decreased in the RF+HS condition when compared to the RF+RS condition (-0.7 ± 0.8 ml/min vs -0.33 ± 0.76 ml/min, P < 0.001) and remained statistically unchanged with the addition of high fructose (-0.33±0.76 ml/min vs -0.54±0.9 ml/min). There was no impact on creatinine clearance due to the diet (Table 3, P = 0.15). The intervention did not induce any changes in hydration status based on the urine specific gravity (Table 3, P = 0.30)

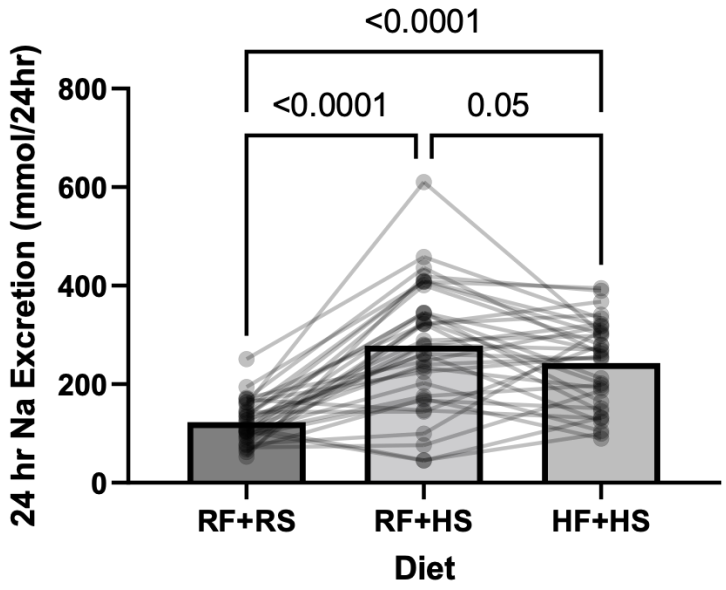

Figure 7 24-hour urinary sodium excretion results following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). N = 35. RM one-way ANOVA: p = <0.0001. Data are presented as individual and mean values. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

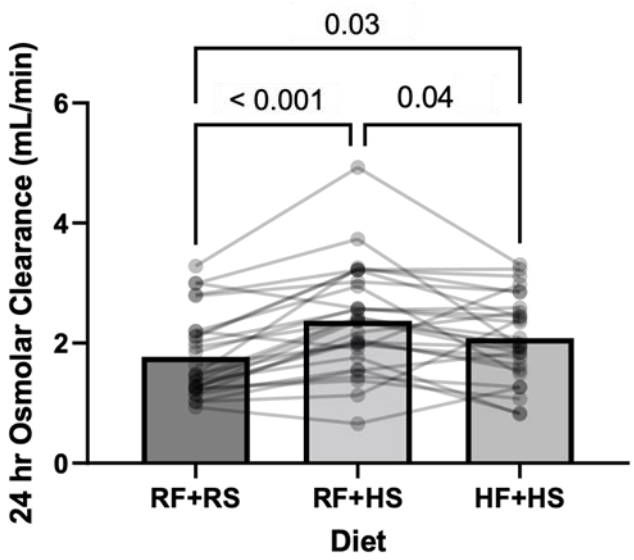

Figure 8 24-hour free osmolar clearance results following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 28. RM one-way ANOVA: p = <0.0001. Data are presented as individual and mean values. Significant differences were followed with post hoc pairwise comparisons using a Tukey's multiple comparison.

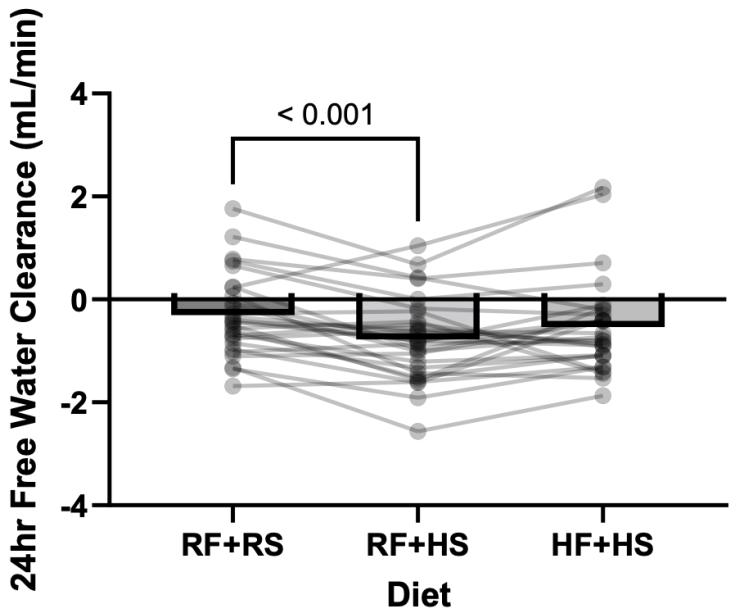

Figure 9 24-hour free water clearance results following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 28. RM one-way ANOVA: p = 0.001. Data are presented as individual and mean values. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

2.3.4 24-hour ambulatory BP monitoring

Participants were asked to wear ambulatory BP monitors for 24 hours. The summary of 24-hr ambulatory BP measures are presented in **Table 4**. Data was excluded from the table due to poor quality or insufficient number of daytime and nighttime readings. Participant data were excluded due to improperly wearing the BP cuff or being non-compliant with the protocol (*N* = 3). We assessed central blood pressure (CBP) for its prognostic value in predicting cardiovascular events and disorders. CBP is more relative to the heart as it measures the pressure in the aorta and carotid artery. We observed a significant increase in systolic CBP (Table 4, P = 0.01)

during the HF + HS condition when compared to the RF + RS condition. We did not observe a statistical difference in the diastolic CBP pressure (Table 4, P = 0.06). 24-hr MAP had a modest increase during the HF+HS condition when compared to the RF + RS condition (Figure 10, P = 0.03). This was not observed in the RF + HS condition when compared to the RF + RS condition (Figure 10, P = 0.25). The change in MAP presented in **Figure 11**, was supported with statistically significant increases in systolic and diastolic BPs. Furthermore, the increased MAP in response to the combination of HF + HS was driven by a modest increase in diastolic pressure (67±6 vs 65±6). Depicted in **Figure 12** is a sex comparison in response to the intervention. There was a significant effect of the diet in both males and females (Figure 12, P = 0.04). Males showed an overall higher BP than females (Figure 12, P < 0.001) however, there were no significant differences in how the diet influenced each sex (Figure 12, Sex x Diet, Interaction, P = 0.90). **Figure 13** presents the effect the diet had on nocturnal dipping. Changes in nocturnal dipping can indicate physiological changes in BP regulation and can serve as a cardiovascular risk marker. Under normal physiological conditions, a dip in nocturnal BP should be between 10-20%. We hypothesized that the combination of fructose and high sodium would impair nocturnal BP dipping when compared to high sodium alone. Nocturnal systolic BP dipping was impaired in the HF + HS group but not the RF + HS group (Figure 13, P = 0.003). There were no differences in nocturnal diastolic dipping. **Figure 14** shows a sex comparison of nocturnal SBP dipping with males being more affected than females in the HF + HS condition (Figure 14, males, P = 0.002). There were no differences observed in the assessment of central hemodynamics.

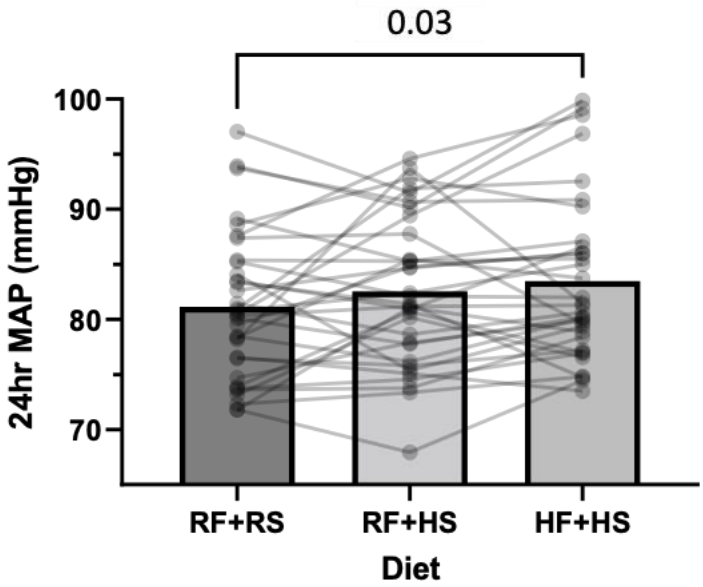

Figure 10 24-hour ambulatory BP results following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 33. Data are presented as individual and mean values. RM one-way ANOVA: p = 0.03. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

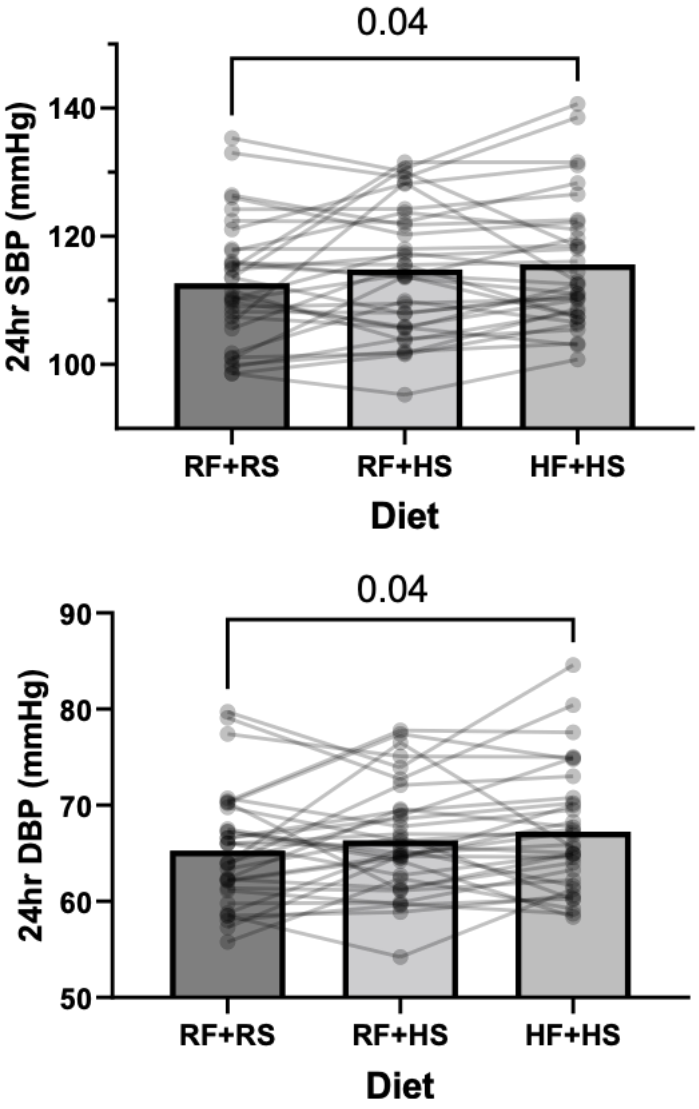

Figure 11 24-hour ambulatory SBP and SBP pressure results following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). N = 33. Data are presented as individual and mean values. (Top)RM one-way ANOVA: p = 0.04. (Bottom). RM one-way ANOVA: p = 0.05 diet. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

