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[Francis Boateng Annor] Date

Psychosocial and Oxidative Stress and Health of Adults

By
Francis Boateng Annor
Doctor of Philosophy

School of Public Health
Division of Epidemiology and Biostatistics

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Psychosocial and Oxidative Stress and Health of Adults
By

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BS, Georgia State University, 2008
MPH, Georgia State University, 2009

Advisor: Ike S. Okosun, MS, MPH, PhD

An abstract of
a dissertation submitted to the School of Public Health at Georgia State University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
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2015

Abstract

Psychosocial and Oxidative Stress and Health of Adults
Francis Boateng Annor

The role of stress (both psychosocial and oxidative) in the pathophysiology of several chronic diseases has been documented and has become a focus for chronic disease prevention and management. Although, psychosocial stress (PS) and oxidative stress (OS) have different mechanisms through which they impact health, they both cause physiological imbalance which might subsequently lead to a disease state. Laboratory and observational studies have linked both stresses to the pathophysiology of diabetes mellitus (DM) and hypertension. However, findings from previous studies have not been entirely consistent and results have varied based on the study population and the stress-measurement tool used. Given the gaps in the literature, three studies were conducted to examine: (1) the relationship between PS and glycemic control; (2) the association between PS and estimated glomerular filtration rate (eGFR); and (3) the association between OS and hypertension among adults.

In the first two studies, a longitudinal data from Kaiser Permanente Georgia (KPGA) survey on Health and Healthy Behaviors linked to patients' laboratory and pharmacy records was used. In the third study a cross-sectional data from Study on Race, Stress and Hypertension was used.

The first study examined the association between baseline measure of work-related PS and glycemic control using both cross-sectional and longitudinal designs. None of the four PS sub-scales or the overall PS measure at the work environment was significantly associated with glycemic control at either study baseline or over time. The second study examined the association between general measures of PS and changes in estimated glomerular filtration rate (eGFR) over time in a structural equation model framework. No significant direct association was observed between general PS measure and eGFR decline. However, age, race, mean arterial pressure and insulin use were found to be associated with eGFR decline. The third study examined the association between hypertension and: 1) four markers of OS (F_2-Isoprostanes, Fluorescent oxidative products, copy number of mitochondrial DNA and Gamma-tocopherol); and 2) plasma nutrient based oxidative balance score (OBS). The OBS was inversely associated with hypertension, but none of the OS markers was significantly associated with hypertension after adjusting for study covariates.

The current work highlights some of methodological issues in the assessment of PS to examine their relationship with DM control and complications. The study also highlights the need for more future studies to be conducted to confirm the association between OBS and hypertension, preferably longitudinal studies. If future studies confirm this finding, then the mechanisms by which OBS may influence risk of hypertension would need to be explored further.

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Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. INTRODUCTION AND BACKGROUND

Introduction

Diabetes mellitus (DM) and high blood pressure/hypertension are two common and costly

chronic conditions that significantly increase the risk of nephropathy, cardiovascular events and

death [1-6]. Although both conditions have several risk factors, an imbalance to the internal

environment or a threat to the balance, known as stress, is considered a factor that impacts both.

In the current study, we will consider psychosocial stress with respect to DM while consideration

will be given to oxidative stress in relation to hypertension.

DM is a chronic metabolic condition associated with elevated blood glucose level. It is usually

caused by insulin deficiency that is often associated with insulin resistance [7]. An estimated 366

million people worldwide, representing 8.3% of the global adult population aged 20 – 79 years

had DM in 2011 [8]. The current prevalence is expected to increase to 10% (552 million) by

2030 [8-11]. The prevalence of DM in the US is not different from the global estimate. An

estimated 8.3% (25.8 million) of adults in the US had DM in 2011 [12]. In addition to the high

DM prevalence, the age of onset, particularly, for type 2 DM has significantly declined in recent

years [13], a development which has a serious implication on quality of life for individuals with

early DM onset, cost associated with management, and complications that may arise from the

disease.

Although strong connection exists between genetic factors and DM etiology [14-16] the evidence

points to the external environmental factors such as diet and sedentary behaviors to be the main

culprits in the current surge in DM incidence and complications [17-19]. Therefore, most DM

management research have focused on these traditional risk factors such as poor dietary habit,

physical inactivity and obesity but these factors have not entirely explained the variability in DM management and complications. Research have also noted an important role of psychosocial stress in DM etiology, management and complications [20, 21], however, research on psychosocial tress and DM management is sparse and results have been inconsistent. Beside their role in DM onset, the DM traditional risk factors as well as psychosocial stress also contribute to poor glycemic control and the risk of complications from the disease [22-24]. In an era of increased life expectancy but decreased age of DM onset [25], the importance of optimal DM control cannot be overemphasized. Good DM management is crucial for improving overall quality of life, while decreasing the long term complications among DM patients. Of importance to the current research is the fact that psychosocial stress in modern society is increasing [26] while the buffering context such as the social support and the physical environment in which they occur is changing [27-29]. Understanding the dynamics of psychosocial stress in relation to glycemic control and subsequent complications from DM will lead to improved DM management and care.

The prevalence of high blood pressure/hypertension is high with approximately 31% of US adults having the condition [30]. The current direct annual medical cost is approximately $70 billion but it is expected to triple by 2030 [31]. The traditional risk factors for hypertension include family history, age, physical inactivity, obesity, tobacco use, and excessive alcohol intake [32-34]. These factors have also not completely explained the pathophysiology of hypertension. Evidence from recent studies have suggested a significant role of oxidative stress (OS) in the pathogenesis of hypertension [35-38]. Although, basic science and animal studies have supported the role of OS in hypertension [39-41], the results from human studies have not

been entirely consistent and protective effects of anti-oxidant supplementation to reduce blood pressure have not proven to be beneficial in clinical trials [42-44].

Based on the considerations of limited research and inconsistent findings from studies that have examined the relationship between psychosocial stress and DM management and complications, as well as the relationship between oxidative stress and hypertension/high blood pressure, the overarching goal of this dissertation is to fill in some of the knowledge gaps. Specifically, the aim of the current study is to examine the relationships between psychosocial stress and DM management and complications on one hand and the relationship between oxidative stress and hypertension on the other hand. The study aim will be addressed through three specific research questions: 1) are high levels of psychosocial stress (at the work environment) associated with poor glycemic control among individuals with DM; 2) are high levels of general psychosocial stress associated with decline in estimated Glomerular Filtration Rate (eGFR); 3) is oxidative stress associated with hypertension? These research questions will be answered using data from two previously conducted studies.

Background

Psychosocial Stress, DM management and complications

Living organisms adapt to and survive in their environment by maintaining a balance that is constantly challenged by complex array of internal and external forces/threats. This balance or equilibrium is maintained by the counteracting forces that may involve both the physical and mental forces to react to the threats in order to establish and re-establish balance/homeostasis [45, 46]. An actual or perceived threat to the maintenance of this balance/homeostasis is what Chrousos referred to as stress [47].

The historical background of psychosocial stress

The concept of psychosocial stress is old and its meaning has changed over time. The contemporary stress concept begun about two millennia ago when Heracleitus suggested that there is an inherent capacity for all things to undergo a constant change [45]. A century later, Hippocrates defined a disease state as a systemic disharmony of systems of balances [45, 48]. Hippocrates system of balance was later extended by Thomas Sydenham who suggested that the adaptive response to those systems could also cause pathological changes[49]. In the early 19th century, the principle of physiological equilibrium was suggested and the term homeostasis was coined to describe the physiologic processes that maintains this balance state [50]. In the 1930s, Selye described the general adaptation syndrome [51] using the term 'stress' to describe the mutual action of forces that take place in the body. In his 'Stress and the general adaptation syndrome' (GAS), Selye identified three phases of the stress response development; the 'Alarm reaction' (AR) that prepares the organism for fight or flight, the resistance stage during which the organism develops resistance to stress if it survived, and the stage of exhaustion, where prolonged stress leads to decreased ability to resist [51, 52]. In the late 80s, Sterling and Eyer (1988) introduced the concept of allostasis to describe the active process by which living organisms adapt to potential threats and changes in their environment in order to maintain homeostasis and promote survival [53]. Five years later, McEwen expanded on the concept of allostasis noting that when the body anticipates a stress response and shifts the homeostatic set point, the shift comes at a cost because it affects other physiological systems and processes. McEwen also coined the term 'allostatic load' to 'refer to the sequelae of overactivity and dysregulation of the network of allostasis' [54, 55]. The concepts of allostasis and allostatic load have implications on the physiological processes that explains the relationship between stress and chronic diseases[56]. It is important to note that stress is the body's mechanism to maintain

homeostasis but chronic activation of the stress process can have serious deleterious effect on the body. Selye made a clarification that not all stressful conditions have deleterious effects on health, by describing 'eustress' as a good stress that could cause good feelings and help in human growth and development [57]. In the early 90s, Chrousos and Gold defined stress as a state of disharmony, or a threatened homeostasis [45]. Most recently, Bao and colleagues have defined stress to be the consequence of the failure of an organism to respond appropriately to emotional or physical threats, whether actual or perceived [57]. The term stress and psychosocial stress have been used interchangeably in the literature.

The stress system and response

The concept of stress is broad but overall, stress may be thought of as (1) a physiological response to an external stimuli, (2) a psychological response to an external stimuli or (3) an encounter of a negative or positive stressful life events [58]. The response to stress comes from the body's reaction to physical, biological and or socio-cultural stimuli that results in adaptive activity [59]. The symptoms of stress may manifest as cognitive, emotional, physical or behavioral responses [51, 57, 60, 61]. Some cognitive symptoms of stress include poor judgment, low self-esteem, and poor concentration, while some emotional signs include moodiness, feeling of anxiety, excessive worrying, irritability, and feeling of loneliness. Physical stress symptoms include aches and pains, diarrhea or constipation, nausea, dizziness, and chest pain while behavioral symptoms may include eating little or too much, sleeping little or too much, social withdrawal, procrastination or neglect of responsibilities [61-63].

Several factors influence individuals' response to a stressful situations, adaptation to stress, and the impact it might ultimately have on health [64, 65]. Factors that might impact stress

experience include culture, religion, race/ethnicity, the number of times experienced the stress, and the source of stress such as poverty, major life events, abuse or trauma [65-68].

Figure 1.1. A schematic representation of the physiological and behavioral response to stress. Reproduced with permission from N. Engl J. Med (1998) [65], Copyright Massachusetts Medical Society
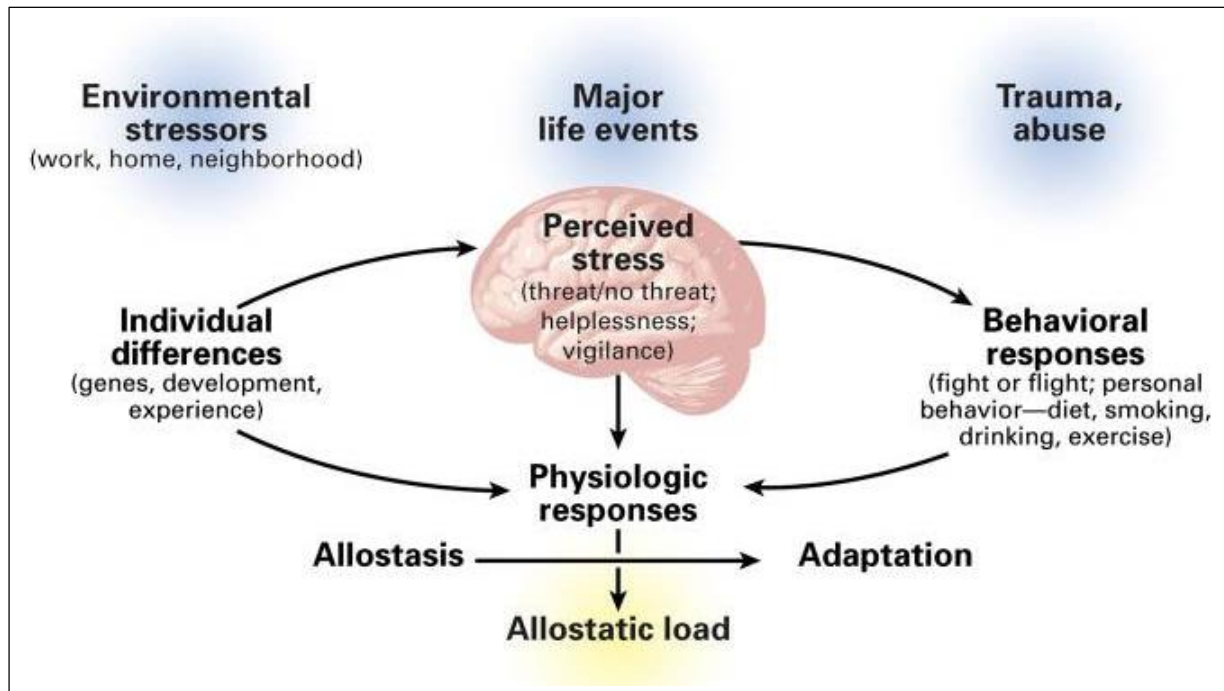
The stress response system is highly complex and involves both the central and peripheral nervous systems. Four components of the central part are involved: the parvocellular neurons which secretes corticotropin-releasing hormone (CRH); the neurons of the paraventricular nuclei (PVN) of the hypothalamus which secrete arginine vasopressin (AVP); the CRH neurons, which form the paragigantocellular and parabrachial nuclei of the medulla and the locus caeruleus (LC) and; other neural groups in the medulla and pons (LC/norepinephrine (NE)) which secret NE. Three components of the peripheral part are also involved: the neuroendocrine hypothalamic-pituitary-adrenal axis (HPA axis); the efferent systemic sympatheticadrenomedullary systems; and the part functioning under the control of the parasympathetic system [49]. Several changes

and interactions occur in the two systems during stressful situations, all of which are geared towards re-establishing a state of homeostasis. Within the central nervous system, the changes that occur activates and enables neural pathways to facilitate functions such as arousal, vigilance, cognition, focused attention, and appropriate aggression while concurrently inhibiting pathways that promotes vegetative functions. The changes to the peripheral system also occur to redirect energy to the central nervous system and the sites under stress [47, 69, 70]. Many sites of these two systems engage in lots of interactions through reciprocal neuronal connections as indicated by Figure 1.2 [49, 71].

Two major components mediates this general adaptation response as described by Selye; the corticotropin releasing hormone (CRH) and the locus ceruleus-norepinephrine (LC-NE)/autonomic nervous system [47, 49]. The paraventricular nucleus of the hypothalamus is mostly associated with the CRH component. The CRH action includes the activation of the HPA-axis and the sympathetic nervous system, thus, increasing the level of glucose, heart rate and blood pressure. The activation of the LC-NE system leads to the release of NE from neurons located throughout the brain to enhance arousal and vigilance, and to also increase anxiety. The sympathetic division of the autonomic nervous system also helps in adaption through its effectors - the sympathetic nerves and the adrenal medulla during response to stress. The parasympathetic division of the autonomic nervous system also produces negative effects to those of the sympathetic nervous system[47].