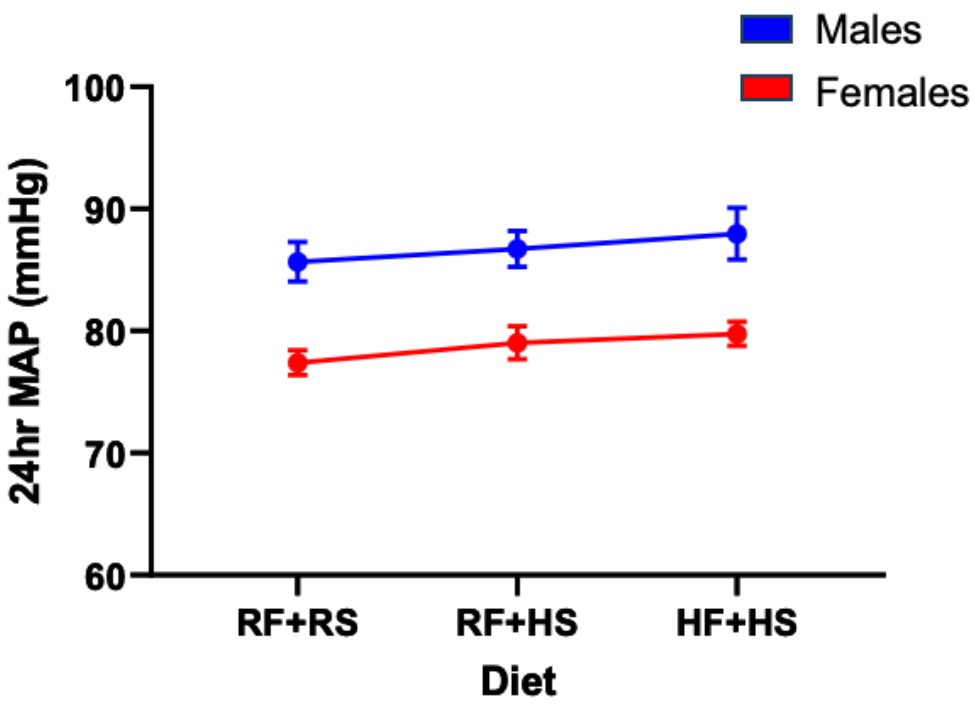

Figure 12 Sex comparison of 24-hour Mean arterial pressure (MAP) results following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). Males, *N* = 15, Females, *N* = 18. Data are presented as mean values. Two-way ANOVA: Sex p = <0.001, Diet p = 0.04, Sex x Diet p = 0.90

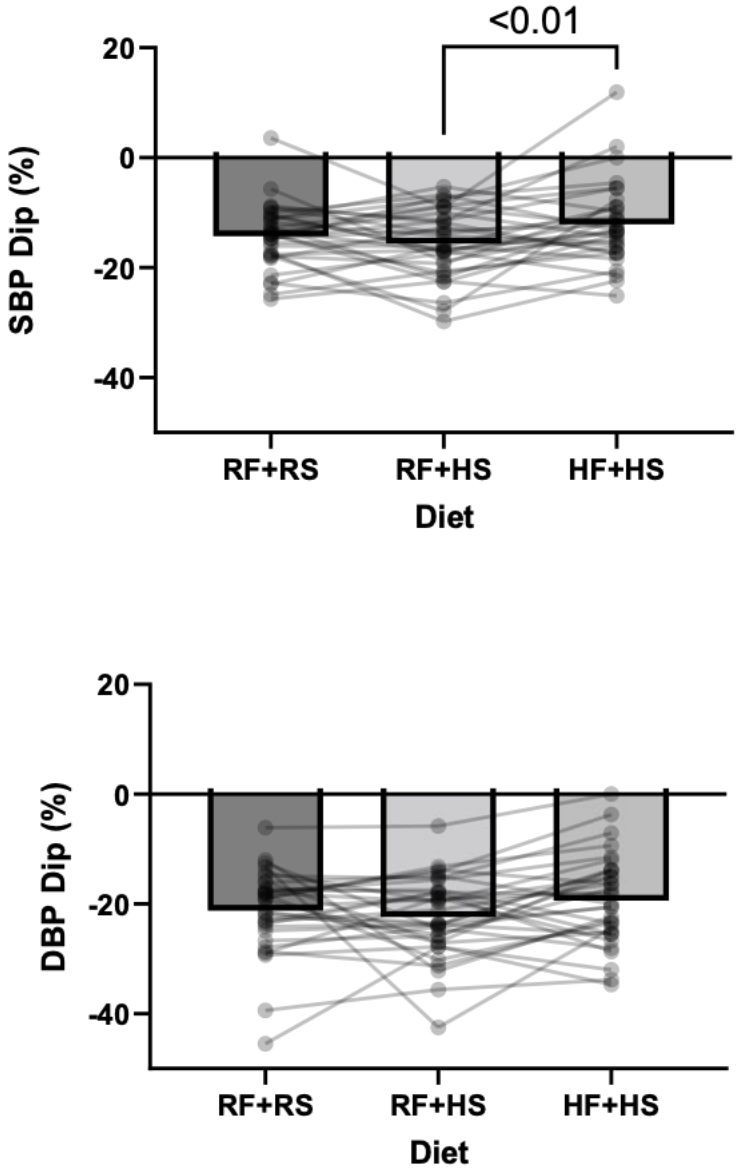

Figure 13 Nocturnal blood pressure dip following dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 33. Data are presented as individual and mean values. (Top)RM one-way ANOVA: P = 0.004. (Bottom). RM one-way ANOVA: p = 0.08 Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparisons.

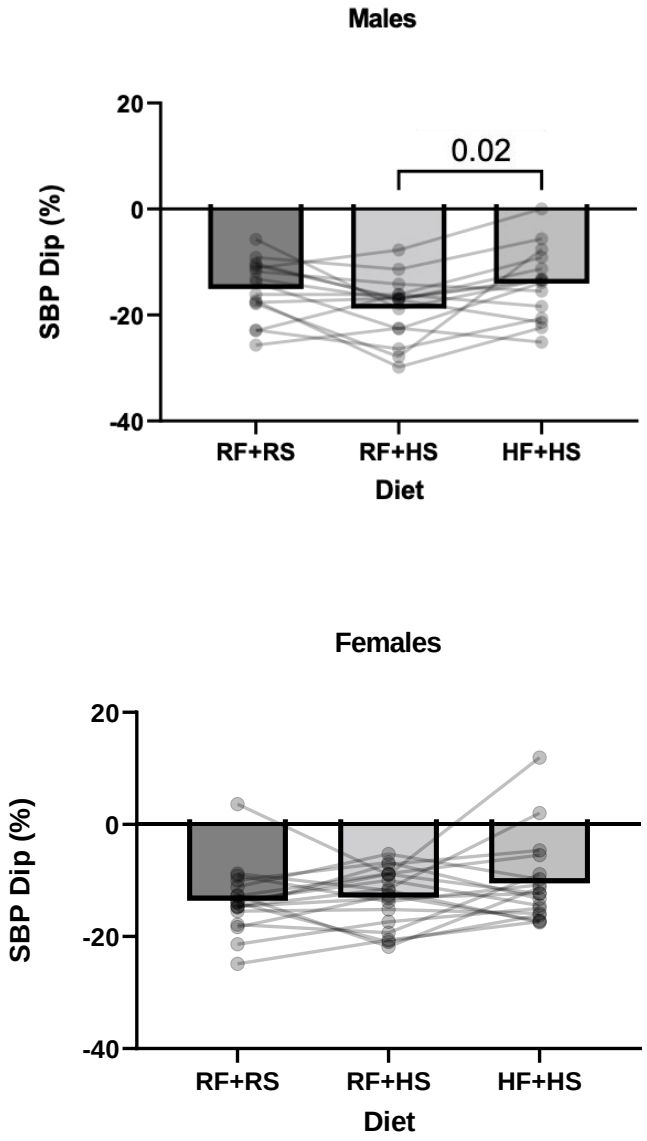

Figure 14 Figure. Sex comparison of nocturnal blood pressure dip following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). Top: Males, *N* = 15 RM one-way ANOVA: p = 0.02. Bottom: Females, *N* = 18, RM one-way ANOVA: p = 0.12. Data are presented as individual and mean values. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

2.4 Discussion

This study utilized a double-blinded, randomized cross-over, controlled trial in humans to investigate the impact of high dietary sodium and fructose on BP and renal function. I hypothesized that increased dietary fructose would impair kidney function in excreting a high sodium load, thereby increasing blood pressure. The primary findings within this cohort of healthy normotensive adults are that increased fructose consumption, when combined with high dietary sodium, results in a modest decline in sodium excretion and an increase in mean arterial blood pressure (MAP). These acute changes were observed with one week of sodium and fructose loading, suggesting that longer-term consumption of high sodium and fructose could have chronic deleterious effects on blood pressure.

Alterations in renal function, whether arising from primary renal pathologies or disruptions in hormonal and neural control mechanisms, contribute to several hypertensive phenotypes[227]. These changes often involve a diminished capacity of the kidneys to effectively excrete sodium, thereby fostering the onset of hypertension [228–230]. Our hypothesis stemmed from prior preclinical investigations suggesting that dietary fructose could induce shifts in renal function and BP[17,18]. Indeed, past studies have elucidated that fructose augments the expression of renal sodium transporters, thereby promoting heightened sodium reabsorption and diminished excretion[217]. This study examines this mechanism in humans by closely assessing changes in sodium excretion across three distinct dietary conditions. The data from this study are consistent with the literature in Sprague Dawley rats where the combination of a high

sodium and fructose diet led to a salt-sensitive blood pressure response in an otherwise salt-resistant strain of rat[17,18].

This study utilized sodium capsules and fructose-sweetened beverages over three 7-day periods. The length of the 7-day intervention was decided based on previous rodent studies that demonstrated changes in BP in as little as a week when dietary sodium and fructose were increased [17,18]. As expected, sodium excretion was significantly increased during the two high sodium conditions, robustly affirming adherence to the protocol. Consistent with our hypothesis, adding fructose reduced sodium excretion. I also observed sodium and fructose-induced alterations in osmolar and free water clearance. Osmolar clearance was increased in the high sodium conditions, while the addition of fructose resulted in a modest decline. There was a significant decrease in the rate of free water clearance with sodium loading, while the addition of fructose resulted in a lesser decrease. There were no differences detected in creatine clearance, our index of glomerular filtration rate.

The second objective of our study was to determine whether an increase in BP could be detected within a 1-week dietary intervention among normotensive adults. We expected an increase in BP in the HF + HS condition since studies conducted on salt-resistant rodents observed significant increases in BP after rodents consumed an increased fructose and sodium diet[17]. The key mechanism driving this elevation primarily involves the upregulation of NHE3 in the proximal tubule, leading to heightened sodium transport (i.e., reabsorption of sodium)[19]. Additionally, NHE3 upregulation concurrently sensitizes the proximal tubule cells to lower concentrations

of Angiotensin II [92]. Within this cohort, we observed an increase in MAP, systolic, and diastolic BP during the HF + HS condition. The magnitude of these changes was modest, but the effect was statistically significant. The clinical significance of these modest observations in 24-hour BP data measures demonstrates that notable changes can be observed within one week, and I would anticipate a more robust effect with a longer intervention period. Furthermore, clinical guidelines can be provided based on this intervention to reduce the risk of healthy adults developing hypertension.

While this study was not statistically powered to investigate sex differences, we explored sex differences within this cohort since prior research has suggested that salt-sensitive blood pressure is more pronounced in females compared to males[170]. When assessing 24hr-MAP, I detected no significant difference based on sex as a contributing factor. Subjects who consumed an increased number of calories, sodium, or fructose in their habitual diet may contribute to the variability of MAP data. However, upon further analysis, I found no correlation between habitual diets, sex, and the change in 24-hour MAP. Furthermore, despite varying levels of habitual sodium or fructose intake within this cohort, I found no significant correlation of responsiveness to the intervention in participants when assessing their habitual diet. Additionally, we explored how this intervention influenced nocturnal BP dipping, since the nighttime dip in BP has prognostic value[231]. A reduction in BP during sleep compared to wake periods typically exceeds 10%. The clinical significance of detecting changes in the nocturnal dip is that it can serve as an early marker for the prognosis of hypertension patients [232]. I observed a decline in nocturnal dipping (i.e., less of a reduction in BP during sleep) due to the combined sodium and fructose

intervention. There is literature that demonstrates increased sodium intake does not impair nocturnal dipping in normotensive adults [224]. Interestingly, the outcomes of this study suggest that the combination of high fructose combined with high salt impairs the nocturnal dip, whereas high salt alone does not have a comparable effect. Moreover, it appears that males exhibit greater impaired nocturnal dipping, highlighting a potential sex-specific effect. Reductions in nocturnal dipping have been observed in previous studies investigating salt-sensitive BP. There is an association between dyslipidemia and impaired BP dipping [233]. Furthermore, SS individuals have been shown to respond to increased sodium intake by excreting more sodium at night to maintain balance [234].

This study has several limitations. I did not include a fructose-only group. There are studies that demonstrate fructose can induce changes in BP, however, these are generally observed over longer periods of consumption[235–237]. While water intake was ad libitum, the recorded intake was not different between the trials. Lastly, we did not control for the menstrual cycle in these premenopausal women. Several references demonstrate differences in the way females respond to hypertensive stimuli when compared to males[238–240].

2.5 Conclusions

In this randomized cross-over, double-blinded controlled trial our goal was to determine if dietary fructose induces salt-sensitive BP in normotensive salt-resistant adults. Our findings reveal that increased dietary fructose, in conjunction with high sodium intake, modestly increased BP. The modest increase in BP was observed in

conjunction with a modest impairment of sodium excretion. Notably, our study provides support that the combination of high fructose and high salt intake impairs nocturnal systolic dipping, a phenomenon absent in the high fructose plus recommended salt condition. This prompts further exploration into the alterations to circadian rhythm induced by high fructose and high salt consumption. This study demonstrates that within a single week high sodium and fructose can alter renal function and BP in healthy adults. Future studies are encouraged to explore how the combination of high fructose and high salt impacts factors influencing BP regulation in humans, such as autonomic regulation and vascular stiffness.

Chapter 3

THE IMPACT OF SODIUM AND FRUCTOSE IN OXIDATIVE STRESS AND INFLAMMATION

3.1 Introduction

The Western diet, abundant in sodium and fructose from processed foods and sugary drinks, contributes to health issues. Excess sodium intake is linked to hypertension and cardiovascular disease (CVD), whereas high fructose consumption is linked to obesity and metabolic syndrome[35,43,241]. Previous research has shown that consuming increased sodium or fructose affects endothelial function, potentially impairing the vasodilation response [94,195]. Elevated sodium consumption is linked to heightened levels of reactive oxygen species (ROS) in the blood vessels, resulting in oxidative stress and inflammation that can induce endothelial dysfunction [41]. Consistent consumption of fructose leads to increased oxidative stress due to abnormal levels of lipids and uric acid, both of which can contribute to impaired functioning of the endothelium[4,187]. Endothelial dysfunction facilitates various phenotypic alterations that contribute to an intensified inflammatory reaction, leading to heightened recruitment and positioning of leukocytes on the endothelium[242].

The regulation of blood pressure and the development of hypertension are strongly tied to endothelial function [243]. There is evidence that suggests increased sodium intake promotes the stimulation and infiltration of immune cells into the vasculature resulting in oxidative tissue damage. In our research, we have observed

heightened ROS activity in endothelial cells taken from salt-resistant adults after consuming a high-sodium diet [244]. This elevated ROS activity, primarily attributed to superoxide, triggers a cascade of immune cell activation, exacerbating endothelial dysfunction. Additionally, our previous findings indicate that increased dietary sodium intake impairs endothelial function, as assessed by flow-mediated dilation (FMD), in salt-resistant adults[245]. This impairment in FMD is likely related to the uncoupling of endothelial nitric oxide synthase (eNOS), leading to reduced nitric oxide release.

The effect of consuming varying amounts of fructose on the function of the endothelium is still a matter of debate in experimental models. This is likely because fructose produces different metabolic byproducts that contribute to the impairment of endothelial function [185]. Studies conducted on rodents suggest that there is a combined effect of sodium and fructose in causing sodium-sensitive BP [17,18,210]. Yang et al., conducted a study in rodents demonstrating that fructose in the diet can impact blood pressure by impairing renal sodium excretion[92]. Furthermore, fructolysis in the nephron results in increased generation of ROS and buildup of uric acid [246]. Research conducted on rodents suggests that fructose-induced hypertension results in renal damage, primarily due to the accumulation of metabolic byproducts and proinflammatory cytokines[247,248].

The link between sodium and fructose as a contributing factor to SSBP remains poorly unexplored. There is evidence in rodents that demonstrates the immune response contributes to the effects of SSBP [143]. Lee et al, demonstrated that salt-sensitive rodent models have increased immune cell activity when compared to

salt-resistant rodents[42]. Furthermore, there is an association between sodium intake and the stimulation of PBMCs resulting in a pathogenic phenotype characterized by upregulated tumor necrosis factor-α (TNF-α) and proinflammatory interleukin 6 (IL6) [113,132]. TNF-α, a multifactorial regulator, including in the expression of endothelial nitric oxide synthase (eNOS), has been shown to decrease eNOS protein levels over time [249,250]. Huang et al. provide evidence that after salt-sensitive rodent models have increased TNF-α localization in the kidney[141]. IL6 is a central cytokine associated with the development of hypertension [135]. Salt-sensitive rodent models fed a high salt diet exhibited a dampened BP response when administered an IL6 inhibitor [251].

While high sodium intake has been established to impair FMD, there has yet to be a study to determine if fructose interacts with sodium to further impair FMD in healthy adults [223]. Therefore, this study aims to investigate the effects of the dietary combination of sodium and fructose in normotensive adults on endothelial function. We hypothesize that healthy non-hypertensive adults consuming increased dietary fructose and sodium will have an impaired FMD function and generate elevated ROS from PBMCs.

3.2 Methods

3.2.1 Study Design

The approach for the research design is an extension of the overall study design previously described in Aim 1. A blood sample was drawn of approximately 2.4 ounces or 70 mL from a vein from the arm of each participant. The sample was

used to assess electrolytes, hemoglobin and hematocrit levels, plasma osmolality, superoxide levels, and purify PBMCs.

3.2.2 Peripheral Blood Mononuclear Cells Isolation

PBMCs were isolated from fresh venous blood collected from subjects participating on the last day of each of the three interventions. PBMCs were obtained from whole blood using two 9 mL EDTA vacutainer tubes. PBMCs were isolated by density gradient centrifugation using the well-established ficoll-plaque method. Briefly, histopaque was layered underneath whole blood, cells with a high density migrated through the ficoll, while cells with a lower density remained on top of the histopaque after centrifugation. Monocytes and lymphocytes, having different densities compared to neutrophils and erythrocytes, were separated to isolate the fraction containing PBMCs from the rest of the blood. The isolated PBMCs had a viability of >80%, assessed using Trypan blue staining and the cell countess. Subsequently, the cells were stored in PBS containing 5% fetal bovine serum and 20% dimethylsulfoxide (DMSO) in concentrations of approximately 2 million cells per 1 mL aliquots. These aliquots were then placed in an isopropanol chamber (Mr. Frosty Freezing Container, Thermo Fisher Scientific) to undergo slow freezing at a rate close to -1°C/minute in a -80°C freezer. After a minimum of 24-72 hours of slow freezing, the cell aliquots were transferred to a liquid nitrogen tank and stored at the gas phase for long-term storage until downstream analysis. A 10% reduction in cell viability after freezing should be expected using this method.

3.2.3 PBMC cell culture

All cells are treated identically throughout the protocol. To thaw the cells, briefly, PBMCs were exposed to a 37-degree water bath. After performing the quick thaw protocol, I washed with PBS containing 2% fetal bovine serum and prepared the culture for an overnight recovery period. This wash is performed to remove all residual DMSO from the frozen cells. For the recovery procedure, PBMCs (2×10^6cells/ml/max 20×10^6cells) the cells were suspended in RPMI 1640 medium containing 1% PenStrep, 2% Glutamine, and 10% FBS. The suspension was placed in a round bottom 5 ml polystyrene culture tube (Stellar scientific, SKU: STS110428) and incubated for 18 hours at 37°C in a humidified atmosphere with 5% CO2. The tube cap was loosened to facilitate gas exchange.

3.2.4 ImageXpress Pico Automated Cell Imaging System—Digital Microscopy (Pico Assay)

Superoxide measurements were performed on cryogenically stored primary PBMCs from participants on each diet. After the overnight recovery period, PBMCs were reanalyzed using the countess and trypan blue stain for accurate cell counts and viability. The cells were then calculated to a relative equal number of cells (300,000) per well based on viability. For the staining process, the washed cell pellet was mixed with a solution containing Hoechst 33342 (Thermo Fisher) at a dilution of 1:2000 (10 mg/mL in PBS) and MitoSox Red (500nM) (ThermoFisher) in a V bottom 5 mL conical tube. The total volume of the mixture was adjusted to 600 uL. Following a 30–40-minute period of incubation, the cells were subjected to centrifugation at a force of 300 times the acceleration due to gravity, washed two times with phosphate-buffered saline (PBS), and subsequently transferred to a 96-well plate. The 96-well plate

underwent a brief centrifugation process lasting 3 minutes. The cells were examined using an ImageXpress Pico automated cell imaging system (Molecular Devices, San Jose, CA, USA) through automated digital microscopy. The cells were examined using the Texas Red and DAPI channels at a magnification of 10×. For each analysis, three identical regions from each well were selected. The focus and exposure time were determined using an automated setup and adjusted by analyzing 3–4 test wells.

3.2.5 Superoxide detection in whole blood

The EMX Nano directly and accurately measures reactive oxygen species in different cellular and tissue compartments in biological settings. Fresh venous blood was collected and incubated with cyclic hydroxylamine (CMH) spin probe (VWR, #89151-138) at 37°C for 10 mins. CMH selectively reacts with superoxide to generate a stable free radical called nitroxide. The CMH-incubated blood samples were then quickly frozen in capillary tubing (#EW-06605-29) in 150uL aliquots using liquid nitrogen and stored in a -80ºC freezer until analysis. Before the analysis, the frozen samples were transported in liquid nitrogen to the EMX Nano spectrometer, where they were placed into the spectrometer apparatus and calibrated. The software preloaded with a spectrum designed for superoxide detection was used to analyze each sample for magnetic field spectrum quality, enabling further quantification of ROS species. This protocol provided a systematic approach for accurately measuring ROS levels in biological samples using the EMX Nano spectrometer.