Figure 1. 2. A schematic summary of the central and peripheral components of the stress system [49].
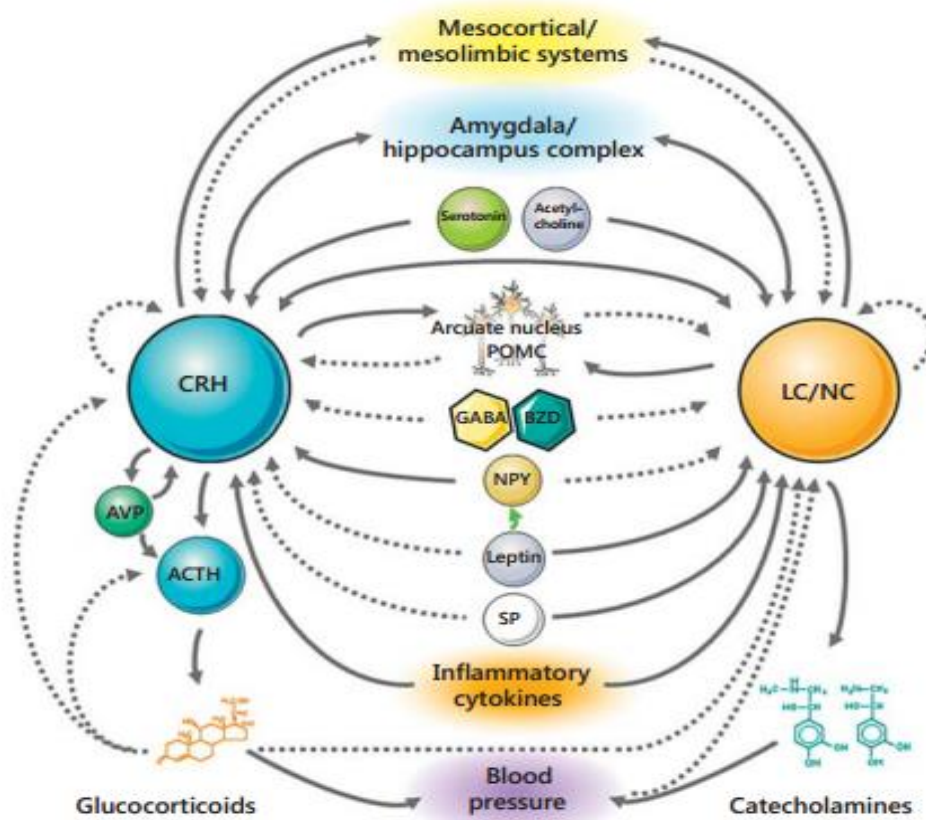
In encountering an acute stressor, the HPA-axis and the sympathetic nervous system (SNS) produce stress hormones. The PVN produce corticotropin releasing factor, which in turn stimulates the pituitary to produce adenocorticotropin. The adenocorticotropin also stimulates the adrenal cortex to secrete cortisol. In parallel, the SNS also stimulates the adrenal medulla to produce catecholamines [72]. The cortisol and the catecholamines together, increases the availability of energy by stimulating lipolysis and glycogen to glucose conversion [72]. The available energy is then distributed to essential organs such as the brain and the skeletal muscles by increasing blood pressure levels. In addition, the immune system gets activated and the cells

of the innate immune system enters the blood stream from the spleen and the lymphatic tissues, migrating to tissues likely to sustain injury during physical confrontation [73]. When a stressor become chronic, the stress system become continuously activated. The response to such chronic stressor can become maladaptive and may lead to a disease state [52].

Psychosocial Stress, DM management and complications – the link

Psychosocial stress impacts DM management and complications through two proposed mechanisms - physiological and behavioral.

The physiologic mechanism

The physiological part of the stress system that affects diabetes management and complications include the autonomic nervous system, the neuroendocrine system, and the immune systems [74]. The cortisol released during stressful situation has been found to antagonize the actions of insulin and fat deposition [58, 75]. Under normal conditions, cortisol production follows a circadian trend where its levels are highest in the morning but subsides as the day goes by. However, when exposures to stress become chronic, excess cortisol get released to maintain homeostasis. The implication is that the constant activation of the biochemical processes that follow the activation of the HPA axis directly affect insulin uptake and fat deposition and thus, impacts blood glucose level. Cortisol is also known to have an immunosuppressive effect and therefore plays a role in the regulation of immune and inflammatory processes [58, 76]. The catecholamines released during stressful situations are believed to promote hyperglycemia among individuals with DM by blocking insulin action and stimulating hepatic glucose production. Thus, stress-related neuroendocrine activity might create or sustain hyperglycemic

conditions [77]. It has also been suggested that stress leads to increased visceral adiposity, a factor that has consistently been associated with insulin resistance [78].

Behavioral mechanism

Psychosocial stress has been indirectly associated with DM control and complications through the difficulties in self-care, inadequate medication adherence, and risky lifestyle behaviors - all of which affect glycemic control and risk of vascular complications [79-83]. It is important to mention that the effect of stressful experiences on behavior to impact diabetes management and complications varies from person to person and it is impacted by several factors [84].

Literature on Psychosocial Stress, DM management and complications

Among DM patients, sustained hyperglycemia or fluctuations in blood glucose, an indication of poor glycemic control have been associated with the initiation, sustenance and progression of DM related complications [85-90].

Initial laboratory studies demonstrated that stressful situations such as unpleasant interviews or impending examinations destabilized blood glucose levels [91]. Later clinical studies corroborated the initial laboratory findings. For instance, Aikens (1992) observed that daily psychosocial stress was significantly associated with poor glycemic control and that the mechanisms of this association included both the direct effect on glucose levels and indirect effect on treatment adherence [77]. Peyrot and colleagues also found poor glycemic control among 105 adult DM patients who had higher levels of stress [92]. Peyrot et al noted that a good stress coping mechanism could offset the deleterious effect of stress on glycemic level [92]. Similarly, Suwit et al. observed that a good stress management program confers clinically significant benefits to individuals with DM through good glycemic control [22]. In assessing a variety of life events as well as long term difficulties, Lloyd found a significant association

between stress and poor glycemic control noting that participants whose glycosylated hemoglobin (HbA1c) level increased or remained high were significantly more likely to have experienced severe personal stressors in the previous three months [23]. A more recent study evaluating the psychosocial burden of Japan's Great East Earthquake found a significant independent association between stress and poor glycemic control among DM patients [93]. Although the majority of the studies noted a significant association between stress and glycemic control, the results have not been entirely consistent. Gois and colleagues, for instance, did not find a significant association between stress vulnerability and poor glycemic control [94]. Similarly, Trief and colleagues also did not find an association between work-related stress and glycemic control [95].

Among DM patients, good glycemic control could delay the onset and slow the progression of complications related to DM such as diabetes nephropathy (DN) [86, 96, 97]. However, some DM patients with poor glycemic control never develop DN while some with good glycemic control progresses to DN [85]. Such occurrence demonstrates that factors other than glycemic control may be important for renal decline and subsequent progression to DN among individuals with DM. Genes have been identified as one factor since there is a strong familial risk for DN; however, there has been a limited success in identifying specific genes that account for such predisposition among large DM population [98, 99]. Other risk factors found to influence the initiation, sustenance, and progression of DN include high blood pressure and smoking but they have not been able to entirely explained the variability in the onset and progression of DN among DM patients [100-102].

Psychonephrologist (social nephrologic scientists), have hypothesized that chronic stress may be may impact the development of CKD [103, 104]. Such reasoning is plausible, the fact that stress

has been found to increase in the engagement of some CKD related risk behaviors such as alcohol use and abuse, tobacco and drug use [81, 105, 106]. The relationship between psychosocial stress and renal decline among DM patients has not been adequately investigated.

The gaps in knowledge

First, as noted in the literature, finding from studies on the association between psychosocial stress and glycemic control remains inconclusive. This calls for more studies to be conducted to further examine this relationship. Secondly, limited studies have examined the relationship between glycemic control and psychosocial stress from a specific source, particularly, from the work environment. The majority of the studies to date have examined the relationship between general psychosocial stress and glycemic control. However, workplace is becoming increasingly relevant to health in the contemporary society where the majority of adults spend eight or more hours a day and five or more days a week. Therefore, understanding the relationship between psychosocial stress at the work environment and glycemic control is crucial for work place policies that might contribute to good DM management. To the best of our knowledge, the research on the association between psychosocial stress at the work environment and glycemic control is limited to the unique study by Trief and colleagues [95]. The study did not find an overall significant association between the factors. However, the study had some limitations; small sample size, cross-sectional study design and the DM patients in the study were all insulin requiring, an indication that the study participants, particularly those with type 2 DM had poor glycemic control and or have had the condition for a long time. Thirdly, although it has been hypothesized that psychosocial stress would be associated with DM complications such as DN, this hypothesis has not been adequately tested. Fourth, the measurement errors associated with the various stress sub-scales is considered a major reason for the limited research in this area.

The use of statistical models that will reduce the measurement errors such as latent variable analysis have not been adequately utilized. Finally, most of the studies published to date had small sample size. For instance all but one of the studies reviewed had a sample size of under 130 participants. This study will address some of these gaps identified in the literature.

Oxidative stress, oxidative balance score and hypertension

Oxidative stress (OS) has been defined as an imbalance between pro-oxidants and anti-oxidants in favor of the former [107]. This imbalance leads to damages in essential biomolecules such as proteins, lipids and DNA [108-110]. Pro-oxidants are factors that promote the generation of reactive oxygen species (ROS) while anti-oxidants counteracts the actions of ROS. Overproduction of ROS or impairment in their removal is the cause of the imbalance. ROS are produced endogenously either as a by-product of aerobic metabolism or oxidative phosphorylation [111-113]. Exogenously, ROS may be produced in response to some environmental exposure such as ionizing radiation, inflammation, alcohol and smoking [114-119]. To maintain homeostasis, living organisms use several strategies, both enzymatic and non-enzymatic, to neutralize the deleterious effect of ROS [120]. Enzymatic anti-oxidants include superoxide dismutase (SOD), catalases (CAT) and glutathione peroxide (GSH-Px) [121]. Non-enzymatic anti-oxidants include a variety of exogenous biological molecules such as gluthathione, vitamin C, vitamin E, flavonoids, polyphenols and carotenoids [122, 123]. An anti-oxidants system may work to either prevent the formation of ROS (primary anti-oxidants) or react with the ROS to neutralize or inhibit their actions (secondary anti-oxidants) [124]. OS have been implicated in several disease states in humans including vascular diseases [125-127]. Hypertension has been associated with higher levels of OS, although the debate still exists whether the increased levels of OS is a cause or a consequence of hypertension [128].

Figure 1.3. Major sources of anti-oxidants in the body and their consequence [121].

Endogenous sources
•mitochondrial leak
•respiratory burst
•enzyme reactions
•autooxidation reactions

Free radical production

O_2^-, H_2O_2

Transition metals Fe^{2+}, Cu^+

$OH^.$

Environmental sources
•cigarette smoke
•pollutants
•UV light
•ionising radiation
•xenobiotics

Lipid peroxidation   Modified DNA bases   Protein damage

Tissue damage
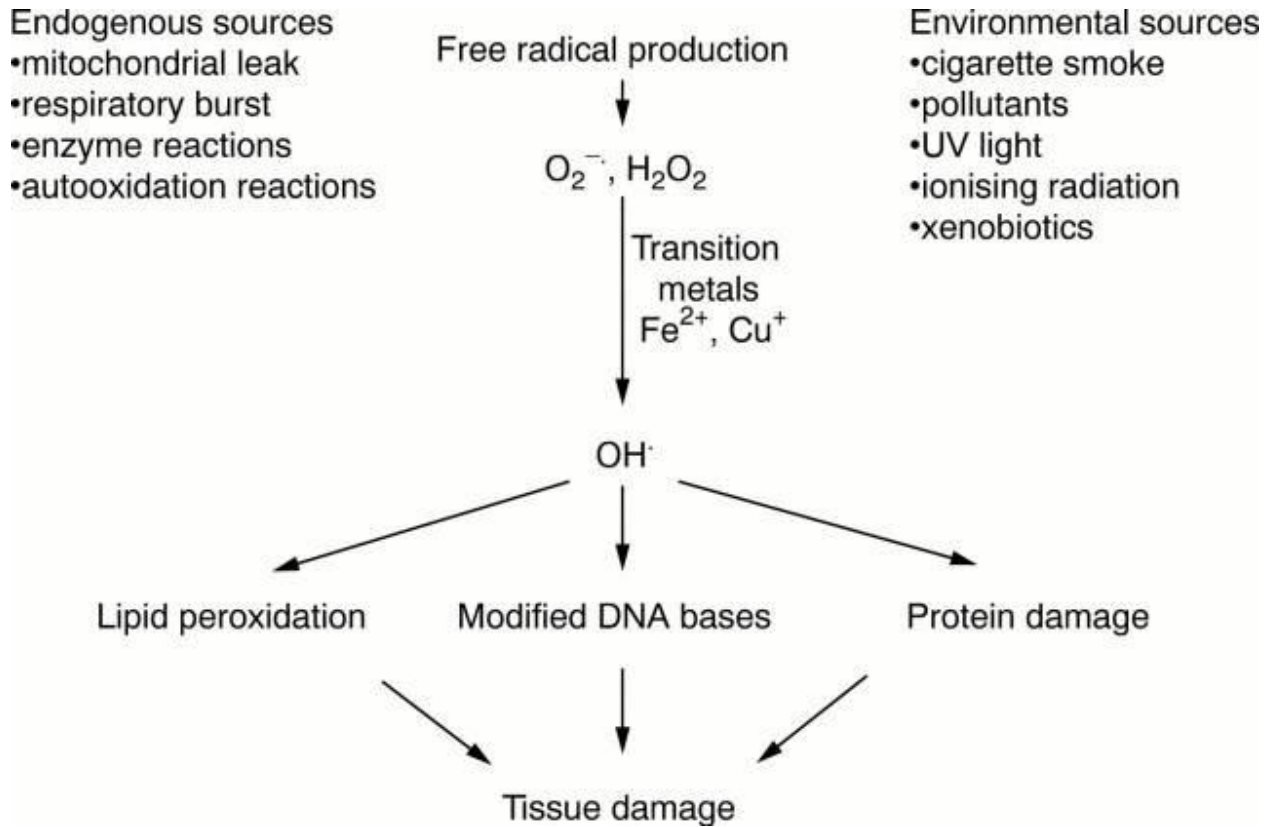
The majority of the evidence supporting the relationship between OS and hypertension are from basic science and animal studies [39-41]. In humans, however, the results have not been entirely consistent and the efficacy of anti-oxidants supplementation in reducing blood pressure have not proven much benefits in clinical trials [42, 43]. Few smaller studies, have however, observed limited benefits of certain anti-oxidants in reducing blood pressure [129, 130].