3.2.6 Superoxide Dismutase Activity Detection

The measurement of SOD activity was conducted using an assay kit specifically designed for detecting Superoxide Dismutase activity (Invitrogen Cat # EIASODC). In summary, a volume of 10 μL of serum was utilized to assess the subject's levels of SOD. Following the completion of the reaction, the absorbance of each sample was quantified at a wavelength of 405 nm using a SpectraMax ID3 plate reader (Molecular Devices). The levels of SOD activity were determined using the formula provided by the manufacturer.

3.2.7 Inflammatory Cytokine detection in serum

The levels of serum IL6 and TNF alpha were quantified using Simoa technology on an SRX analyzer, following the guidelines provided by the manufacturer (Quanterix, Billerica, MA). The Simoa IL6 and Simoa TNF alpha Kits (Quanterix) were utilized. In summary, the serum samples were defrosted at a temperature of 22°C, carefully stirred, and then subjected to centrifugation at a speed of 2000 RPM for a duration of 5 minutes at the same temperature. The samples were diluted at a ratio of 1:4 with a sample diluent and then attached to paramagnetic beads that were coated with a capture antibody that specifically targets human IL6 or TNF alpha. The beads were coated with antibodies and then incubated with biotinylated detection antibodies specific to IL6 or TNF alpha. These detection antibodies were labeled with a complex of streptavidin and β-galactosidase.

3.2.8 Endothelial Function Measurements

Endothelial function was evaluated using a non-invasive technique called flow-mediated dilation (FMD). To perform this technique, an ultrasound probe is used to quantify the diameter of the brachial artery and the velocity of blood flow. Following a period of establishing a baseline, we promptly increased the pressure of an occlusion cuff to a level above the normal systolic pressure (approximately 200 mmHg) on the upper forearm. This was done to block the flow of blood to the forearm and hand. The occlusion was sustained for a duration of 5 minutes. The cuff was promptly depressurized, facilitating the restoration of blood flow to the forearm and hand. We measured the diameter and blood flow of the brachial artery for a duration of 3 minutes following the release of the cuff. The placement of the ultrasound probe was recorded to ensure consistent examination of the identical region of the brachial artery in each situation. I calculated the percent vasodilation of the brachial artery from baseline to peak diameter (FMD % = (Peak Diameter- Baseline Diameter)/(Baseline Diameter)).

3.3 Results

3.3.1 Baseline Subject Characteristics

This study utilized subjects who were screened as part of **Aim 1** of this dissertation. The screening characteristics of the participants are presented in **Table 1.** The participants were young, normotensive, and non-obese. Most participants were Caucasian and Asian. Refer to **Table 2** for information of this cohort's habitual dietary intake.

3.3.2 Intervention Adherence Measures

Table 3 shows the standard perturbation data collected at the end of each diet. For a detailed summary of findings refer to the aim 1 results section.

3.3.3 Quantification of Superoxide's

We measured PBMCs and whole blood for changes in superoxide. PBMCs were extracted from whole blood after participants completed each condition and stained for intracellular superoxide. **Figure 15** displays the data obtained from participants' PBMCs stained with Mitosox Red at the conclusion of each dietary regimen. The intervention induced an increased cellular fluorescence indicating elevations of intracellular superoxide in the HF + HS group when compared to the RF+RS and RF+HS and (Figure 15, P = 0.01)). We assessed for changes in PBMC derived ROS due to sex differences **(**Figure 16**)**. Superoxide increased during the HF + HS condition compared to the RF + HS condition in men (Figure 16, 138.2±84 vs 100.5±37 RFU, P = 0.04), but not in women (138.7±121.8 vs 123.7±84 RFU, P=0.11). We assessed whole blood for changes in superoxide using electron paramagnetic resonance (EPR) (**Figure 17)**. Each participant had an aliquot of blood stored with a spin trap probe (CMH) specific for detecting superoxide. The number of electron spins correlates with the molarity of free radicals detected in each sample. We did not detect differences when assessing whole blood for superoxide using EPR (Figure 17, P = 0.83). Furthermore, we found that the intervention induced no change in free radical scavenging when measuring superoxide dismutase activity (SOD) (Figure 18, P = 0.40)

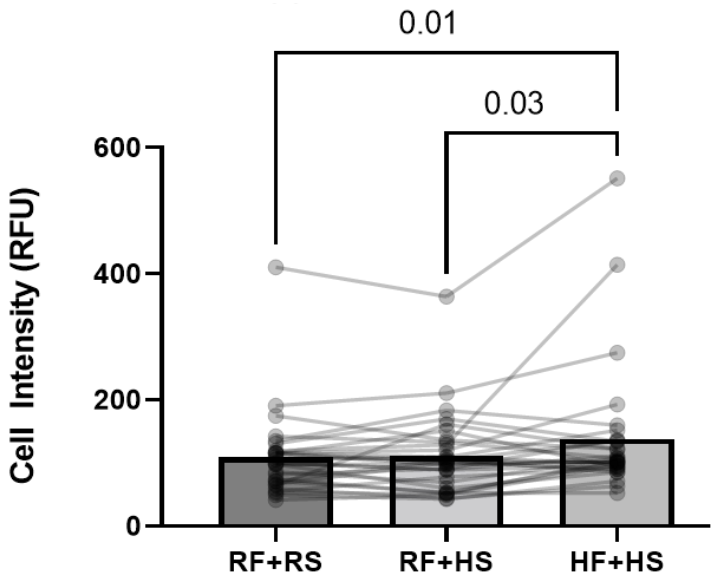

Figure 15 Superoxide intensity in PBMCs following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). Relative Fluorescent Units (RFU). *N* = 30. Data are represented as individual and mean values. RM one-way ANOVA: P = 0.01. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

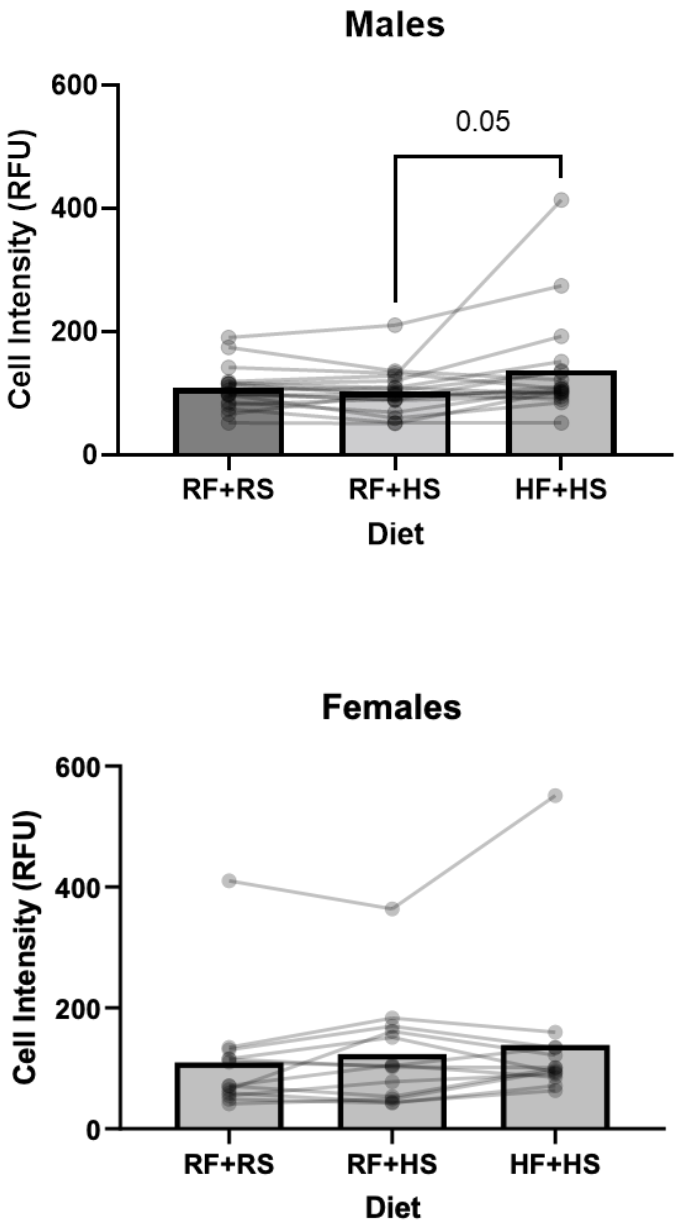

Figure 16 Sex comparison of superoxide intensity in PBMCs following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). Relative Fluorescent Units (RFU). Top: Males, *N* = 18 RM one-way ANOVA: P = 0.04, Bottom: Females, *N* = 13, RM on-way ANOVA: p = 0.12. Data are represented as individual and mean values. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

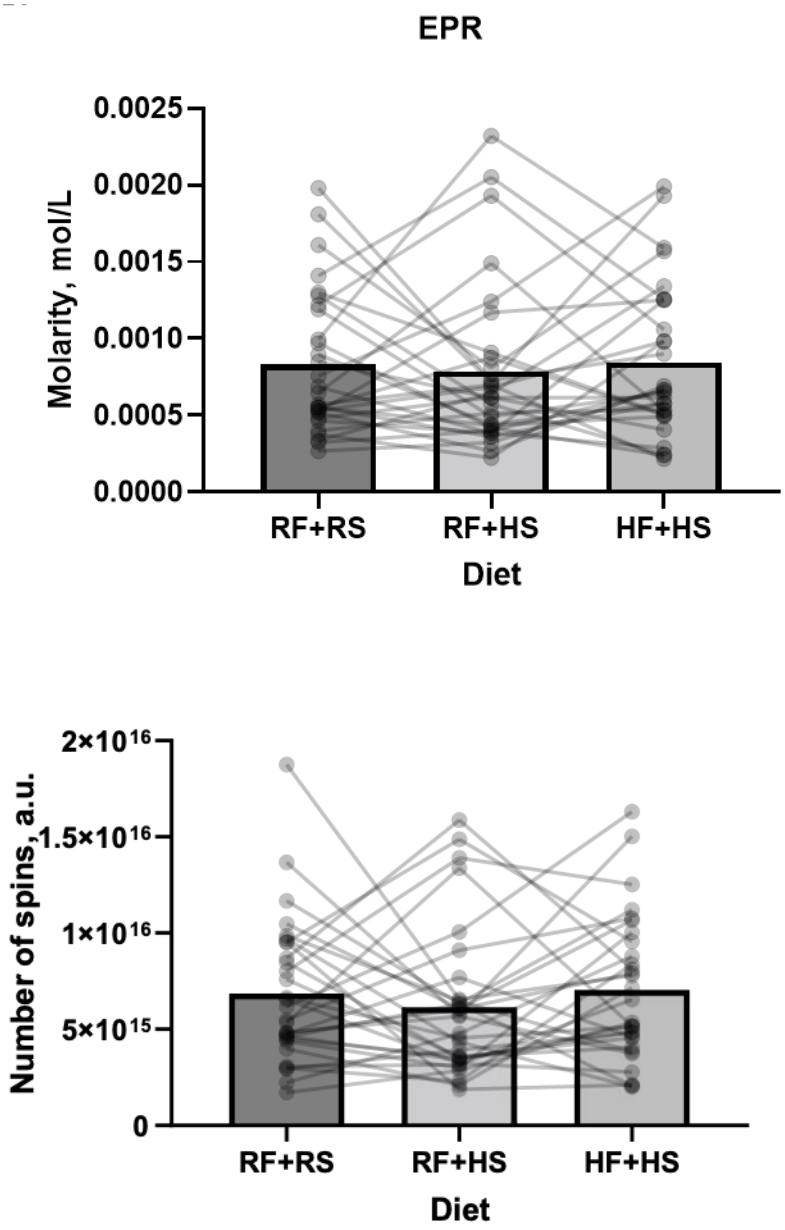

Figure 17 Free radicals in whole blood following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). (Top) Molarity value of the total concentration of free radicals. N = 30, RM one-way ANOVA: p = 0.83.(Bottom) Number of electron spins indicating the number of free radicals. N = 30, RM one-way ANOVA: P = 0.52. Data are represented as individual and mean values.

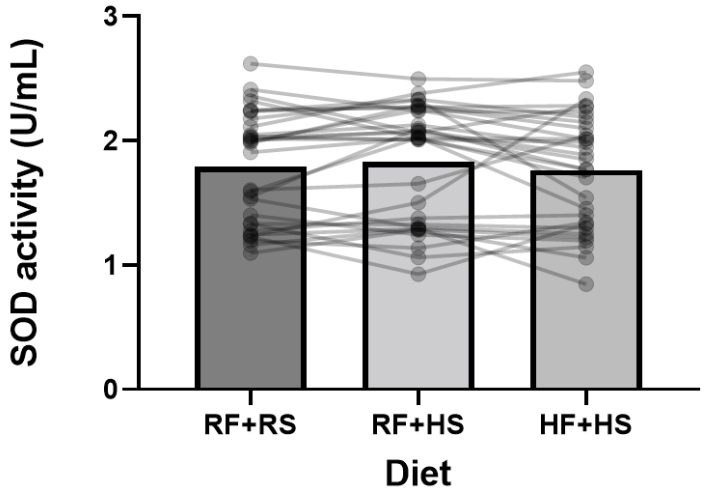

Figure 18 Superoxide dismutase measurements in PBMCs. blood following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 31. RM one-way ANOVA: P = 0.40 Data are represented as individual and mean values.

3.3.4 Inflammatory Cytokine Levels in Serum

Serum was collected from each participant at the end of each diet to assess markers of inflammation. **Figure 19** presents the levels of Interleukin 6 (IL-6) detected in serum following each diet. The IL-6 concentration in serum increased slightly after the HF + HS condition compared to the RF + RS condition (Figure 19, 0.31±0.2 vs 0.24 ± 0.19 pg/mL, P = 04). **Figure 20** shows the concentration of Tumor Necrosis alpha (TNF α) in serum from participants following each diet. Like the findings observed in assessing IL6 levels, TNF α also demonstrated a slight increase during the HF + HS diet when compared to the RF + RS diet (Figure 20, 0.42 ±0.2 vs 0.38±0.2 pg/mL P = 0.03).

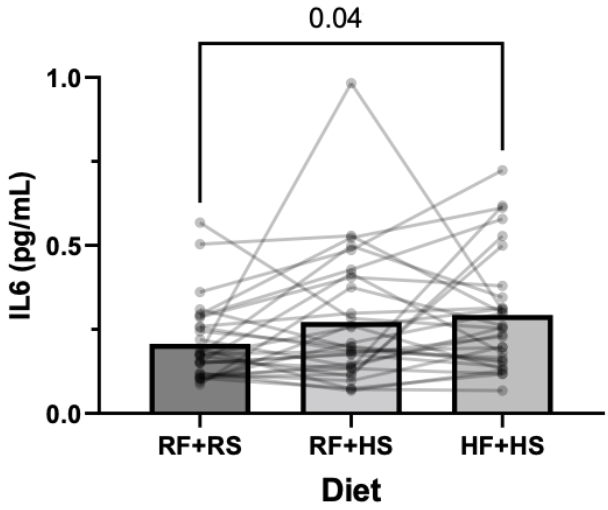

Figure 19 Interleukin 6 levels detected in serum following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 30, RM one-way ANOVA: p = 0.05. Data are represented as individual and mean values. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

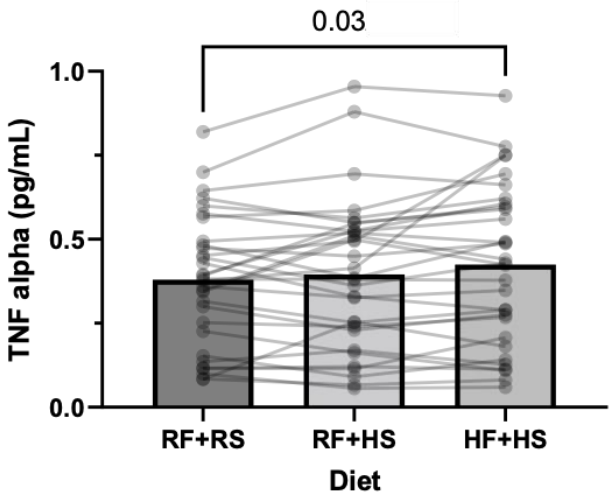

Figure 20 Tumor Necrosis alpha levels detected in serum following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 31, RM one-way ANOVA: p = 0.04. Data are represented as individual and mean values. Significant differences were followed with post hoc pairwise comparisons using Tukey's multiple comparison.

3.3.5 Endothelial Function Assessment

Participants were evaluated for changes in endothelial function after each diet using flow-mediated dilation (FMD), **Figure 21**. The shear stress stimulus of each participant was not different (Table 7, P = 0.59). There were no differences in baseline or peak diameter. Endothelial function assessed using FMD was not different across the diets.

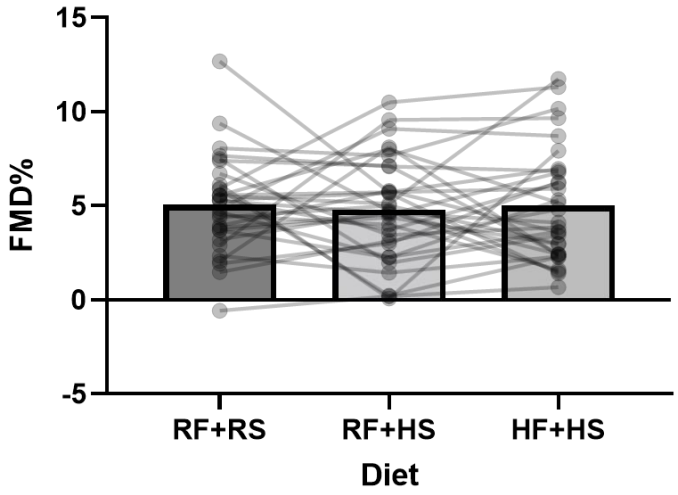

Figure 21 Endothelial function assessed using flow-mediated dilation following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). *N* = 31, RM one-way ANOVA: p = 0.90. Data are represented as individual and mean values.

Measure	RF+RS	RF + HS	HF + HS	P
Baseline diameter	3.76±0.7	3.75±0.7	3.77±0.7	0.59
Peak diameter	3.93±0.7	3.92±.07	3.95±0.8	0.49
FMD%	4.65±2.6	4.71±2.5	4.91±2.9	0.89
Shear rate (AUC)	20209.4±6648.7	20081.1±7417.5	20423.8±10072.1	0.59

Table 7 Brachial Artery Flow-Mediated Dilation Results following each dietary intervention of recommended fructose plus recommended salt (RF + RS), recommended fructose plus high salt (RF + HS), and high fructose plus high salt (HF + HS). Flow mediated dilation percent (FMD %). Area under curve (AUC). *N* = 31.

3.4 Discussion

This study utilized a double-blinded, randomized cross-over, controlled trial in humans to investigate the impact of dietary sodium and fructose on oxidative stress, inflammation, and endothelial function. I hypothesized that increased dietary sodium and fructose would be more proinflammatory and lead to a greater impairment of endothelial function when compared to salt loading. The primary findings within this cohort demonstrate that HF + HS induces a modest increase of pro-inflammatory cytokines, an effect that was absent with sodium loading alone. Furthermore, the generation of superoxide from PBMCs was greater in the HF + HS condition compared to the RF + HS condition. While ROS and proinflammatory cytokines were increased in the HF + HS condition, counter to our hypothesis, I did not observe differences in endothelial function due to the dietary intervention.

The hypothesis, supported by evidence from prior animal models and human trials, proposes that the combination of high fructose and high sodium intake would trigger a more substantial inflammatory response than high sodium intake alone [6,8,179]. We expected this to lead to a reduction in endothelial function, attributable to increased levels of oxidative stress and inflammation. This study utilized sodium capsules and fructose-sweetened beverages over three 7-day periods. As expected, sodium excretion was significantly increased in both high sodium conditions, and fructose loading resulted in a significant increase of serum triglycerides and VLDL, confirming participant adherence. Fructose metabolism stimulates uric acid production [252]. Hyperuricemia is linked to increased superoxide production via xanthine oxidase and NADPH oxidase activation, which can impair endothelium-dependent vasodilation by reducing nitric oxide bioavailability [253,254]. Interestingly, serum uric acid levels were significantly decreased in both sodium-loading conditions. The observation that increased sodium intake decreases serum uric acid levels aligns with other sodium-loading studies [252,255,256]. The evidence suggests that increased sodium intake increases uric acid excretion, thereby lowering serum levels[255,256]. This should be considered in future studies investigating the combination of fructose and sodium.

This study was designed to investigate whole blood, serum, and PBMCs for changes in ROS and proinflammatory cytokines. Whole blood analysis using EPR provides insight into the changes of extracellular superoxide secreted by the cell [257]. Changes in extracellular superoxide are valuable as this would suggest increased signaling mechanisms and promote the migration of immune cells[258]. The assessment of whole blood using EPR demonstrated superoxide levels were not different.

Furthermore, there were no differences observed in measuring free radical scavenging when assessing superoxide dismutase activity. However, tumor necrosis alpha and interleukin 6 were increased in the combined high sodium and fructose trial. There is an association between dyslipidemia, oxidative stress, and inflammation [259–261]. Contrary to prior evidence, this study demonstrates that high sodium alone does not result in significant differences in TNF alpha or interleukin 6, only the combination of high sodium and fructose increases inflammation and oxidative stress. Although we don't expect severe consequences from the modest increases in TNF alpha or interleukin 6, longer exposure to these proinflammatory cytokines due to chronic consumption of sodium and fructose can lead to deleterious effects on the vasculature.

There is evidence to support that SSBP is exacerbated due to increased ROS from PBMCs [143,158]. This is mostly mediated by NADPH oxidase 2 or altered mitochondrial function[153]. Increases in intracellular ROS signaling can promote infiltration of PBMCs into the vasculature and the kidney resulting in changes in vascular function[154,262]. We assessed for changes in intracellular superoxide by performing mitosox red staining which indicates changes in mitochondrial function. Within our cohort, we observed a significant increase in mitochondrial-derived ROS in PBMCs due to the fructose and salt loading. Furthermore, our data suggests that males are more susceptible to diet-induced oxidative stress than females. Our study detected no changes in FMD suggesting endothelial function was not impaired with our intervention in young healthy adults, despite prior literature showing that oxidative stress and chronic low-grade inflammation are contributors to endothelial dysfunction[115,159].

There are some study limitations associated with this study design. I decided not to control the menstrual cycle in female participants. This was due to (1) the fact that we were not adequately powered to make this dissertation a sex-focused study and (2) the complexity of scheduling in combination with this study design. There is evidence that demonstrates females have decreased levels of oxidative stress likely due to differences in hormone regulation. However, FMD is unaffected by the menstrual cycle or oral contraceptive pill[263]. This study was designed to investigate the addition of high fructose to high-sodium diets, so it lacks a fructose control group. Previous research has shown that increased dietary sodium impairs endothelial function when measured with FMD, whereas the effect of fructose has received less attention[41,192]. This aids the field, but it also creates challenges when attempting to compare our findings to the relative data of this study.