14

Oxidative Stress and Hypertension

ROS has been identified as a major player in blood pressure regulation through their activities in the homeostasis of the vascular wall [131, 132]. A variety of sources of ROS exists in the blood vessel including NOX and NO synthase [133]. ROS effect on vascular tone are mediated through redox-sensitivity regulation of multiple signaling pathways and second messengers [134]. OS relates to hypertension through an impaired endothelium-dependent vasodilation with inactivation of endothelium-derived nitric oxide by oxygen free radicals [128, 133].

The endothelial cells are important in the relaxation of arterial walls [135] through the release of NO by agents such as acetycholine in the vascular vessel to cause vascular relaxation [136, 137]. The biosynthesis and bioavailability of NO to perform this function is mostly dependent on the oxygen derived free radical superoxide anion which rapidly degrades them [138]. In addition, the nitric oxide synthase (NOS), specifically, the endothelial isoform (eNOS) which generates NO under normal cellular functioning also generate superoxide [139] in response to artheriogenic stimuli in a process termed "NOS uncoupling", which refers to the decrease in NO enzyme activity due to increase in NOS-dependent superioxide activity [140]. NOS uncoupling could also occur under conditions of depleted tetrahydrobiopterin (BH_4), a cofactor and L-arginine, a substrate to eNOS [140]. There is an evidence of the depletion of these cofactors during OS which results in eNOS uncoupling [37, 140]. Beside their role in degrading the NO, superoxide anions are also vasoconstrictors and may therefore modify the endothelial function [37].

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a major source of ROS in the vasculature [141, 142]. The activity of NADPH oxidase is upregulated by hormones such as Angiotensin II (AT-II), endothelin-1 (ET-1) and urotensin II (UT-II), thus, increasing the production of ROS [131]. These hormones are also capable of exerting an array of physiological

15

effects by mediating other processes that have the potential to alter arterial pressure as indicated

by Figure 1.4 [143]. Ang II also stimulates hypertension by decreasing NO biosynthesis through

the down-regulation of soluble guanylyl cyclase which impair NO/cyclic guanosine

monosphosphate signaling [137]. NADPH oxidase-driven generation of ROS leads to the

formation of peroxynitrate that causes eNOS uncoupling to produce more ROS. Peroxynitrate

also causes the inactivation of the NO [144]. NO also not only suppress the effect of AT-II, it

also down regulate the activity of angiotensin converting enzymes (ACE) and AT_1 [141].

Figure 1.4. Schematic summary of the role of vascular oxidative stress in pathogenesis of
hypertension [141]. Used with permission from Macmillan Publishers Ltd: Journal of
Hypertension Research.
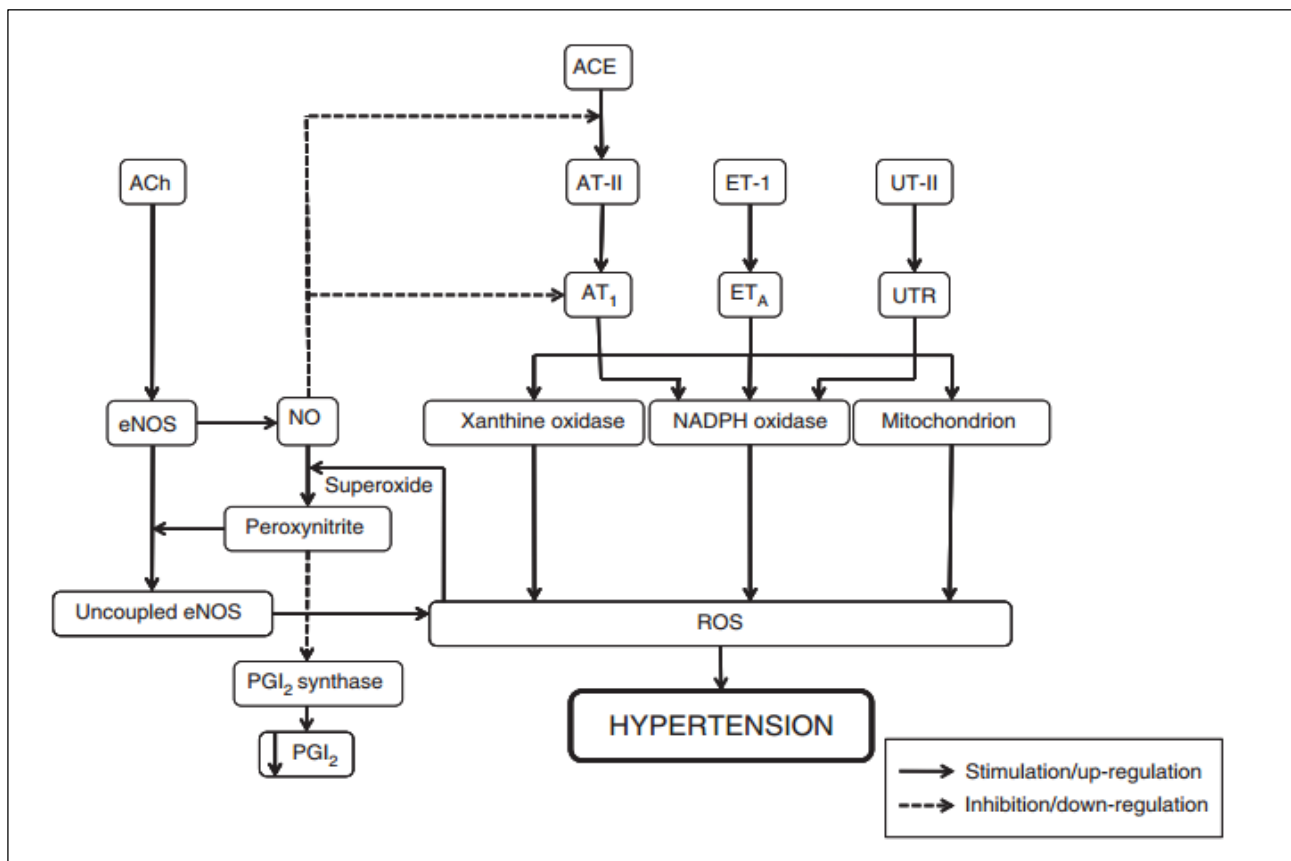
Anti-oxidants, oxidative balance score and hypertension

Anti-oxidants are compounds that are able to trap ROS and thus may be capable of reducing oxidative damages and possibly, blood pressure [138]. They scavenge on free radicals. They act as reducing agents (so they get oxidized) to terminate the actions of ROS by removing free radical intermediates, and inhibiting other oxidation reactions [37, 145]. Antioxidants include agents such as carotenoids, vitamin E, vitamin C, and selenium [146]. Dietary carotenoids are obtained from consuming green and yellow vegetables such as sweet potatoes, spinach and carrots. The major carotenoids include α-carotene, β-carotene, lycopene, leutin, β-cryptoxanthin and zeaxanthin [147].

The intake of diets rich in anti-oxidants have been found to reduce blood pressure in both normotensives and hypertensives and resulted in increased blood anti-oxidants capacity [148]. The Dietary Approaches to Stop Hypertension (DASH) study for instance, found diet rich in fruit and vegetable to reduce clinical and ambulatory blood pressure in hypertensive and normotensive subjects than the control diet [149]. A follow-up to the DASH study also noted that the modified diet increased serum anti-oxidants capacity and decreased malondialdehyde, a biomarker of OS [150]. A 6-months blood pressure intervention among hypertensives using fruits and vegetables (consumption of 5 servings of fruits and vegetables daily) found a reduction in both systolic and diastolic blood pressure, and noted an increase in plasma level α- and β-carotene, lutein, β-crytoxanthin, and vitamin C [151].

While dietary antioxidants seem to have beneficial effects on blood pressure, antioxidant supplementation have shown to be ineffective or even dangerous in clinical trials [152]. The reasons for this discrepancy include: 1) the trial designs; 2) patients cohorts; 3) type of anti-oxidants; 4) and the dosage of anti-oxidants [36]. One other possible reason is that in diets,

antioxidants are mixed and work as continuous chain while supplementation are usually one or two specific anti-oxidants and therefore, lacks this anti-oxidants chain. Also, if an antioxidant is not restored by the next in the chain after scavenging ROS, it begins to act as a pro-oxidant [153]. The evidence therefore suggests that biochemical interactions exists in dietary antioxidants which may be lacking in supplements due to the use of one or two individual antioxidants [154, 155].

To account for the complex relationship that may exist among various pro- and anti-oxidants and to overcome the limitations of analyzing independent OS exposures, some researchers have proposed combining known individual pro- and anti-oxidants available to a score, termed by van Hoydonck and colleagues as 'Oxidative Balance Score' (OBS) [156]. The OBS has been associated with the risk of cancer mortality [157-159]. Studies on the relationship between OBS and hypertension is sparse.

Measure of Oxidative Stress

OS cannot be directly observed *in vivo*, due to the short lifespan of reactive oxygen species. They can be assessed using biomarkers [160]. Numerous biomarkers of OS have been proposed. While some are non-specific, others measure specific biochemical aspect of the process [161, 162]. Four OS markers that are important to hypertension studies include F_2-isoprostanes (F_2-isoP), fluorescent oxidative products (FOP), copy number of mitochondrial DNA (MtDNA) and γ-tocopherol (γ-Toc).

F_2-isoP is a validated biomarker of OS and considered a gold standard OS marker [163]. They are prostaglandin-like substances that are produced in vivo, primarily by free radical-induced peroxidation of arachidonic acid, independently of cyclooxygenase enzymes [164]. The product

represents the level of lipid peroxidation in OS. High levels of F_2-isoP has been associated with cardiovascular disease [135].

FOP is considered a non-specific marker of OS that measures a mixture of analytes resulting from reactions of reactive oxygen species with lipids, proteins and or DNA [165]. It is comprised of fluorescent conjugated Schiff bases that are formed when malonaldehyde, a byproduct of lipid peroxidation, reacts with amino groups [165]. Its use in human studies is relatively new. A study found FOP to be an independent predictor of coronary heart disease in humans [166].

The use of MtDNA as an OS marker is also relatively new. The copy number of MtDNA has been found to alter in response to OS [167], increasing its copy numbers during OS [168, 169]. The MtDNA has a limited repair capability and compensate for damage by increasing its copy numbers [170]. Higher copy numbers of MtDNA has been associated with the risk of developing prostate cancer [171].

γ-Toc is an isomer of vitamin E with two methyl groups on the phenol ring[172]. It has been characterized as an antioxidant defense indicator whose level increases to reflect metabolic response to OS [173]. Plasma levels of γ-Toc has been inversely associated with cardiovascular disease [174].

OBS has also been used to represent the overall oxidative burden as initially proposed by van Hoydonck and colleagues [156]. It has been assessed by assigning a score to the known pro- and –anti-oxidant and summing the scores to determine the OBS. The elements that have been included in the scoring and the methodology used in assigning the scoring has differed from study to study [156, 159, 175].

The gaps in knowledge

Although findings from laboratory studies and basic biology supports the association between OS and hypertension, results from human studies have been entirely inconsistent. The results from clinical studies have been driven by the type of OS biomarker used and population being studied [35]. Few studies have examined multiple markers of OS and hypertension among the same study population. To the best our knowledge, participants included in studies that have examined the relationship between OS and hypertension tend to be homogeneous, usually from same race and or ethnicity. Finally, although some studies have demonstrated the utility of OBS as representing the overall oxidative burden and have found the score to be associated with some health outcomes, to the best of our knowledge, the association between OBS and hypertension has not been examined.

Research Plan

Objectives, Specific aims and study hypotheses

The primary objective of this dissertation is to examine the associations of stress (psychosocial and oxidative) with diabetes management, diabetes complication and hypertension. The association between OBS and hypertension will also be investigated.

Aim 1: Using data from Kaiser Permanente Georgia (KPGA) survey on Health and Healthy Behaviors that has been linked to patients clinical and pharmacy information (n=537), investigate the association between work-related psychosocial stress and glycemic control among working adults with DM. I hypothesized that adults with DM who experience more strain and less support at the work environment will have poorer glycemic control.

Aim 2: Using the same KPGA data as in study aim 1 above, examine the association between general psychosocial stress and changes in eGFR over time among DM patients. I hypothesized that higher general psychosocial stress will be associated with decline in eGFR.

Aim 3: Using data from the cross-sectional study on Race, Stress and Hypertension (SRSH), examine the association between oxidative stress (F_2-isoP, FOP, MtDNA and g-Toc), oxidative balance score (OBS) and hypertension. I hypothesized that oxidative stress will be positively associated with hypertension while OBS will be inversely associated with hypertension.

Methods for Aims 1 and 2

To address the first two study questions (study aims 1 and 2), data from the 2005 Kaiser Permanente Georgia (KPGA) Survey on Health and Healthy Behaviors will be utilized.

Data Source

Sample Selection and Survey Administration

Study participants were working adults who at the time of the data collection in 2005 met the following inclusion criteria: (1) age 25-59 years; (2) diagnosed with DM but without major micro or macrovascular complications; (3) employed by one of the 100 largest private or public employer groups offering KPGA as an insurance option; (4) enrolled in KPGA; and (5) subscribed within the enrolled family. Stratified randomized design was used to collect relatively well balanced samples of respondents by condition cohort and by primary care practice. In the initial data collection, three conditioned cohorts were identified for sampling: adults with diabetes, adults with elevated lipids but no history of advanced coronary artery disease (CAD), and adults without any identifiable major physical or mental morbidities (i.e. "low risk" adults). The current study used data on only the diabetes conditioned cohorts. Only individuals who reported their race as African American (black) or Caucasian (white) will be included in the

current study because other racial/ethnic groups represented a very small proportion of KPGA enrollees. There were a total of 625 participants in the diabetes conditioned cohort but study specific sample size will vary due to different exclusionary criteria that will be implemented. The sample size for study 1 will be 537 and that of study 2 will be 575. KPGA Institutional Review Board reviewed and approved the study protocol.