3.5 Conclusions

In this randomized cross-over double-blinded controlled trial, we provide evidence that increased fructose intake combined with increased salt induces higher levels of proinflammatory cytokines and oxidative stress than increased salt alone. Additionally, this study provides support that changes in inflammation and oxidative stress occur independent of changes in FMD. Notably, this study demonstrates that within a single-week diet, combined high sodium and fructose increase circulating lipids within a healthy population. Interestingly, uric acid was significantly decreased in the fructose loading group. This prompts further exploration into the synergistic effects that salt loading has on serum uric acid levels. In conclusion, after 7 days of

increasing fructose and sodium intake, healthy adults demonstrate increased levels of oxidative stress and inflammation, which may contribute to longer-term vascular health declines.

Chapter 4

Conclusion

4.1 Summary

In this study, I hypothesized that fructose would impair kidney function leading to decreased sodium excretion thus increasing BP [3,18,19]. I found that when healthy adults consumed increased sodium and fructose BP increased. Additionally, I hypothesized that the combination of increased sodium and fructose would generate an increase in ROS and proinflammatory cytokines resulting in impaired endothelial function. In this cohort, I observed an increase in oxidative stress and inflammation when participants consumed increased sodium and fructose. The data reported in this study supports my hypothesis that the combination of fructose and sodium increases BP, oxidative stress, and inflammation while sodium loading alone does not. The hypothesis that the combination of sodium and fructose would impair endothelial function, however, was rejected.

My hypotheses were based on key findings from the literature, including both in vitro and in vivo models [17–19,178,217,218]. Research in humans demonstrates the BP response to increased dietary sodium varies, with some individuals being more sensitive than others [1]. Multiple systems have been implicated in SSBP, yet the precise underlying mechanisms remain incompletely understood [39,40,42,143]. In Sprague Dawley rats, fructose and sodium increased BP over 7 days, but fructose alone did not[17]. Evidence shows that fructose enhances the expression of sodium transporters in the

proximal tubule[92]. Gonzalez-Vicente et al. demonstrated that dietary fructose mediated SSBP by enhancing angiotensin II activity, increasing the ability of low Ang II concentrations to stimulate proximal tubule sodium reabsorption [19]. This mechanism can increase extracellular fluid volume, thus raising BP. Supporting our second aim, Ramick et al. provided evidence that oxidative stress increases with high-sodium diets [244]. Mazzoli et al. demonstrated that increased fructose consumption in rodents led to mitochondrial dysfunction, inflammation, and oxidative stress [264]. Superoxide impairs endothelial function by decreasing endothelial nitric oxide synthase activity[103]. Furthermore, increased sodium or fructose intake elevates proinflammatory cytokine levels, stimulating immune cells and leading to further ROS production [6,8,195]. There is evidence in rodent models that proinflammatory cytokines can regulate renal function, and the infiltration of immune cells into the kidney has been implicated in mediating the SSBP phenotype [132,138,168].

Despite these findings in the literature, gaps remain in our understanding of the effects of fructose-induced SSBP in humans. There is a lack of information within humans about how fructose impacts kidney function. Additionally, the role of endothelial function in fructose-induced SSBP has not been thoroughly investigated in humans. Furthermore, there is a lack of studies in humans linking sodium and fructose intake to immune cell function. Addressing these gaps is crucial for a comprehensive understanding of fructose-induced SSBP and its underlying mechanisms in the human body. Our research study focused on these areas to elucidate the interactions between dietary factors, kidney function, endothelial function, and immune responses in the regulation of BP.

I made several observations to note during our investigation of how healthy adults' BP and renal function respond to increased fructose and sodium intake. In this cohort, participants demonstrated significant levels of increased circulating lipids and triglycerides. Hypertriglyceridemia and dyslipidemia contribute to chronic inflammation and atherosclerosis [265]. I observed an increase in sodium excretion in both sodium loading conditions with fructose having a modest impairing effect. Furthermore, we observed modest increases in BP when normotensive participants consumed increased sodium and fructose whereas sodium loading did not. I conducted a correlation analysis to identify any association between the increased BP and increased VLDL and triglycerides in the HF+HS condition. There was no significant correlation identified when performing that analysis. The data reported in this investigation supports our hypothesis that fructose impairs renal sodium handling by observing modest declines in sodium excretion.

I made several observations during our investigation of how increased sodium and fructose impact inflammation and endothelial function in healthy adults. I previously stated observing significant increases in serum triglycerides and lipids which may contribute to the inflammatory response. When directly measuring serum for markers of inflammation we observed a modest increase in circulating proinflammatory cytokines when dietary sodium and fructose were increased. I investigated immune cells and observed increased levels of oxidative stress, likely due to mitochondrial dysfunction. This did not translate to free radicals present in whole blood. Our observation of increased inflammation and intracellular oxidative stress

was exclusive to the HF + HS condition. While others have demonstrated sodium loading is indeed proinflammatory, the data from this cohort contradicts those findings indicating that sodium loading for 7 days is insufficient to stimulate a similar response [266]. I conducted a correlation analysis to identify any association between the increased BP and increased inflammatory cytokines in the HF+HS condition. There was no significant correlation identified when performing that analysis. Furthermore, I conducted an analysis to identify sex differences in the inflammatory response. There was no significant difference in the inflammatory response due to sex. Additionally, this cohort demonstrated no change in endothelial function in response to the dietary intervention. The evidence from this investigation demonstrates that a diet of increased sodium and fructose induces inflammation and oxidative stress but does not impair endothelial function.

This study can serve as a platform for several future studies interested in how fructose can induce sodium sensitivity in a healthy population. Future studies should observe the synergistic effects of this dietary combination over longer interventions. Another area of interest is the effect this has on different populations. How would an older vs younger population respond to this intervention design? Based on the outcomes of this investigation, we would hypothesize that a longer dietary intervention would provide an even greater response than this 7 day intervention. Additionally, we would hypothesize that the diet would impact the older population more than the younger population.

4.2 Perspective

The mechanisms underlying the Western diet and the development of BP-related diseases are poorly understood. Americans continue to overconsume dietary sugar and sodium even though the Western diet is known to increase the risk of BP-related diseases. This study aimed to fill a gap by investigating the impact of increased fructose and sodium intake on BP. Additionally, we sought to assess potential changes in oxidative stress, inflammation, and endothelial function. Previous studies have investigated this in rodents yet there have been few studies in humans [19,143]. A strong evidence base includes mechanistic rodent studies that are ultimately translated to human-based clinical trials (i.e., coupling pre-clinical and clinical studies). In isolation, there are advantages and disadvantages to each approach, which are summarized below.

Pre-clinical approaches allow for a deep mechanistic explanation of physiological phenomena in a highly controlled setting in genetically similar animal strains. The disadvantage is that it is not always clear if the findings translate to humans, therefore it is important to couple pre-clinical finding of basic biological mechanisms with clinical/human studies to fully understand a topic. The focus on this dissertation has been on what impact does too much sodium and fructose have on blood pressure and markers of inflammation in healthy adults. Our hypotheses were formed based on pre-clinical studies that have addressed these questions in rodents. Ultimately, our primary interest lies in human health, and clinical/human studies get us closer to answering questions that directly impact the general population. For any clinical/human study, there are inherent limitations. For instance, participant compliance to a diet (in this case pills and drink) can vary, as can their levels of

physical activity and eating patterns during the study period. Furthermore, human studies tend to exhibit more variability than in vitro and rodent studies.

To summarize, the collection of in vitro and in vivo studies provides distinct benefits and is equally necessary to address physiological questions such as SSBP. The fructose and sodium loading studies conducted in rodents served as a foundational premise for this study, and the findings of this dissertation extend and translate the findings observed in rodents to humans. Furthermore, this study demonstrates how the Western diet contributes to the development of BP-related disease through renal and inflammatory mechanisms.

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Appendix A

IRB APPROVAL LETTER

Institutional Review Board
210H Hullihen Hall
Newark, DE 19716
Phone: 302-831-2137
Fax: 302-831-2828

DATE:	September 19, 2023
TO:	Ronald McMillan, MS
FROM:	University of Delaware IRB
STUDY TITLE:	[1617405-11] The impact of sodium and fructose on blood pressure and inflammation
SUBMISSION TYPE:	Amendment/Modification
ACTION:	APPROVED
APPROVAL DATE:	September 19, 2023
EXPIRATION DATE:	June 16, 2024
REVIEW TYPE:	Expedited Review
REVIEW CATEGORY:	Expedited review per 45 CFR 46.110 (b)(1) (ii)

Thank you for your Amendment/Modification submission to the University of Delaware Institutional Review Board (UD IRB). The UD IRB has reviewed and APPROVED the proposed research and submitted documents via Expedited Review in compliance with the pertinent federal regulations.

As the Principal Investigator for this study, you are responsible for and agree that:

- All research must be conducted in accordance with the protocol and all other study forms as approved in this submission. Any revisions to the approved study procedures or documents must be reviewed and approved by the IRB prior to their implementation. Please use the UD amendment form to request the review of any changes to approved study procedures or documents.
- Informed consent is a process that must allow prospective participants sufficient opportunity to discuss and consider whether to participate. IRB-approved and stamped consent documents must be used when enrolling participants and a written copy shall be given to the person signing the informed consent form.
- Unanticipated problems, serious adverse events involving risk to participants, and all non-compliance issues must be reported to this office in a timely fashion according with the UD requirements for reportable events. All sponsor reporting requirements must also be followed.

Oversight of this study by the UD IRB REQUIRES the submission of a CONTINUING REVIEW seeking the renewal of this IRB approval, which will expire on June 16, 2024. A continuing review/progress report form and up-to-date copies of the protocol form and all other approved study materials must be submitted to the UD IRB at least 45 days prior to the expiration date to allow for the required IRB review of that report.

If you have any questions, please contact the UD IRB Office at (302) 831-2137 or via email at hsrb-research@udel.edu. Please include the study title and reference number in all correspondence with this office.

Appendix B

Informed Consent

UNIVERSITY OF DELAWARE

UD IRB Approved: 07/13/2022
IRBNet ID#: 1617405-7

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title of Study: The impact of sodium and fructose on blood pressure and inflammation

Principal Investigator(s): Ronald McMillan, MS and William B. Farquhar, PhD

KEY INFORMATION

Important aspects of the study you should know about first:

- **Purpose**: The purpose of this research study is to determine if the combination of sodium and fructose increases blood pressure and inflammation.
- **Procedures**: You will be asked to complete a screening session and three different 1-week interventions, separated by at least one month. For one of the weeks, you will be asked to consume placebo (sugar) pills and a low fructose drink, another week you will be asked to consume sodium pills and a low fructose drink, and the other week you will be asked to consume sodium pills and a high fructose drink. The diets will be randomized, and you will not be told which pills (placebo or sodium) or drink (low or high fructose) you are given. You will prepare and eat your own foods, but we will provide guidance on what you should/should not be eating during the trial. Your blood pressure will be assessed, urine collected, and assessments of your blood vessels will be performed during each diet.
- **Duration**: This study will span at least 3 months and include 8 (7 if you decide to consent virtually) total visits and approximately 9 hours total time commitment.
- **Risks**: Consuming the sodium pills or the low/high fructose drink may cause you to experience slight hand or foot swelling, frequent urination, feel bloated, feel nauseous, or experience diarrhea. Spacing out the pills and drink throughout the day should minimize these possible side effects. We expect blood pressure to increase modestly during the high salt-high fructose drink condition.
- **Benefits**: There will not be a benefit to you for participating in this research study. The data collected during the screening session includes blood work and blood pressure data, which can be provided to you upon completion of the study.
- **Alternatives**: There are no known alternatives available to you other than not taking part.
- **Costs and Compensation:** If you decide to participate there will be no additional cost to you and you will be compensated $300 if you complete the entire study. Partial compensation will be provided for partial completion.
- **Participation**: Taking part or not in this research study is your decision. You can decide to participate and then change your mind at any point.