Study Variables

Psychosocial Stress – Work environment. Work-related stress was assessed by the 4 MIDUS subscales of work decision latitude (6 items), job demands (5 items), coworker support (2 items), and supervisor support (3 items). Each item was assessed using a 5-response Likert scale: "All of the time", "Most of the time", "Sometimes", "Rarely", "Never". Each subscale was scored from 0 (most strained, least supportive work climate) to 100 (least strained, most supportive work climate) by transforming each item response from 0-100 (and reverse coding where necessary). An overall work-related psychosocial stress score was computed as the mean of these 4 subscales. The Cronbach's alphas for work decision authority, job demands, coworker support, and supervisor support subscales were 0.88, 0.78, 0.73, and 0.89, respectively – similar to the MIDUS subscale alphas (0.68 for work decision authority, 0.74 for job demands, 0.74 for coworker support, and 0.87 for supervisor support) [176].

Psychosocial Stress- Social Settings. This was assessed by 2, 4-item subscales of friend/family support and friend/family strain, which was adapted from the MIDUS survey [176]. The MIDUS scales for family and friends were essentially identical except for the reference (e.g. "How much do members of your family really care about you?" and "How much do your friends really care about you?"); therefore, we chose to combine the references to create a measure of psychosocial stress in the social climate (e.g. "How much do your friends and family members

22

really care about you?"). Like the work-related psychosocial stress, the friends/family related psychosocial stress was scored from 0 (most strained, least supportive social climate) to 100 (least strained, most supportive).

Dietary Intake. Percent calories from fat, fruit and vegetable (F/V) servings per day, and daily fiber intake (grams per day) were derived from responses to the Block fat and F/V screeners [177].

Leisure Physical Activity. Leisure physical activity (LPA) was ascertained from responses to the Behavioral Risk Factor Surveillance Survey (BRFSS) physical activity items in the survey and measured using 2 dichotomous variables: physical activity at the recommended level and physical inactivity [178, 179]. Physical activity at the recommended level was defined as moderate physical activity (leisure activities of moderate intensity for a minimum of 30 minutes per day, 5 or more days per week) or vigorous physical activity (leisure activities of vigorous intensity for a minimum of 20 minutes per day, 3 or more days per week). Physical inactivity was considered to be <10 minutes per week of moderate or vigorous physical activity.

Physical and laboratory examinations. The physical and laboratory examination measures were extracted from KPGA's electronic medical record (EMR) data system. Height and weight, systolic blood pressure (SBP), and diastolic blood pressure (DBP) associated with primary care visits were collected. Height and weight were used to compute body mass index (BMI). SBP and DBP were used to compute mean arterial pressure (MAP). Other component measures including HbA1c, total cholesterol, LDL, HDL and serum creatinine were obtained from laboratory results.

For each respondent, component measures were summarized into an annual measure. If a respondent had more than one result for a component measure in a year, median of the results for the respondent in the year was retained for the component measure. Since most respondents had none, one, or two results on a component measure in a year, the mean and median were equivalent for most respondents.

Covariates. Respondent-level covariates included: age group, gender, race/ethnicity, and level of education. Age (25-34, 35-39, 40-44, 45-49, 50-54, 55-59), and gender were assessed from KPGA computerized data; race/ethnicity (African American, white, other/unknown) and level of formal education (high school education or less, some college, college graduate, post-graduate) were assessed from survey.

Calculated Variables

Estimate Glomerular Filtration Rate. The main dependent variable for the second study will be eGFR. Using the serum creatinine (SCr) measures, the annual eGFR will be estimated using the Modification of Diet in Renal Diseases (MDRD) equation [180].

$$\boldsymbol{eGFR} = 186 * \boldsymbol{SCr}^{-1.154} * \boldsymbol{Age}^{-0.203} * [1.210 \; if \; black] * [0.742 \; if \; female]$$

Laboratory factor. The following baseline measures were obtained from participants' laboratory records; low density lipoprotein (LDL), high density lipoprotein (HDL) and cholesterol. Using the lab measures and BMI values, we will calculate a *laboratory factor* using principal component analysis to reduce the number of parameters to be estimated in the model. The reciprocal of HDL will be taken to make the direction of all the factors consistent before

performing principal component analysis. We will retain the first factor only if it explains more than 50% of the variance among the variables, otherwise, will retain the second factor as well.

Dietary Intake and Physical Activity factor. We will derive the percent calories from fat, the number of fruit and vegetable (F/V) servings per day, and daily fiber intake (grams per day) from the responses to the Block F/V screener [177] from the 2005 survey. Using the Behavioral Risk Factor Surveillance Survey (BRFSS) physical activity items, we will categorize participants as meeting the physical activity as recommended by the CDC [178, 179]. Using a principal component analysis, we will create a *dietary and physical activity factor* using the dietary and physical activity variables to reduce the number of parameters to be estimated in the model. We will retain the first factor only if it explains more than 50% of the variance among the variables, otherwise, will retain the second factor as well.

Neighborhood-based Socio-economic status (SES) index. Individual level SES were generally not available so will not include in this study as a covariate, rather we will use the neighborhood-based SES, a validated scale comprising of 7 measures from the US Census at the census track level [181].

Use of insulin and oral hypoglycemic agents. A binary variable will be created to indicate insulin use versus insulin non-use. For individuals using oral hypoglycemic (OH) agents, we will estimate the proportion of days with OH coverage in 2005.

Data Analyses Plan

The study aim #1 will be addressed using linear regression model (PROC REG procedure) and individual growth model approach (PROC MIXED procedure) in SAS [182] to examine the relationship between work-related psychosocial stress and glycemic control. The linear

regression model will be used to assess the relationship between HbA1c and work-related psychosocial stress sub-scales and their two-way interactions at study baseline (2005). The linear growth model will be used to examine the relationship between work-related psychosocial stress in 2005 and HbA1c measures from 2005 to 2009 while centering time at 2005. Different covariance matrices will be explored to identify the matrix that best fit the data. Both the linear and the growth model will be fit in a hierarchical fashion: model #1 will not include any covariate; model #2 will adjust for socio-demographic variables (age, sex, race/ethnicity, neighborhood-based SES, marital status and education level); model #3 will adjust for the diet and physical activity factor; model #4 will adjust for the laboratory factor, MAP, insulin use and proportions of days covered by oral hypoglycemic agents in 2005. Statistical significance for all analyses will be determined at $p<0.05$.

The study aim #2 will be addressed using a confirmatory factor analysis (CFA) and a linear growth model in a structural equation model (SEM) framework. Latent psychosocial stress variable will be specified using CFA by loading the four work-related psychosocial stress indictors and the social environment stress indicator on the latent stress variable. Without an a priori hypothesis about the functional form of the relationship between stress and eGFR over time, in the final conditional growth model, stress will be specified with direct effects on the repeated measures to allow the greatest flexibility to obtain a time-varying effect estimates. In the final growth model, we will controll for the annual HbA1c measures as a time varying covariate while socio-demographic variables (sex, age, race, education, neighborhood-based SES), smoking, BMI, insulin use, medication coverage (proportion of days covered by oral hypoglycemic agents), and MAP will be controlled for as time invariant covariates. The robust

26

maximum likelihood estimator will be used. Statistical significance will be determined at a two

sided alpha level of 0.05.

Methods for Study Aim 3

Data Source

To address study aim #3, data from the Study on Stress, Race and Hypertension (SRSH) will be

used. SRSH was designed to assess dietary, lifestyle and psychosocial exposures, in relation to

blood pressure and presence of arterial hypertension in three groups of subjects: Caucasians,

African-Americans and West African immigrants. The study included individuals aged 25-74

years who self-identified as Non-Hispanic Whites (NHW), African Americans (AA) or West

African Immigrants (WAI) and who were residents of Georgia. NHW and AA subjects were

selected from among 800 participants in a previously completed feasibility phase of the Georgia

Cohort Study (GCS). The WAI subjects were recruited *de novo* using previously established ties

with Atlanta churches that included large proportions of WAI. The sample of GCS participants

was selected after the completion of the WAI recruitment and frequency matched to WAI

participants on age and sex. All study protocols were reviewed and approved by the Institutional

Review Boards of the Emory University and the Georgia State University.

Variables and Measures

Blood Samples

All participants provided blood samples that were drawn into five 10mL vacutainer tubes (2

sodium heparin tubes, 1 EDTA tube, and 2 red top tubes for serum collection) and immediately

plunged into ice and protected from direct light. Plasma, serum, and buffy coat specimens were

separated within 4-8 hours by centrifugation under refrigeration, aliquoted, frozen and stored at -80°C. The aliquots were then shipped overnight on dry ice for molecular analysis by the Molecular Epidemiology and Biomarker Research Laboratory (MEBRL) at the University of Minnesota, Minneapolis, MN.

Laboratory Analysis

Plasma lycopene, α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, α-tocopherol, and γ-tocopherol were measured by a high performance liquid chromatography (HPLC) assay using previously described methods [183-185]. Serum ferritin was measured by an antibody-based method using Roche 911 analyzer.

F_2-Isoprostanes (F_2-isoP). Gas chromatography-mass spectrometry (GCMS) [161], a gold standard for the measurement of F_2-isoP, was used to measure plasma free F_2-isoP. The F_2-isoP were extracted from the plasma sample with deuterium (4)-labeled 8-iso-prostaglandin F_2 alpha as an internal standard. Unlabeled, purified F_2-sioP was used as a calibration standard.

Fluorescent Oxidative Products (FOP). The measurement of FOP was performed using a modified Shimasaki method [186], which has been previously described elsewhere [187]. A mixed solution was centrifuged for 10mins at 3000rpm, 1mL of supernatant was added to cuvettes for spectrofluorometric readings, and a relative fluorescence intensity unit per milliliter of plasma was estimated using the spectrofluorometer [187]. Calibration was performed using standard quinine diluted in 0.1 NH_2SO_4.

The copy number of mitochondrial DNA (MtDNA). MtDNA was determined using real-time quantitative PCR described by Shen et al [188]. Two primers, one for MtDNA and the other for DNA were used in the two steps of relative quantification for MtDNA content: one for the amplification of the MT-ND1 gene in MtDNA, and another for the amplification of the single-

copy nuclear gene human globulin (HGB). The ratio of MtDNA and nuclear DNA was determined using serially diluted genomic sample DNA of a healthy referent [188].

Oxidative Balance Score (OBS)

The OBS will be estimated using *a priori* selected 13 pro- and anti-oxidants components according to our previous study [175] and those of others [189, 190] as listed in Table 1.1. The score will combine plasma micronutrient measurements and lifestyle behaviors. The plasma level of pro- and anti-oxidants, will be divided into sex and race/origin specific tertiles. The number of minutes of physical activity per week will also be divided into tertile. For anti-oxidants (α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, α–tocopherol) and physical activity, the first to third tertiles will be assigned scores of 0-2. For pro-oxidants (ferritin), the first to third tertile will be assigned a score of 2-0 respectively. To maintain scoring consistency, we will assign a scores of 0-2 to the other categorical OBS components. We will assign a score of 0-2 for obese (BMI $\geq 30 kg/m^2$), overweight (BMI=25-29.99kg/m^2) and normal weight (BMI <25kg/m^2) respectively. For smoking or alcohol use: never-smokers or never-drinkers will receive a score of two; former smokers and former drinkers or those with missing information a score of one; and current smokers and current drinkers a score of zero. For NSAIDs and aspirin, zero points will be assigned to participants reporting no regular use of these medications, one point to those reporting no usage or missing information, and two points to those reporting regular use. The points assigned to each component will be summed up to represent the overall OBS. OBS will then be categorized into three approximately equal intervals; 3-10, 11-17 and 18-25 to represent low, medium and high OBS, respectively. OBS will be used in a separate analysis as a continuous variable.

Table 1.1. OBS Assignment

Component	Score Assignment
Plasma Zeaxanthin	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma Cryptoxanthin	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma Lycopene	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma α-carotene	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma β-carotene	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma α-tocopherol	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Serum Ferritin	2=low (1st tertile), 1=medium (2nd tertile), 0=high (3rd tertile)
Physical Activity	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Alcohol use	0=current drinker, 1=former drinker/missing, 2=never drinker
Smoking	0=current smoker, 1=former smoker/missing, 2=never smoked
Aspirin use	0=no regular user, 1=unknown/missing, 2=regular user
NSAID use	0=no regular user, 1=unknown/missing, 2=regular user
Obesity	0=obese, 1=overweight, 2=normal weight

NSAID = Non-steroidal anti-inflammatory drugs. Normal weight=BMI<25kg/m^2, overweight=BMI between 25.0-29.9kg/m^2, Obese=BMI ≥30kg/m^2.

Blood Pressure and Hypertension

Trained and certified staff took all blood pressure measurements. After participants had rested about five minutes seated, three blood pressure measures were taken with at least a minute interval using mercury sphygmomanometry and *appropriately sized arm cuffs. The mean of the three blood pressure measures will be estimated and used in this study.* Systolic and diastolic blood pressure (SBP and DBP) measures will be expressed as a separate continuous variables. Individuals will be considered hypertensive if they meet any of the following conditions; (a) ever been told by a health care professional that s/he has hypertension, (b) on a blood pressure lowering medications, (c) had systolic blood pressure (SBP) equal or greater than 140mmHg (c) had diastolic blood pressure (DBP) equal or greater than 90mmHg.

Statistical Analysis

All analyses will be performed using SAS version 9.3 [182].The F_2-isoP, FOP, MtDNA and γ-Toc will each be dichotomized into a 'low' and 'high' using their respective sex and race/origin specific median as the cut-off. SBP and DBP will be modeled as continuous variables. Hypertension will be included as a dichotomized (hypertensive and normotensive). OBS will be used both as a continuous and a three level categorical variable.

The first series of statistical analyses will examine the association between SBP, DBP and each of the OS markers and OBS entering them as continuous variables in a linear regression. The second set of analyses, will examine the association of hypertension with OBS and with each biomarker of OS. The odds ratios (OR) for the continuous OS variables in the logistic equation will be scaled to their respective one standard deviation. Except for associations involving OBS, each linear and logistic regression analysis will adjust for race/origin, age, sex, education and BMI. Analyses involving OBS will not control for BMI because it was included in the scoring. To estimate the effect of missing data, a sensitivity analysis will be performed by imputing the missing values in two different fashions: 1) using five times multiple imputation method available in SAS and 2) by replacing missing values with sex and race specific mean. All measures of association will be accompanied by 95% confidence intervals (CI). Statistical significance will be determined at two sided p-value of <0.05.

Significant and impact of the study

The purpose of the current study will be to address several of the gaps identified in the literature with respect to psychosocial stress and DM. This study is important because the findings from

previous studies have been inconsistent, inundated with small sample sizes, the use of general measures of psychosocial stress and difficulty measuring the stress concept.

The innovative feature of the current study is the use of a unique study population, longitudinal data, and the use of latent analysis approach. To the best of my knowledge, this is the first study to investigate the association between psychosocial stress at the work environments and glycemic control among working adults with DM who had no major DM related complications. The sample size of the study sample is also adequate. Another strength of the study is the use of confirmatory factor analyses in the measurement and quantification of psychosocial stress which will explicitly account for differential measurement error related to the different stress sub-scales, thus, yielding a more accurate and precise assessment of the underlying psychosocial stress construct.

In relation to OS, OBS and hypertension, although, basic biology and some observational studies have found association between OS and hypertension, the use of anti-oxidants supplementation to reduce blood pressure have not yielded expected results. Part of the reasons for the inconsistencies between anti-oxidants supplementation and blood pressure reduction identified include: 1) the trial designs; 2) patients cohorts; 3) type of anti-oxidants; 4) and the dosage of anti-oxidants. To overcome the problem of using one OS exposure variable at a time, the use of OBS has been proposed but its use has been limited to cancer research. The current study will examine the association between OBS and hypertension using plasma levels of micro-nutrients which may accurately represent current intake and availability of pro- and anti-oxidants compared to food frequency questionnaire-derived measures. Another important methodological feature of the present study will be the use of a racially and ethnically diverse population. This will allow for assessing multiple biomarkers of OS and their relation to each other and to

hypertension in US born whites and blacks and in West African immigrants. One of the limitations of OS and hypertension research is that study findings have been driven by the population being study and the type of OS marker used. Therefore our use of racially ethnically diverse population while utilizing multiple markers of OS makes this study a unique.

The results of this study will help direct future epidemiologic studies that will examine the association between psychosocial stress and DM, particularly, by identifying the best statistical methods to use to combine psychosocial stress from multiple sources so as to control for measurement errors. Secondly, the results from the association between OS, OBS and hypertension will help re-direct the research in this area as few studies on the topic exixts.

CHAPTER 2. WORK-RELATED PSYCHOSOCIAL STRESS AND GLYCEMIC CONTROL AMONG WORKING ADULTS WITH DIABETES MELLITUS

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Abstract

Objective: To examine the association between work-related psychosocial stress and glycemic control among patients with diabetes mellitus at both study baseline and over time.

Materials and Methods: We used data from Kaiser Permanente Georgia (KPGA) 2005 survey on Health and Healthy Behaviors linked with patients' clinical, pharmacy and laboratory records for the period 2005-2009. Study participants (n=537) consisted of working adults aged 25-59 years, diagnosed with diabetes mellitus (DM) but without advanced micro or macrovascular complications at the time of the survey. Four work-related psychosocial stress sub-scales were used for the study. We estimated the baseline (2005) association between each work-related psychosocial stress sub-scales as well as their two way interactions and HbA1c in linear regression analyses. Using individual growth model approach, we estimated the association between each work-related psychosocial stress subscale and HbA1c over time. Each model controlled for socio-demographic variables, diet and physical activity factors, laboratory factors, physical examinations variables and medication use in a hierarchical fashion.

Results: After adjusting for all study covariates, we did not find a significant association between work-related psychosocial stress and glycemic control either at baseline or over time.

Conclusion: Among fairly healthy middle aged working adults with DM, psychosocial stress at the work environment was not directly associated with glycemic control.

Introduction

Diabetes mellitus (DM) is a major public health problem. It significantly increases the risk of micro-vascular complications such as retinopathy, nephropathy and neuropathy, and macro-vascular damages including myocardial infarction and stroke [86, 191, 192]. Currently, an estimated 8.3% American adults have overt DM while about 35% have pre-diabetes [12]. In the past three decades, DM prevalence has more than doubled and related complications have significantly increased [22-24]. Although a strong connection exists between genetic factors and DM etiology [14-16], recent increase in DM prevalence and its related complications have mostly been attributed to internal environmental factors such as stress [20, 21] and the external environmental factors such as diet and sedentary behaviors [17-19]. Long term complications from DM are primarily the results of chronic elevation and/or fluctuations of blood glucose level, which in turn damage blood vessels resulting in micro and macro-vascular complications [193, 194]. With increasing life expectancy but reduced age of DM onset in the US [13], the importance of good glycemic control to prevent and or delay the onset and progression of long term DM related complications cannot be overemphasized.

Proper DM management is demanding and involves adherence to multiple activities including diet, physical activity, medication use, and self-monitoring of blood glucose level [195]. Each of these activities is affected by multiple factors including: socio-demographic characteristics such as age, race, and socio-economic status [196, 197]; the presence of other chronic conditions such as obesity and hypertension [198]; and psychosocial stress [92, 199].

The relationship between general measures of psychosocial stress and glycemic control is well established [77, 200-203]. Laboratory studies have demonstrated that stressful situations such as unpleasant interviews or impending examinations destabilized blood glucose levels [91, 204]. Studies in real life settings, including meta-analysis corroborated the initial laboratory findings

[22, 23, 77, 92, 93, 199, 201]. Although the exact mechanism through which psychosocial stress may impact diabetes management is not very well understood, the underlying pathway has been hypothesized to involve physiological and/or behavioral mechanisms [77, 78, 81, 205]. Physiologically, psychosocial stress has been proposed to impact glycemic control through series of processes involving the hypothalamic-pituitary-adrenal axis (HPA) that leads to accumulation of visceral fat due to altered energy homeostasis and increased insulin resistance due to persistently higher levels of cortisol [78, 206]. The HPA is the major controller of hormones involved in the regulation of peripheral insulin sensitivity [207]. The behavioral aspect comes from increased engagement in risky lifestyle behaviors (such as smoking, excessive alcohol use), decreased capacity to make modifications to lifestyle behaviors (such as healthy eating and physical activity), medication adherence and difficulties in self-care among individuals with higher levels of psychosocial stress [82, 83, 205, 208].

Despite several studies investigating the relationship between general measures of psychosocial stress and glycemic control, limited studies have examined this association using psychosocial stress from a specific source, particularly at the work environment. Work-related psychosocial stress has been associated with general ill health [209, 210]. The job strain model has been used to explain the association. Individuals working in jobs that have high demand and low control are at greater risk of stress-related ill health and diseases [95]. The American Institute of Stress has noted that job stress is by far the major source of stress among American adults [211]. A report by the National Institute for Occupational Safety and Health (NIOSH) included a finding from a prior study that noted that stress at the work environment is strongly associated with health complaints than any other life stressors [212]. Spending eight or more hours a day and five or more days a week, several American adults spend more time at the work environment than they

do with family and friends. It is therefore important to understand stress at the work environment and how it relates to health, particularly, DM and its management. Research on stress at the work environment and glycemic control appears to be limited to the work by Trief and colleagues [95]. Trief et al study did not find a significant association between psychosocial stress at the workplace and glycemic control [95]. The current study was therefore designed to further examine the relationship between work-related psychosocial stress and glycemic control while addressing the limitations of the unique study; cross-sectional study design, small sample size, and inclusion of only insulin requiring DM patients. Given that 64.5% of American adults are in the work force [213], 8.3% diabetes prevalence [12], and the work environment has an impact on overall health, we conducted a study of both cross-sectional and longitudinal analyses of the association between work related psychosocial stress and glycemic control. The study had two objectives: (1) to examine the association between four sub-scales of work-related psychosocial stress as well as their two-way interactions and HbA1c at study baseline, and (2) to examine the association between four work-related psychosocial stress subscales and glycemic control over time; while adjusting for socio-demographic variables, diet and physical activity factor, laboratory and physical examinations variables and medication use in a hierarchical fashion.

Methods

Study population

We utilized data from Kaiser Permanente Georgia (KPGA) 2005 Survey on Health and Healthy Behaviors. Study participants consisted of working adults who at the time of the data collection in 2005 met the following inclusion criteria: (1) aged 25-59 years; (2) diagnosed with diabetes but without advanced micro or macrovascular complications; (3) employed by one of the 100

largest private or public employer groups offering KPGA as an insurance option; (4) enrollee of KPGA; and (5) subscriber within the enrolled family. Only individuals who reported their race as African American (black) or white were included in the current study due to the small sample size of other racial groups. KPGA IRB reviewed and approved the study protocol.

Data and Measures

The survey instrument included items and scales that had previously been used in other studies and which had demonstrated reliability and validity [176, 181]. Data obtained from the participants' survey was linked to their clinical information including pharmacy and laboratory records from 2005 through 2009.

The dependent Variable: The dependent variable was glycemic control assessed using HbA1c measures from participants' laboratory results from 2005 through to 2009. HbA1c measures within a calendar year were summarized into an annual measure and where a respondent had more than one result within a calendar year, the median was retained. Since most respondents had one or two results on HbA1c per year, the mean and median were equivalent for most respondents.

The main independent variable: The main independent variable was work-related psychosocial stress. This was assessed using 4 stress subscales from the Midlife in the United States (MIDUS) study [176]; work decision authority (6 items), job demands (5 items), coworker support (2 items), and supervisor support (3 items). Each item was assessed using a 5-response Likert scale: "All of the time", "Most of the time", "Sometimes", "Rarely", "Never". Each subscale was scored from 0 (most strained, least supportive work climate) to 100 (least strained, most supportive work climate) by transforming each item response from 0-100 (and reverse coding

where necessary). An overall work-related psychosocial stress score was computed as the mean of the 4 subscales. The Cronbach's alpha for the decision authority, job demands, coworker support and supervisor support subscales were 0.88, 0.78, 0.73, and 0.89 respectively.

Covariates:

Physical Examinations: Data on height, weight, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained from medical records associated with participants' primary care visits. Height and weight were used to compute body mass index (BMI). SBP and DBP were used to compute mean arterial pressure (MAP).

Laboratory factor: The following baseline measures were obtained from participants' laboratory records; low density lipoprotein (LDL), high density lipoprotein (HDL) and cholesterol. Using the lab measures and BMI values, we created a *laboratory factor* using principal component analysis to reduce the number of parameters to be estimated in the model. The reciprocal of HDL was taken to make the direction of all the factors consistent before performing principal component analysis. We retained the first factor which explained more than 50% the variance among the variables.

Dietary Intake and Physical Activity factor: Percent calories from fat, the number of fruit and vegetable (F/V) servings per day, and daily fiber intake (grams per day) were derived from responses to the Block F/V screener [177] from the 2005 survey. Using the Behavioral Risk Factor Surveillance Survey (BRFSS) physical activity items, we assessed physical activity at the recommended level [178, 179]. Recommended physical activity level was defined as moderate physical activity (leisure activities of moderate intensity for a minimum of 30 minutes per day, 5 or more days per week) or vigorous physical activity (leisure activities of vigorous intensity for a minimum of 20 minutes per day, 3 or more days per week). Using principal component analysis,

we created *dietary and physical activity factor* using the dietary and physical activity variables to reduce the number of parameters to be estimated in the model. A single factor that explained more than half the variance among the variables was retained and included in the model.

Neighborhood-based Socio-economic status (SES) index: Individual level SES were generally not available so we did not include in this study as a covariate, rather we used the neighborhood-based SES, a validated scale comprising of 7 measures from the US Census at the census track level [181].

Use of insulin and oral hypoglycemic agents: A variable was created for insulin use (1=use insulin, 0=not using insulin). For individuals using oral hypoglycemic (OH) agents, we estimated the proportion of days with OH coverage in 2005.

Other Socio-demographic measures: Participants age (in years) and sex (males, female) were assessed from the KPGA computerized data. Race/ethnicity (Caucasian (white), African American (black)), level of formal education (high school education or less, some college, college graduate, post-graduate), and marital status (married and not married) were assessed from the survey.

Statistical analysis

We addressed the first study objective using a linear regression model in SAS software version 9.3 [182] to assess the relationship between HbA1c and work-related psychosocial stress sub-scales and their two-way interactions at study baseline (2005). We fit four regression models for each of the work-related psychosocial stress subscales and their two way interactions in a hierarchical fashion: model 1 did not include any covariate; model 2 adjusted for socio-demographic variables (age, sex, race/ethnicity, neighborhood-based SES, marital status and education level); model 3 adjusted for the diet and physical activity factor; model 4 adjusted for

the laboratory factor, MAP, insulin use and proportions of days covered by oral hypoglycemic agents in 2005.

The second study objective was addressed using the individual growth model approach in SAS using the PROC MIXED procedure [182] to examine the relationship between work-related psychosocial stress and in 2005 and HbA1c from 2005 to 2009. Time from 2005-2009 was measured as 0-4 respectively in the model. We used the unstructured variance covariance matrix for the intercepts and the slopes in the mixed model. Like the first study objective, we fit four hierarchical models entering the variables in the same order as the linear regression models. Statistical significance for all analyses was determined at $p<0.05$. We hypothesized a significant association between HbA1c and the four sub-scales of work-related psychosocial stress as well as their two way interactions at baseline. We also hypothesized that higher work-related psychosocial stress at baseline would be associated with poor glycemic control over time.

Results

Characteristics of Study Population

Overall, 652 participants met the original study inclusion criteria. We excluded 115 (17.6%) individuals who had no measure for HbA1c in any year from 2005-2009, bringing the sample size to 537. Of this, 58% were females (Table 1). Age ranged between 27 and 59 years with mean age of 49.7 (SD= 6.9) years. About 55% were blacks, the vast majority (76%) had some college education or were college graduates and about 60% were married or with a partner. Approximately 23% of participants were on insulin while the remaining were using other types of diabetes management regimen including OH agents. Those on OH agents had average coverage of 75.3% of the days in 2005. At the baseline in 2005, the mean HbA1C of the

participants was 8.1% SD=1.8). HbA1c values were relatively constant between 2005 and 2009 with mean annual values ranging between 7.9 and 8.1% (Table 2). The mean score for work-related psychosocial stress subscales ranged between 47.1 (work demands) and 63.1 (supervisor support). The mean BMI, HDL, LDL, MAP and cholesterol for participants at baseline were 34.3 (SD=7.3), 48.0 (SD=13.0), 113.7 (SD=36.1), 115.0 (SD=13.4), and 188.7 (SD=41.1) respectively. At baseline, 65% of participants were meeting the CDC fruits and vegetable consumption recommendation (of five or more servings per day). Participants mean fiber consumption in grams per day at baseline was 20.8 (SD=5.2) while the percent calories obtained from fat averaged about 44.2% (SD=5.2) (Table 2).

Results from cross-sectional analysis

In the series of linear regression analyses for the cross-sectional data, none of the work-related psychosocial stress sub-scale or the overall score was significantly associated with the baseline HbA1c level after adjusting for socio-demographic variables and other covariates in the models. Despite the non-significant relationship between the individual sub-scales and HbA1c, we tested for the two way interaction between the subscales and their relationship with HbA1c. This approach was taken due to the job strain model which suggests interaction between job demand and decision control. We found the interaction between job demand and supervisor support to be marginally significant with HbA1c in the crude model (model 1) but significance disappeared after adjusting for the study covariates. None of the other interaction terms was significant either in the crude model or in the adjusted models (Table 3).