Please carefully read the entire document. You can ask any questions you may have before deciding if you want to participate.

You are being invited to participate in a research study. This consent form tells you about the study including its purpose, what you will be asked to do if you decide to take part, and the risks and benefits of being in the study. Please read the information below and ask us any questions you may have before you decide whether you want to participate.

PURPOSE OF THE STUDY

Excess dietary salt is associated with an increased risk of cardiovascular diseases. Some healthy adults have been determined to be more sensitive to dietary salt than others, which can result in a 10% or more increase of blood pressure. The mechanisms underlying salt sensitivity of blood pressure are not fully understood. Previous research has suggested excess intake of added sugars, especially fructose, can increase salt sensitivity of blood pressure. Chronic elevated consumption of salt and fructose independently have been shown to have damaging effects on the body, however, the combination of both salt and fructose has not been fully studied in humans. The purpose of this research study is to determine if increased dietary fructose consumption will stimulate salt-sensitive hypertension and induce proinflammatory cytokines through activation of the immune system. Understanding how the body regulates blood pressure during times of elevated salt and fructose is important for determining cardiovascular risk in a population with normal blood pressure.

WHO IS BEING ASKED TO PARTICIPATE?

You will be one of approximately 100 participants to be recruited in this study.

You are being asked to participate because you are between 18 and 45 years old and have normal blood pressure. There are several factors that may result in your exclusion from the study if they are discovered during the screening visit described below. These include blood pressure greater than 140/90 mmHg; history of cardiovascular disease, cancer, diabetes, or kidney disease; obesity (BMI > 30kg/m^2); smoking or tobacco use; current pregnancy; nursing mothers. You should not participate in this study if you have donated blood or had a large volume of blood drawn within 4 weeks of study participation. In addition, you should not donate blood in the 4 weeks following study participation.

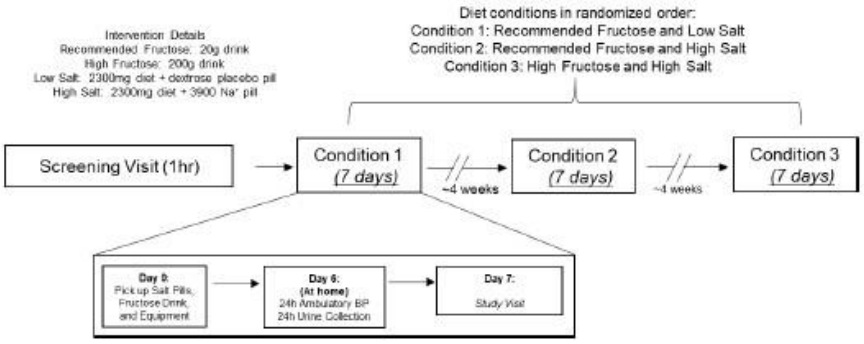
PROCEDURES: WHAT WILL YOU BE ASKED TO DO?

Total Visit Time Summary (8 total visits (7 if you decide to consent virtually); 9 hours total time commitment)

As part of this study you will be asked to report to the STAR Campus, Newark, DE for a total of no more than 8 study visits over the course of up to 3 months. Prior to your participation within the study you will be scheduled for a consenting visit either in person or a zoom web conference. During this visit, we will review and ask that you sign the consent form. You will have the opportunity to ask any questions regarding the study. You will then be given the USDA Food Model for Estimating Portions Booklet and written instructions on how to complete a 3-day food diary

while consuming your typical diet. You will also complete a physical activity questionnaire (PAQ) for us to assess your level of daily physical activity. Upon completion of the diet record, you will send this to us via email so we can analyze and store the information regarding your habitual diet, this information will be collected but is not part of the exclusion criteria for your eligibility to participate in this study. Following the informed consent, you will be screened for inclusion and exclusion criteria (described below) at the Cardiovascular Research Core Laboratory at STAR Campus, Newark, DE (approximately 30 minutes). Following the screening visit, we will schedule you for three study visits each separated by at least 4 weeks. If you are a female participant, an attempt will be made to schedule you during the early follicular phase of your menstrual cycle or the placebo (sugar pill) phase if you are taking hormonal birth control. Each study visit will require approximately 2 hours. All study visits will be completed within approximately 3 months.

Full participation in this study involves completion of 2 phases: (#1) subject screening and (#2) a diet supplementation. These 2 phases will be separated by approximately 1 week and each diet supplementation will be separated by approximately four weeks. The total number of visits will not exceed more than 8 separate visits to the Physiology Core Laboratory. A visual study outline of each phase and a description of each visit is described below.



Telephone Pre-Screening Initial contact
Visit 1 Informed Consent and Screening (60 minutes)
Visit 2 Pick up capsules, BP monitor, urine container, and pregnancy test (women only) (15 minutes)
Pre-Visit 3 Wear 24-hour blood pressure monitor and collect urine for 24 hours
Visit 3 Randomized Study Visit 1-Bring BP monitor and urine to lab; Pregnancy test (women only), vascular function testing, blood collection, additional urine sample collection for urine specific gravity (all) (2 hours)
Visit 4 Pick-up capsules, BP monitor, urine container, and pregnancy test (women only) (15min)
Pre-Visit 5 Wear 24-hour blood pressure monitor and collect urine for 24 hours
Visit 5 Randomized Study Visit 2-Bring BP monitor and urine to lab; Pregnancy test (women only), vascular function testing, blood collection, additional urine sample collection for urine specific gravity (all) (2 hours)
Visit 6 Pick-up capsules, BP monitor, urine container, and pregnancy test (women only) (15min)

Pre-Visit 7 Wear 24-hour blood pressure monitor and collect urine for 24 hours
Visit 7 Randomized Study Visit 3-Bring BP monitor and urine to lab; Pregnancy test (women only), vascular function testing, blood collection, additional urine sample collection for urine specific gravity (all) (2 hours)

Screening:

During this visit we will review and ask that you sign the consent form. All questions regarding the study will be answered. This visit will either be held in person or via zoom. If held via zoom the session will not be recorded. We will ask you to complete the session in a private space to minimize the conversation being overheard. You will sign the consent securely in a RedCap database that is separate from the location where research data is stored. As such, personal identifiable information will not be linked to research data. To determine if you qualify to be in the study, the following information will be obtained: a complete medical history using a questionnaire, a physical activity questionnaire, height, weight, waist circumference, hip circumference, resting blood pressure, and a body fat assessment. All information will be reviewed by the study investigators, and you will only be accepted into the study if you show no signs or symptoms of disease (example: chest pain, dizziness, or shortness of breath, etc.). Also, the medical history form will be reviewed by the study investigators to determine if you qualify for the study. In addition, you will be excluded if you are obese (body mass index greater than 30 kg/m^2), if you use any tobacco products, or – for women – if you are pregnant or taking hormone therapy. If you are taking hormonal contraceptives you will be allowed to participate in the current study. All medications will be reviewed on a case-by-case basis by the study investigators to determine if you are eligible for study participation. The investigator(s) will discuss the results of these tests with you and upon your request, make copies of these results available. If one of the test results is abnormal, you will be referred to your personal health care provider to follow-up. In addition, you will be asked to record and submit a 3-day diet inventory prior to the study. This will be documentation of your usual daily diet.

7-day intervention

For 7 days, you will be advised to limit consumption of products containing added sugars outside of what is provided and maintain a diet that does not exceed the daily recommended dietary sodium intake (2300mg). Additionally, you will be asked to maintain a similar diet during the 3 study trials, and you will be provided with a diet intake log to keep a record of your diet and water intake for the duration of each 7-day intervention. You will be randomly assigned to a diet of a daily low or high fructose drink in combination with placebo or salt pills. We will not know what the content of the pills are until the end of the study, meaning the researchers and you will be "blind" to which pills you are consuming. During the 7-day intervention you will consume a daily low fructose drink (20g fructose) or a high fructose drink (200g) in combination with an additional 3900 mg sodium in the form of pills (9 daily), or an equal number of placebo pills containing dextrose (sugar). Throughout each day of the diet, you will be asked to consume small portions of the 500mL fructose drink and salt pills with food. The fructose drink and salt pills will be made in the metabolic kitchen in the Tower at STAR.

Physical activity monitoring:

We will provide you with a device called an accelerometer and instruct you on how to use the device. The accelerometer will monitor your physical activity levels during each of the diets. The accelerometer is like popular activity trackers like Fitbit®. You will be asked to wear the accelerometer for the duration of the 7-day study. Your moderate and vigorous physical activity levels will be assessed for 7 days before the study visit. You will receive the accelerometer when you come to pick up their diet supplementation for the respective supplement. You will also receive a log to record times when the accelerometer was worn and not worn. We will ask that you return the accelerometer at the conclusion of your study.

24-hour blood pressure:

On the last day of each diet, you will be asked to wear a blood pressure monitor on your upper arm for 24 hours. This blood pressure cuff will be set to automatically take your blood pressure every 20 minutes during the day. You will continue to wear the monitor at night while you sleep; it will automatically take your blood pressure every 30 minutes. The monitor records and saves each blood pressure measurement automatically. Additionally, you will record on a provided data sheet the time you were awake and asleep.

24-hour urine collection:

During the same 24 hours that you are wearing the blood pressure monitor on your arm, you will also be asked to collect all your urine into a plastic container. The urine sample will be analyzed for salt content, as well as for the concentration of several hormones that control salt / water balance and blood pressure. Some of these analyses will be done immediately in the lab, and some of the urine sample will be stored in a freezer for future analysis. Your name will not appear on the stored urine sample; rather, the sample will be coded with a number. These samples will be stored indefinitely.

Measurement of blood pressure and heart rate:

During your visit, you will have 4 sticker electrodes placed on your chest to monitor heart rate, a blood pressure cuff around the upper arm and finger of the non-dominant arm will be attached (left arm if you are right-handed) to measure blood pressure, an elastic-like band will be placed around the chest and stomach to measure breathing rate. An inflatable cuff will be placed around the upper arm on the dominant side (right side if you are right-handed).

Artery function measurements:

During your visit, artery function will be assessed non-invasively using flow-mediated dilation (FMD). We will measure the brachial artery with ultrasound and then blood flow to the forearm and hand will be cut off with and inflatable cuff. This occlusion will be maintained for 5 minutes, and then the cuff will be deflated and blood flow to the forearm and hand will be restored. Blood flow will be measured with ultrasound throughout this test

Blood draw:

During the experimental visit, a blood sample will be drawn from your arm vein (approximately 2.4 ounces of blood, or 70 mL, will be removed). Only trained personnel will take blood. The blood sample will be used to assess electrolytes, hormones that control salt and water balance in the body, hemoglobin and hematocrit levels, and osmolality. Frozen samples of blood will be kept indefinitely for analysis in sample batches later of additional hormones and/or inflammatory markers associated with cardiovascular disease.

WHAT ARE POSSIBLE RISKS AND DISCOMFORTS?

There are no known risks associated with obtaining your height, weight, resting blood pressure.