Results from Mixed Models

In the unconditional mixed model, we found the mean HbA1c value at baseline to be 8.0%. Although not statistically significant, there was a marginal decline in HbA1c value at an average of 0.022% per year during 2005-2009. In the set of the mixed models, we examined the relationship between the baseline measures of each of the four subscales of work-related psychosocial stress, the overall score and HbA1c over time. None of the subscales or the overall score was significantly related to glycemic control over time after adjusting for socio-demographic factors, diet and physical activity factor, laboratory factor, MAP, insulin use and proportions of days covered by oral hypoglycemic agents.

Although there was no significant association between work-related psychosocial stress and glycemic control, four of the covariates were significantly associated with HbA1c; race, insulin use, percent oral hypoglycemic coverage and laboratory factor. On average, blacks had significantly higher mean HbA1c at baseline than whites (0.7%, p=0.001), and insulin users had significantly higher HbA1c than non-insulin users (1.0%, p<0.001) at baseline. At baseline, increasing oral hypoglycemic coverage was significantly associated with lower HbA1c (-1.5%, p=0.001) and a unit increase in the laboratory factor was associated with 0.3% increase in HbA1c (p=0.004).

Discussion

In the current study of relatively large sample (N=537) of adults with DM, who were using different DM management regimen, we aimed to examine the relationship between HbA1c and work-related psychosocial stress sub-scales as well as the overall score at baseline in two different set of analyses - cross-sectional and longitudinal. The focus of the study was to examine whether or not stress at the work environment was significantly associated with glycemic control among working adults with DM. The results from the analyses did not support either of our

hypotheses. We did not find a significant association between any of the baseline measures of work-related psychosocial stress sub-scales or the overall score and glycemic control at baseline or over time among study participants. However, in an uncontrolled model, we found an interaction between job demand and supervisor support subscales to be significantly associated with HbA1c. The interaction term suggested that job demand was related to HbA1c conditioned on the level of supervisor support. This finding warrants further research.

The finding from the current study is consistent with that of Trief and colleagues[95] in specific and other studies that have examined the association between work-related psychosocial stress and DM in general [214-216]. While Trief's study included only insulin requiring DM patients, the current study included patients that were using different diabetes management approach including OH agents, yet both studies arrived at similar conclusions.

General measures of psychosocial stress have mostly been associated with DM onset and management [93, 217]. The results have, however, been unclear for the association between work-related psychosocial stress and diabetes (incidence or control). It appears stress at the work environment has very limited relationship with DM in general. For instance, a large French study did not find work-related psychosocial stress (psychosocial demands, decision latitude and social support) to be associated with DM incidence [215]. Similarly, a Canadian study did not find a significant association between any of the sub-scales of psychosocial stress at the work environment and incident DM among men, although in women, low levels of job control was associated with increased risk of DM incidence [214]. A meta-analysis of nine studies concluded 'the evidence does not support the hypothesis that work-related psychosocial stress increases the risk for DM' [216].

Although our study findings are consistent with the other prior study, our study population may be different from the general DM patients and may partly explain the recent findings. First our study population was young, aged 25-59 years without advanced micro or macrovascular complications at the time of the survey. Secondly, participants were insured within KPGA system, an integrated delivery system of well-established DM management program [218]. Access and coverage for DM management may be better in the study population than the general diabetes population, thus, work-related psychosocial stress as an isolated factor might not significantly impact glycemic control among this group. For instance, less than a quarter of the study participants were on insulin and those on OH agents had an average coverage of 75.3% of the days in 2005. The percent medication coverage may even be higher considering the fact that some individuals may have filled their prescriptions outside the KPGA system and may not have been captured by the study as filling their prescription. Although not statistically significant, we observed HbA1c decline among study population during the period under consideration, an indication this population may be different than the general DM population.

The limitations of the current study need to be noted. First, we did not have information on two important covariates; occupation information and length of time since participants have had DM. Although stress is highly personalized and its perception can vary depending on personality type, interpretation of life events and cultural context [84], the nature of some occupations may be more stressful than others. Similarly, length of time since DM onset has been directly related to glycemic control [219] so we recognize the need to have included these two variables but they were not available. Secondly, participants were enrollees of KPGA and results may not be generalizable to uninsured patients, those in other health insurance system or patients in other geographic locations.

Conclusion

The current study did not find a direct significant association between HbA1c and any of the work-related psychosocial stress sub-scale or the overall score after adjusting for study covariates. The study finding is consistent with a prior study by Trief and colleagues. Since only two studies have been conducted in this area, although, each with a unique study population, more studies need to focus on this topic.

Tables and figures

Table 2.1 Demographic Characteristics of Study Population

Demographic Variable	Percent (n)
Age (Years) - 2005	49.7(7.0)*
Sex	
Male	42.5% (228)
Female	57.5% (309)
Race	
White	44.9% (241)
Black	55.1% (296)
Education	
Less than HS	4.8 % (26)
HS Grad	19.2% (103)
Some College	36.3% (195)
College Grad	39.7% (213)
Marital Status	
Married	59.4% (319)
Not Married	40.6% (218)
Area based SES Quartiles	
1st Quartile	31.3% (169)
2nd Quartile	25.4% (136)
3rd Quartile	23.6% (126)
4th Quartile	19.4% (104)

*Mean and standard deviation

Table 2.2: Distribution of Study Variables among Study Participants

Variable	Mean (SD)
Work-Related Stress (2005)	
Overall Score	57.5 (14.8)
Decision latitude	59.2 (24.3)
Work demands	47.4 (18.4)
Coworker Support	60.3 (22.2)
Supervisor Support	63.2 (24.3)
HbA1c Measures	
Year 2005	8.1 (1.8)
Year 2006	8.0 (1.9)
Year 2007	7.9 (1.7)
Year 2008	8.0 (1.7)
Year 2009	7.9 (1.5)
BMI (2005)	34.3 (7.4)
HDL (2005)	48.0 (13.0)
LDL (2005)	113.7 (36.1)
MAP(2005)	115.0 (13,4)
Cholesterol (2005)	188.7 (41.1)
Fiber Consumption per day(grams)	20.8 (5.2)
Percent Calories obtained from fat	44.2 (5.2)
Proportion of days covered by oral agents in 2005	0.75 (0.26)
Physical activity recommendation	35%
Fruit/vegetable consumption recommendation	65%
Percent on insulin	23%

Table 2.3: Covariates with Significant Association with HbA1c

Variable	Estimate	P-Value
Race		
White (0), Black (1)	0.7	0.001
Insulin Use		
No (0), Yes (1)	1.0	<0.001
Laboratory Measures		
Continuous	0.3	0.004
Oral Hypoglycemic Coverage		
Continuous	-1.5	0.001

CHAPTER 3. PSYCHOSOCIAL STRESS AND CHANGES IN ESTIMATED GLOMERULAR FILTRATION RATE AMONG ADULTS WITH DIABETES MELLITUS

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Abstract

Background: Psychosocial stress has been hypothesized to impact renal changes but this hypothesis has not been adequately tested. The aim of this study was therefore to examine the relationship between psychosocial stress and estimated glomerular filtration rate (eGFR) and to examine other predictors of eGFR changes among persons with diabetes mellitus (DM).

Materials and Methods: We used data from Kaiser Permanente Georgia (KPGA) 2005 survey on Health and Healthy Behaviors linked to patients clinical and pharmacy records (n=575) from 2005-2008. Study participants were working adults aged 25-59 years, diagnosed with DM but without advanced micro or macrovascular complications at the time of the survey. eGFR was estimated using the Modification of Diet in Renal Disease equation. A latent psychosocial stress variable was created from four work-related psychosocial stress subscales and a social stress subscale. Using a growth factor approach in structural equation model framework, we estimated the association between psychosocial stress and eGFR. In the final model, we controlled for socio-demographic variables, HbA1c, smoking, BMI, insulin use, and diabetes medication coverage.

Results: The psychosocial stress variable was not directly associated with eGFR after adjusting for study covariates. Factors found to be associated with eGFR were age, race, insulin use and mean arterial pressure. The model indices suggested adequate model fit.

Conclusion: Among DM patients with no major micro- or macro-vascular complications, we did not find an evidence of a direct association between psychosocial stress and eGFR after controlling for covariates. Predictors of eGFR changes included age, race, insulin use, and mean arterial pressure.

Background

Reduced renal function, which may progress to diabetes nephropathy (DN), is a major cause of mortality among diabetes mellitus (DM) patients [220, 221]. It is estimated that mortality rate among type 1 DM patients without kidney disease approaches individuals free of the condition [222]. Between a quarter and a third of individuals with DM may develop DN, usually as a result of decline in renal function [85, 223, 224]. It is therefore crucial to understand the predictors of renal decline in order to minimize their occurrence and ultimately, reduce chronic kidney diseases (CKD) among individuals with DM.

Among DM patients, tight glycemic control decreases the risk of renal decline and slows the progression of DN [86, 193]. However, some DM patients with poor glycemic control never develop DN while some with good glycemic control progresses to DN [85]. Such occurrence demonstrates that factors other than glycemic control may be important for renal decline and subsequent progression to DN. One obvious candidate has been genetic factors since there is a strong familial risk for DN; however, there has been a limited success in identifying specific genes that account for such predisposition among large DM population [98, 99]. Other traditional risk factors identified to influence the initiation, sustenance, and progression of DN include high blood pressure and smoking [100-102]. Hypertension, for instance, is estimated to be present in about 80% of patients with kidney diseases [225]. However, the variability in the onset and progression of DN have not been fully explained as a function of the group differences in these traditional risk factors alone – glycemic control, high blood pressure, and smoking [85]. In search for other factors to explain this variability, some non-traditional risk factors have been proposed to influence the renal decline in the general population, including psychosocial stress, oxidative stress, advanced glycation end products and activation of protein kinase C [226-228].

The relationship between psychosocial stress and renal decline among DM patients has not been adequately investigated; thus, the reason for the current study.

Psychosocial stress, defined as demanding conditions that exceed the behavioral resources of an individual [229] has been suspected as a potential factor in renal decline due to its established relationship with glycemic control, hypertension, and smoking [200, 230-232]. Another proposed link between psychosocial stress and renal decline is through the increased engagement in behaviors that may increase the risk of renal damage such as alcohol abuse, smoking and drug abuse [81, 105, 106, 226]. Although higher levels of psychosocial stress has been associated with overall poor health, high blood pressure, poor glycemic control, and smoking [22, 230, 233-236], the direct association between psychosocial stress and decline in renal function has not been adequately examined. Part of the reason for the limited research in this area is the difficulty in operationalizing the concept of stress. Psychosocial stress is broad and may originate from multiple sources such as constant exposure to socio-economically challenging environments, social relationships, and work environment [237-239], presenting challenges to comprehensively assess psychosocial stress and appropriately combining the stress subscales from multiple sources.

The primary aim of the current study is therefore to examine the direct relationship between psychosocial stress and renal function over time among individuals with DM. Although factors including glycemic control, blood pressure, smoking and other socio-demographic factors have been associated with renal decline, the course of eGFR among DM patients can be complex and heterogeneous, and may be affected by multiple factors including existing comorbid conditions [240]. For instance, albuminuria was identified as the strongest predictor of eGFR decline among Caucasians with DM [241], while among Japanese with DM, higher glycemic levels was the

strongest predictor of eGFR decline [242]. In the light of the variability in eGFR trajectory among different study population, our secondary study aim was to explore other documented predictors of renal decline among this unique study population (White and Black working adults with DM but without a major micro- or macro-vascular complications). In this study, we use multiple indicators of psychosocial stress including stress from the work environment, family, and friends to operationalize psychosocial stress using a confirmatory factor analysis (CFA). The use of CFA in the measurement and quantification of psychosocial stress is preferred over the use of an aggregate score because this approach explicitly accounts for differential measurement error related to the different stress sub-scales yielding a more accurate and precise assessment of the underlying constructs [243, 244].

Methods

Study population

We utilized data from the 2005 Kaiser Permanente Georgia (KPGA) Survey on Health and Healthy Behaviors. Study participants were working adults who at the time of the data collection in 2005 met the following inclusion criteria: (1) age 25-59 years; (2) diagnosed with DM but without major micro- or macro-vascular complications; (3) employed by one of the 100 largest private or public employer groups offering KPGA as an insurance option; (4) enrolled in KPGA; and (5) subscribed within the enrolled family. Only individuals who reported their race as African American (Black) or Caucasian (White) were included in the current study because other racial/ethnic groups represented a very small proportion of KPGA enrollees. KPGA Institutional Review Board reviewed and approved the study protocol.

Data and measures

The survey instrument included items and scales that had previously been used in other studies and which had demonstrated reliability and validity [176, 181]. Data obtained from the participants' survey were linked to their clinical information and pharmacy records from 2005 to 2008.

Dependent variable: The main dependent variable was eGFR. Using the serum creatinine (SCr) measures, the annual eGFR was estimated using the Modification of Diet in Renal Diseases (MDRD) equation [180].

$$eGFR = 186 * SCr^{-1.154} * Age^{-0.203} * [1.210 \; if \; Black] * [0.742 \; if \; female]$$

Main independent variable: The main independent variable was psychosocial stress, assessed from two major sources; social settings (family and friends) and the work environment. Social stress was assessed by two 4-item subscales: one reflecting friend/family support and the other measuring friend/family strain. These subscales were adapted from the Midlife in the United States (MIDUS) study [176]. The MIDUS scales for family and friends are identical except for the reference (e.g., "How much do members of your family really care about you?" and "How much do your friends really care about you?"); therefore, we combined the references to create a single measure of social climate (e.g. "How much do your friends and family members really care about you?"). Each subscale was averaged and scaled from 0 (most strained, least supportive) to 100 (least strained, most supportive). The work-related psychosocial stress was assessed using the following 4 subscales from the MIDUS [176] study: work decision authority (6 items), job demands (5 items), coworker support (2 items), and supervisor support (3 items).

Each item was assessed using a 5-response Likert scale: "All of the time", "Most of the time", "Sometimes", "Rarely", "Never". Each subscale was averaged and scaled from 0 (most strained, least supportive) to 100 (least strained, most supportive) by transforming each item response from 0-100 (and reverse coding where necessary).

Health-related covariates: Glycemic control was assessed using HbA1c measures from participants' laboratory results from 2005 through 2008. Data on height, weight, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were obtained from medical records associated with participants' primary care visits. Height and weight were used to compute body mass index (BMI). SBP and DBP were used to compute mean arterial pressure (MAP). A binary variable was created to indicate insulin use versus insulin non-use. For individuals using oral hypoglycemic (OH) agents, we estimated and included the proportion of days in 2005 with OH coverage.

*Other socio-demographic measures***:** Participants age (ranging between 25 and 59 years), and sex (male=0, female=1) were assessed from the KPGA computerized data. Race/ethnicity (Black=0, White=1), level of formal education (high school education or less=0, some college=1, college graduate=2, post-graduate=3), and marital status (married=0 and not married=1) were assessed from the survey. Individual level income information was generally not available and was not included in this study as a covariate. Instead, we used the neighborhood-based socioeconomic status (SES), a validated census track-level scale comprised of 7 measures from the US Census as described by Roblin [181].

Statistical analyses

Descriptive statistics was performed in SAS software version 9.3 [182]. The percent missing on covariates ranged between 0 and 41% while the percent pairwise coverage for the covariates ranged between 0.39 and 1.00. The percent missing for the stress indicators ranged between 0.5% and 1.6% with covariance coverage ranging between 0.98 and 1.00. For eGFR measures, 49% had a measure on all four waves while 91% had at least a measure on two waves. To address the missingness on exogenous predictors, we performed multiple imputations (10 times) in SAS. We used this imputed data for both the measurement and the growth models. Other than the descriptive statistics, all analyses were performed in Mplus statistical software version 6.1 [245]. Latent psychosocial stress variable was specified using confirmatory factor analysis (CFA) by loading the four work-related psychosocial stress indictors and the social environment stress indicator on the latent stress variable (Fig 1). An unconditional (no covariate) growth model was fit to the four eGFR waves. Without an a priori hypothesis about the functional form of the relationship between stress and eGFR over time, in the final conditional growth model, stress was specified with direct effects on the repeated measures to allow the greatest flexibility to obtain a time-varying effect estimates. We controlled for the annual HbA1c measures as a time varying covariate while socio-demographic variables (sex, age, race, education, neighborhood-based SES), smoking, BMI, insulin use, medication coverage (proportion of days covered by oral hypoglycemic agents), and MAP were controlled for as time invariant covariates. The robust maximum likelihood estimator was used. Statistical significance was determined at a two sided alpha level of 0.05.

Results

Descriptive statistics

The study included 575 participants with the mean age of 49.6 years (6.9 years). As shown on

Table 1, slightly higher proportions of females and blacks made up the study sample. Individuals

included in the study were highly educated and the majority were married. The prevalence of

current smoking (16%) in the study sample was lower than the state smoking prevalence of 22%

during 2005 [246]. The baseline mean eGFR was 83.2ml/min/1.73m^2 (SD=21.3) while the mean

psychosocial stress for the subscales ranged between 47.1 and 66.0.

The measurement and the growth models:

Measurement Model

Using supervisor support to scale the factors, the unstandardized factor loadings ranged between

0.106 and 0.787 (Table 3). The mean fit indices for the CFA model were: χ^2 p-value <0.001,

root-mean-square error of approximation (RMSEA) = 0.072 (90% CI=0.041-0.107), comparative

fit index (CFI) = 0.951, Tucker Lewis index (TLI) =0.902 and Standardized Root Mean Square

Residual (SRMR) =0.037. The mean factor score determinancy coefficient was 0.873 with

values ranging between 0.869 and 0.878. The mean standardized residual variances of the stress

sub-scales was each significant. Values ranged between 0.35 (Supervisor support) and 0.99

(work demand) (Table 3).

Structural Model

The baseline model estimated an intercept parameter with time centered at 2005 (baseline) and a

slope parameter that represented the annual mean rate of eGFR change during the four year study

period. The model fit was adequate: χ^2 p-value >0.001, RMSEA = 0.058 (90% CI=0.061-0.094), CFI = 0.94, TLI = 0.938, TLI=0.926 and SRMR=0.037. Significant variance existed in the intercept (σ^2 = 360.77, p<0.001) and the slope (σ^2 = 10.49, p<0.016) parameters. The mean intercept was 82.62 while the mean slope was 0.88 (p = 0.003) which was significantly different from zero. Table 3 contains both the unstandardized and the standardized estimates of the CFA model.

The fit for the final conditional model to estimate the direct association between psychosocial stress and eGFR was also adequate: χ^2 p-value >0.001, RMSEA = 0.048 (90% CI=0.041-0.055), CFI=0.916, TLI = 0.893 and SRMR=0.037. Psychosocial stress was not directly associated with any of the four measures of the eGFR. At the study baseline, age, race, MAP and insulin use were significantly associated with eGFR. Over time, MAP was associated with eGFR decline. Table 4 contains the estimates of the final growth model.

Discussion

Changes in renal function have been associated with increased risk of mortality [247, 248]. Adverse clinical outcomes such as cardiovascular events have also been associated with decline or rapid improvements in eGFR [249] [248]. Great variability exists in eGFR changes and may reflect in the variation in the onset and progression of DN [96]. Factors such as obesity, hypertension and dyslipidemia are associated with changes in renal function and psychosocial stress [250]. In the current study, we examined the direct association between changes in eGFR and psychosocial stress. We also examined other documented predictors of eGFR decline in this unique study population - white and black working adults with DM but without major micro- or macro-vascular complications.

Our primary hypothesis that psychosocial stress would be associated with eGFR was not supported in the final growth model. We did not observe an evidence of a direct association between psychosocial stress and eGFR. This null finding is consistent with the findings from the unique study by Tsurugano and colleagues, who did not find a direct association between job stress and CKD (eGFR <60mL/min/1.73 m^2) [250]. A number of reasons may partly explain the null finding in the current study. First, psychosocial stress is a broad concept, spanning multiple facets of life including major life events, financial circumstances, perceived discrimination, social circumstances and the work-environment [237-239]. The current study included stress from two main sources – the work environment and social settings in creating the latent psychosocial stress factor. It is therefore possible that the current measure had underestimated the level of psychosocial stress in this population. The measure might not have been comprehensive enough to assess all stressful situations in individuals that might predispose them to a decline in eGFR. Secondly, although our study participants were DM patients, they were relatively young (mean age of 49.9 years, SD=6.9) and healthy without a major micro or macrovascular complications at the time of the study in 2005 so changes in renal function may be slow. The rate of renal decline increases with age but the decline has been noted to accelerate after the age 50-60 years [251, 252]. Less than half of the study population was between the ages 50-60 years old. Thirdly, a major predictor of renal decline among DM patients is poor glycemic control. Goel and Perkins have demonstrated that higher HbA1c increases eGFR loss, and observed the greatest decline among individuals with albuminuria [253]. The Diabetes Control and Complications Trial (DCCT), the Epidemiology of Diabetes Intervention and Complications (EDIC) study and a number of other studies have also made similar findings of the relationship between HbA1c and eGFR [86, 254-256]. During the four year study period, the mean HbA1c

remained virtually constant with values ranging between 7.9% and 8.1%. A marginal but significant improvement in eGFR was also observed among the study population. Although unexpected, particularly, among our population of DM patients, kidney function can be highly variable and may improve over time [209, 257]. Finally, the study participants were in an integrated delivery system of well-established DM management program and might have received special care to prevent or slow down eGFR decline [218].

Although, no direct association was observed between psychosocial stress and changes in eGFR, some of the study covariates were significantly associated with eGFR in the expected direction. These significant associations were important for two reasons; 1) validation of the variables in the data and 2) identification of factors that are important to changes in eGFR among this unique study population which could provide information that could guide prevention efforts, particularly, for factors that could be modified. At baseline, race, age, insulin use and MAP was each significantly related to eGFR. Blacks had lower eGFR values compared to their white counterparts. Racial differences in renal decline have been reported, with blacks experiencing the greatest disparity compared to whites [258, 259]. Increasing age has been associated with eGFR decline among adults with DM as has been adequately captured in the introductory part of this paper. The relationship between insulin use and renal decline is consistent with the literature as well [240]. Insulin use may be related to having had DM for a long time, and or poor glycemic control, particularly, among type 2 DM patients. Both factors have been associated with decline in renal function among DM patients [193, 260-262]. Increasing MAP was found to be associated with eGFR at both study baseline and over time, a finding that is consistent with several studies that have found high blood pressure to be closely associated with renal decline among DM patients [263-265]. Some interventional studies have also demonstrated that

antihypertensive treatment among DM patients may reduce the incidence or slow the progression of renal decline [266, 267]. As shown by Figure 1, the effect of MAP on eGFR trajectory over the four year period indicated that not only was people with higher MAP value started with lower eGFR value but also their rate of eGFR decline was faster.

The strengths of the study need to be noted. First, to the best of our knowledge, this is the first study to examine the direct association between psychosocial stress as a latent factor and renal function over time among individuals with DM. The use of latent psychosocial stress factor reduced the measurement errors associated with the items used to create the latent factor [243, 244]. Secondly, the study controlled for several covariates that could have an impact on renal function among individuals with DM including HbA1c level, blood pressure, smoking, medication coverage and demographic variables. Despite the study strengths, the following points need to be considered as study limitations – 1) our measure of psychosocial stress may be limited by the inclusion of fewer major sources; work and social environments; and 2) participants were enrollees of KPGA and results may not be generalizable to uninsured patients, those in other health insurance system or patients in other geographic locations.

Conclusion

In a study of fairly healthy adult DM patients, we did not find a direct association between psychosocial stress and eGFR. However, predictors of changes in eGFR among our study population were age, race, insulin use and blood pressure. Interventions to address renal decline among DM patients should address high blood pressure. Considering the inclusion of limited sources of psychosocial stress in creating the stress factor, future studies that would use a comprehensive measure of psychosocial stress are needed.

Competing interests

Authors have no conflicts of interest.

Acknowledgements

Tables and figures

Table 3.1 – Selected characteristics of study sample

Demographic Variable (n=575)	Percent (n)
Age (Years)- 2005	49.6(6.9)*
Sex	
Male	40.7% (234)
Female	59.3% (341)
Race	
White	45.9% (264)
Black	54.1% (311)
Education	
Less than HS	5.0 % (29)
HS Grad	19.1% (110)
Some College	36.4% (209)
College Grad	39.5% (227)
Marital Status	
Married	59.5% (342)
Not Married	40.5% (233)
Current Smokers	
Yes	15.8%
No	84.2%

 *Mean and standard deviation

Table 3.2. Health status-related characteristics of study sample

Variable	Mean (SD)
Stress Sub-scales (2005)	
Decision latitude	58.5 (24.4)
Work demands	47.1 (18.6)
Coworker Support	60.3 (21.8)
Supervisor Support	62.5 (24.1)
Social Stress	66.0 (17.8)
eGFR	
Year 2005	83.2 (21.3)
Year 2006	82.5 (25.2)
Year 2007	81.8 (22.0)
Year 2008	82.3 (23.3)
HbA1c	
Year 2005	8.1 (1.8)
Year 2006	8.0 (1.9)
Year 2007	7.9 (1.7)
Year 2008	8.0 (1.7)
BMI (2005)	34.3 (7.3)
MAP (2005)	114.3 (13.5)
Proportion of days covered by oral agents in 2005	0.79 (0.3)

Table 3.3 Estimates from the CFA and the unconditional growth models

	Estimate	S.E.	P-Value	Standardized Estimate
Stress-subscale				
Supervisor Support	1.00	0.00	NA	0.81
Coworker Support	0.79	0.09	< 0.001	0.71
Decision Latitude	0.55	0.08	< 0.001	0.44
Work Demand	0.11	0.05	0.046	0.11
Social Stress	0.26	0.06	< 0.001	0.29
Residual Variances				
Supervisor Support	203.91	44.35	<0.001	0.35
Coworker Support	239.39	26.47	<0.001	0.50
Decision Latitude	479.12	33.85	<0.001	0.81
Work Demand	340.04	17.71	<0.001	0.99
Social Stress	270.85	19.06	<0.001	0.92
eGFR Intercept Factor				
Mean	82.62	0.88	< 0.001	4.351
Variance	360.77	4.34	< 0.001	1.00
eGFR Slope Factor				
Mean	0.88	0.30	0.003	0.28
Variance	10.49	4.34	0.016	1.00
Intercept/Slope Covariance	2.63	8.29	0.751	0.05

Mean Fit Indices for CFA Model: χ^2 p-value >0.001; RMSEA 0.072 (90% CI=0.041-0.107); CFI=0.951; TLI=0.902; SRMR=0.037

Mean Fit Indices for Unconditional growth model: χ^2 p-value >0.001; RMSEA= 0.058 (90% CI=0.061-0.094); CFI=0.938; TLI=0.926; SRMR=0.037

Table 3.4. Covariates in the final model

Variable	Intercept (p-value)	S.E (I)	Slope (p-value)	S.E (S)
SES Quartile	-0.01 (0.994)	1.078	0.27 (0.398)	0.316
Education	-1.29 (0.298)	1.242	0.04 (0.902)	0.344
Marital Status (0=NM)	-0.91 (0.671)	2.129	-0.69 (0.274)	0.63
Smoking	1.03 (0.724)	2.925	1.19 (0.184)	0.897
BMI	-0.10 (0.486)	0.138	-0.02 (0.592)	0.039
Insulin (0=non-use)	-6.99 (0.003)	2.326	-1.09 (0.127)	0.714
Medication Coverage	0.71 (0.871)	4.39	1.28 (0.290)	1.208
MAP	-0.24 (0.003)	0.08	-0.10 (<0.001)	0.026
Age	-1.22 (<0.001)	0.159	0.02 (0.634)	0.048
Sex (0=Male)	0.66 (0.732)	1.913	0.30 (0.585)	0.545
Race (0=black)	7.45 (<0.001)	2.088	-0.05 (0.933)	0.614

NM=Not Married; S.E (I) = Standard Error of the Intercept; S.E (S) = Standard Error of the Slope
***Mean Fit Indices**: χ^2 p-value >0.001; RMSEA=0.048 (90% CI=0.041-0.055); CFI=0.902; TLI=0.876; SRMR=0.037*

Figure 3.1. Changes in eGFR at different values of MAP, controlling for other covariates
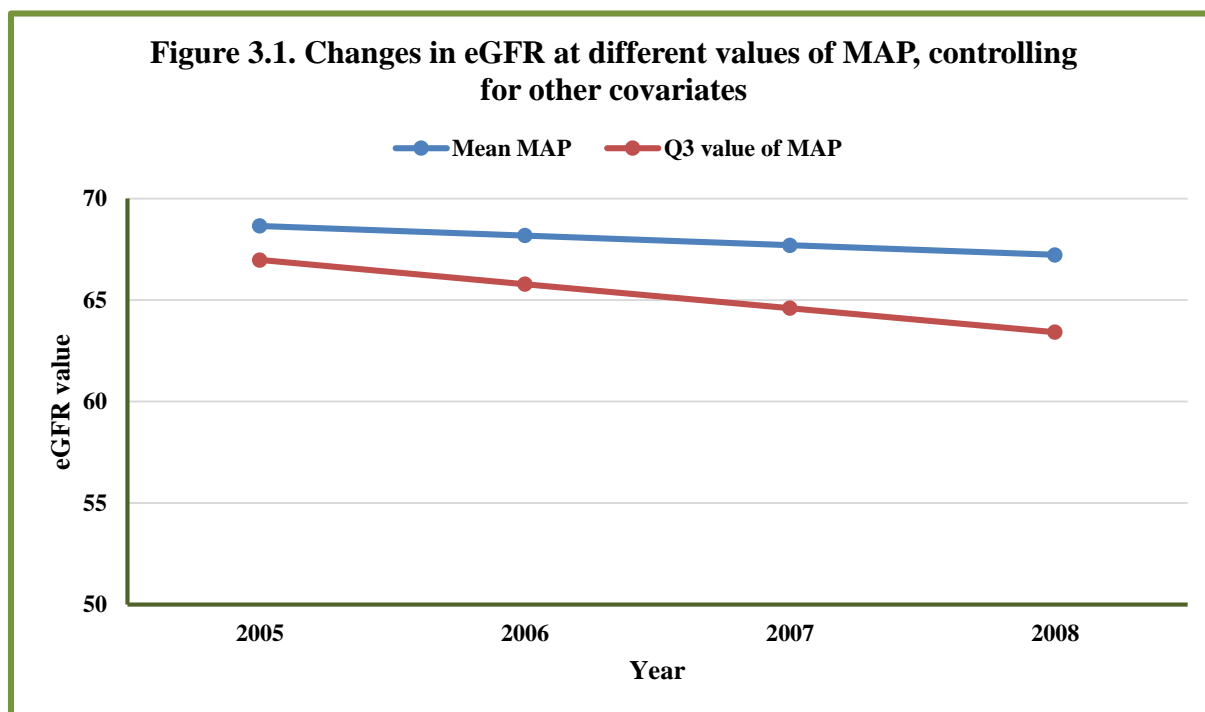
Adjusted for age, sex, race, education, BMI, smoke, medication coverage, insulin use, psychosocial stress and glycemic control. Both the intercept and the slope were significantly different from each other.