7-Day Intervention

There are minimal risks associated with consuming 7-days of high salt with fructose and 7-days of low salt with fructose. During the high salt with fructose diet, blood pressure may rise to higher-than-normal levels, slight hand swelling, frequent urination from increased water intake and occasionally feeling bloated. There could also be other symptoms that you experience during these diets. It is likely that you will experience some gastrointestinal-related discomfort while completing this study which could include diarrhea and/or nausea. Due to the manipulation of dietary salt and fructose it is also possible that you may experience symptoms such as increased bowel movements, dizziness, headaches, breathing problems, and bloating. If you experience symptoms that are bothersome, please relay this information to the investigators. In response to any GI discomfort, you may note while on the diet, we may request that you temporarily reduce the daily dosage of the intervention supplements. This temporary modification is done in order to alleviate your discomfort due to the diet. You may stop the diet anytime without penalty. We will keep track of your blood pressure by checking it every time you come into the lab. If your lab blood pressure rises above 140/90 mmHg, we will have you discontinue the diet.

Physical activity monitoring, 24-Hour Blood Pressure & 24-Hour Urine

There are no known risks to wearing a physical activity monitor or continuous blood pressure monitor on the upper arm. During waking hours, blood pressures are taken every 20 minutes, while at night they are taken every 30 minutes. You may experience minor discomfort when the cuff inflates, and it may wake you during sleep. The 24-hour blood pressure cuff and 24-hour urine collection may disrupt normal daily living. There are no known risks to providing a urine sample.

Blood draw

This risk of infection during the blood draws is minimized by using only sterile, single-use blood collection materials and using sterile technique. Universal precautions mandated by the Center for Disease Control and OSHA will be used in the handling of human blood. The total volume of venous blood drawn is modest (<100 mL at each of the

study visits. Subjects may experience some pain and/or bruising at the site where blood is taken, and there is a small risk of infection. Fainting can sometimes occur during or shortly after blood is drawn.

Artery function measurements

There are no known risks associated with the use of ultrasound probe placed on your upper arm to measure femoral artery blood flow. There may be slight discomfort to the area during the protocol and immediately following the study visit. The ultrasound testing involves the inflation of a blood pressure cuff that may result in some temporary mild discomfort. This is like the feeling of an individual's experience when an arm feels numb if it was slept upon.

WHAT ARE POTENTIAL BENEFITS FROM THE STUDY?

There will not be a benefit to you for participating in this research study. The data collected during the screening session includes body composition assessment (i.e., percent body fat), blood work and blood pressure data during the 24-hour blood pressure measurements. This information will be provided to you upon completion of the study. In addition, the study may provide new, useful information regarding the control of blood pressure in normal healthy adults and those diagnosed with high blood pressure.

NEW FINDINGS THAT COULD AFFECT YOUR PARTICIPATION

During the course of this study, we may learn new important information. This may include information that could cause you to change your mind about participating in the study. If any new important information becomes available while you are a participant we will let you know.

CONFIDENTIALITY: WHO MAY KNOW THAT YOU PARTICIPATED IN THIS RESEARCH?

Information obtained from this study will be kept strictly confidential. You will not be individually identified, except by subject number known only to the investigators. All data stored as paper files or on computer disk may be kept indefinitely, but your identifying information will no longer be linked to the data 3 years after study closeout (that is, the file that links your name/identifying information to the data we collect will be destroyed). Data may be used for retrospective analysis. The paper files are stored in a locked cabinet, and the computers are password protected. While the results of this research may be published, your name or identity will not be revealed

The confidentiality of your records will be protected to the extent permitted by law. Your research records may be viewed by the University of Delaware Institutional Review Board, which is a committee formally designated to approve, monitor, and review biomedical and behavioral research involving humans. Records relating to this research will be kept for at least three years after the research study has been completed.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

USE OF DATA COLLECTED FROM YOU IN FUTURE RESEARCH:

Identifiers about you might be removed from the identifiable private information and after such removal, the information could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you or your legally authorized representative

COSTS AND COMPENSATION

There are no costs for participating in this study. However, your participation requires several visits to the University of Delaware campus. You will be responsible for the cost of transportation on these occasions. Compensation will be provided to help offset these costs.

You will receive $300.00 for full completion of all three 7-day trials (phase #1 the screening and phase #2 the dietary intervention) to offset the cost of transportation, etc. Those who complete only the screening and only one of the dietary salt trials, but not the remaining two salt dietary trials, will receive $100.00. Those who complete only the screening and two of the three salt dietary trials will receive $200.00.

WHAT IF YOU ARE INJURED DURING PARTICIPATION IN THE STUDY?

In the event of an emergency during the daytime, you can contact the following telephone number for the Nurse Managed Primary Care Center (302) 831-3195. If you are injured during research procedures, you will be offered first aid at no cost to you. If you need additional medical treatment, the cost of this treatment will be your responsibility or that of your third-party payer (for example, your health insurance). By signing this document, you are not waiving any rights that you may have if injury was the result of negligence of the university or its investigators.

DO YOU HAVE TO TAKE PART IN THIS STUDY?

Taking part in this research study is your decision. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide later not to participate, or if you decide to stop taking part in the research, there will be no penalty or loss of benefits to which you are otherwise entitled. Your decision to stop participation, or not to participate, will not influence current or future relationships with the University of

Delaware. As a student, if you decide not to take part in this research, your choice will have no effect on your academic status or your grade in the class.

OPTIONAL CONSENT TO BE CONTACTED FOR FUTURE STUDIES:

Do we have your permission to contact you regarding participation in future studies? If you agree to being contacted in the future, we will keep your contact information. Please write your initials next to your preferred choice.

_____ YES _____ NO

Appendix C

Recruitment Flyer

Cardiovascular Research Laboratory

Volunteers Needed for Research Study

Examining the effects of dietary salt and fructose on blood pressure and inflammation

You may be eligible to participate if you are:

- Between 18-45 years old
- Have normal blood pressure
- Non-smoker and in reasonably good health
- Willing to undergo 7-day diet intervention

Participants will receive compensation for participating

Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030	Ronald McMillan/Salt-Fructose Study rmcmill@udel.edu 302-544-0030

Appendix D

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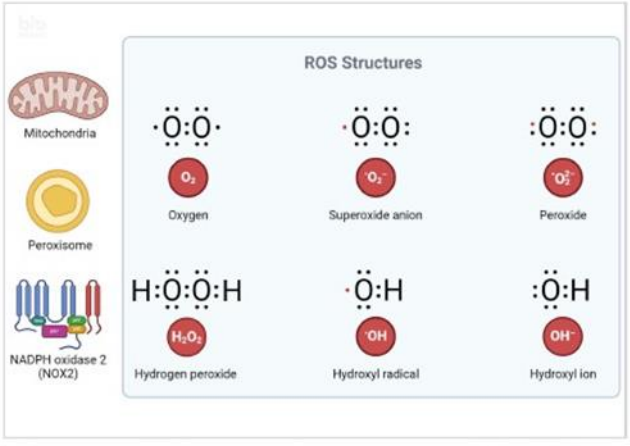

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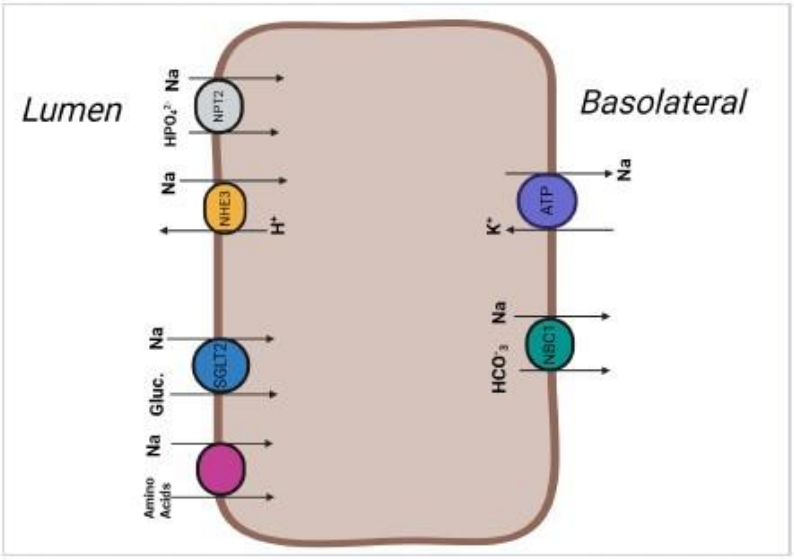

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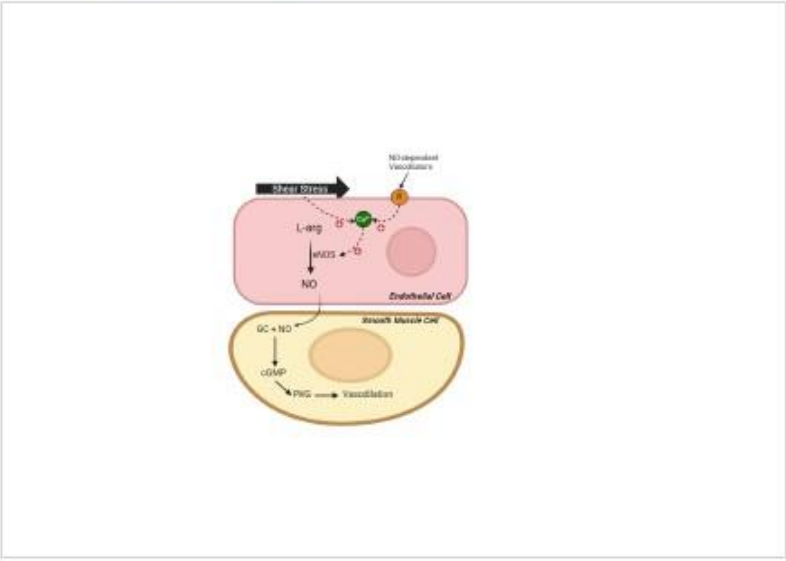

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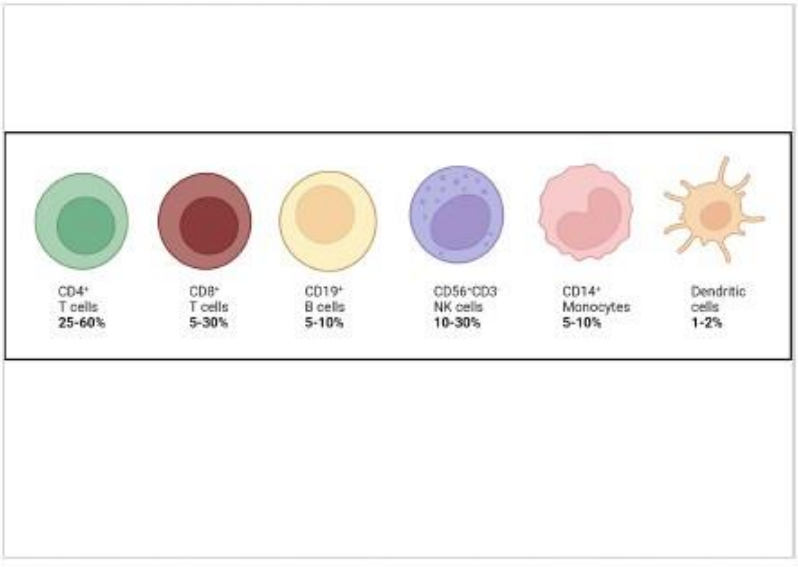

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