Figure 3.2.S Changes in eGFR over time, Insulin users vs non-users, controlling for other covariates
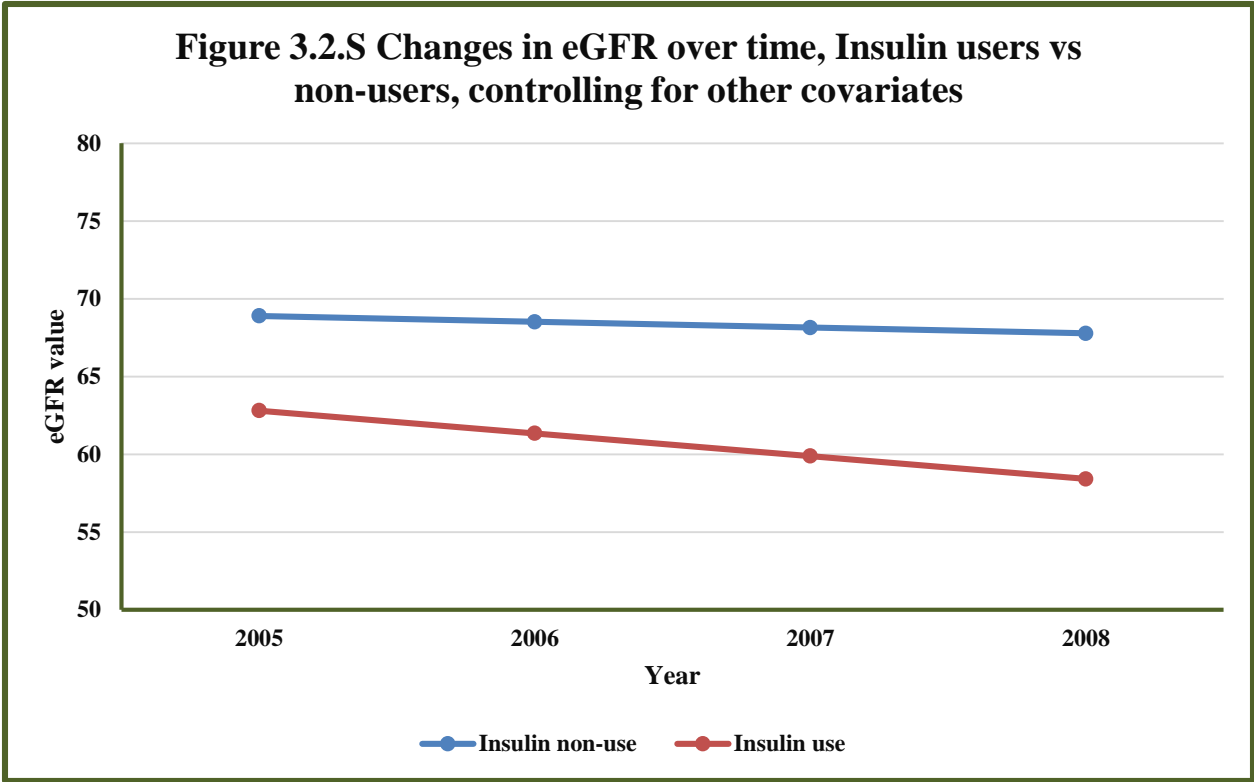
Adjusted for age, sex, race, education, BMI, smoke, medication coverage, MAP, psychosocial stress and glycemic control. Only the intercept was significantly different from each other.

Figure 3.3.S Changes in eGFR over time, White vs Black, controlling for other covariates
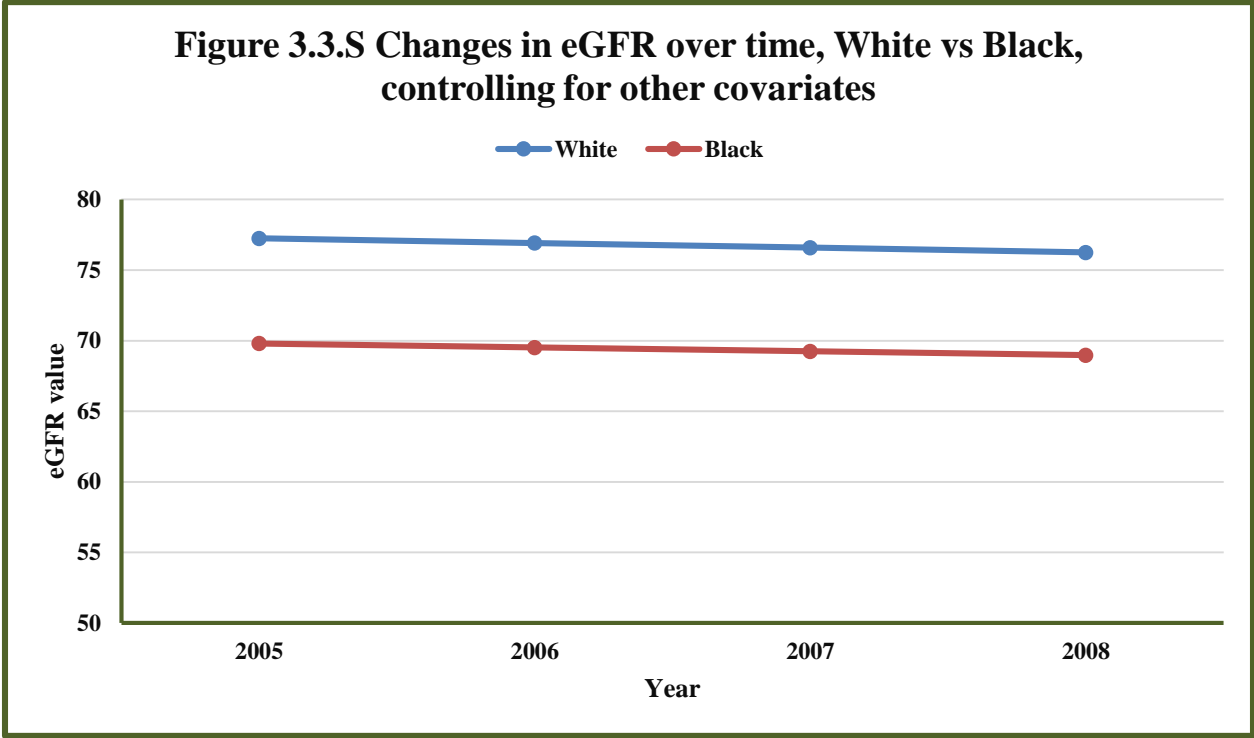
Adjusted for age, sex, education, BMI, smoke, medication coverage, insulin use, MAP, psychosocial stress and glycemic control. Both the intercept and the slope were significantly different from each other.

Figure 3.4. Graphical representation of the final growth model

al
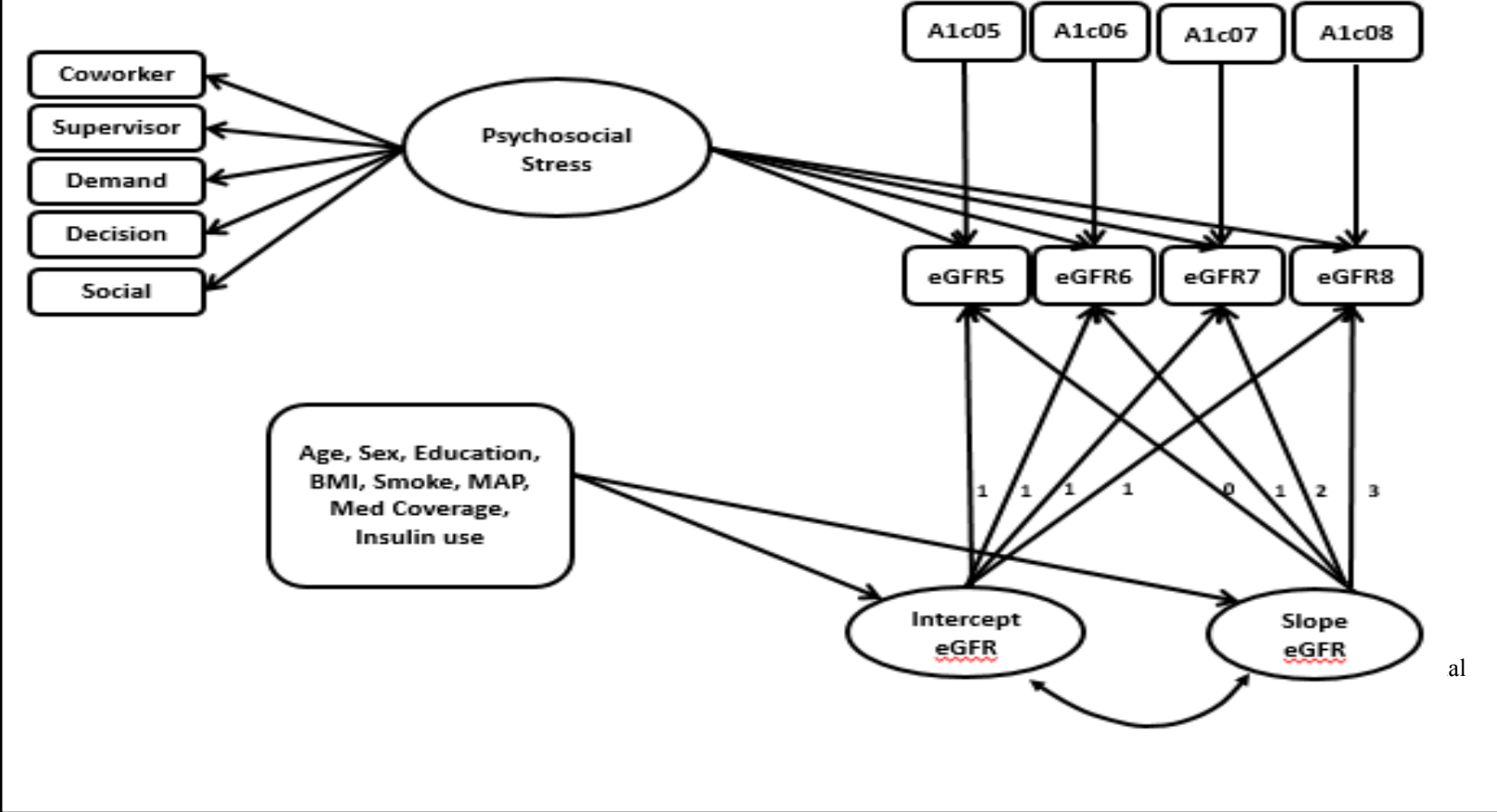
CHAPTER 4. OXIDATIVE STRESS, OXIDATIVE BALANCE SCORE AND HYPERTENSION AMONG RACIALLY ETHNICALLY DIVERSE POPULATION

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Conflict of interest

Authors declare that they do not have any conflict of interest.

Abstract

Objectives: In a racially diverse population, examine the association between: 1) blood pressure/hypertension and four markers of OS and 2) blood pressure/hypertension and oxidative balance score (OBS)

Method: Using data (n=317) from the cross-sectional Study on Race, Stress and Hypertension (SRSH), an OBS was constructed from various measures of pro- and anti-oxidant exposures. OS was assessed by 4 biomarkers: fluorescent oxidative products, F_2-Isoprostanes, mitochondrial DNA copy number and γ-tocopherol. Multivariable linear and logistic regression analyses were used to estimate the associations of interest.

Results: In the final adjusted model, none of the OS markers was significantly associated with blood pressure/hypertension. OBS was inversely associated with hypertension after adjusting for study covariates.

Conclusion: Persons with higher OBS are less likely to have hypertension; however the evidence on the relationship between OS markers and blood pressure remains unconvincing.

Key Words

Oxidative stress
Oxidative balance score
Hypertension
Blood pressure
Anti-oxidants

Introduction

Hypertension is a common and costly disease [30, 268]. It significantly increases the risk of death and cardiovascular events such as stroke and heart attack [31, 269-272]. It is estimated that hypertension accounts for about 54% of all strokes and 47% of all ischemic heart disease cases worldwide [273]. In the US, the prevalence of hypertension is approximately 31% [30] with the annual direct medical cost of about $70 billion. This cost estimate is expected to triple by 2030 [31]. The traditional risk factors for hypertension include family history, age, physical inactivity, obesity, tobacco use, and excessive alcohol intake [32-34]. Evidence from recent studies has suggested the role of oxidative stress (OS) in the pathogenesis of hypertension [35-37]. OS has thus, become a therapeutic target in hypertension treatment [161].

OS is defined as an imbalance between pro-oxidants and anti-oxidants in favor of the former [107]. OS results from overproduction of reactive oxygen and nitrogen species, which in turn damage essential biomolecules such as proteins, lipids and DNA. OS-induced damage has been implicated in several human illnesses [125-127]. Hypertension has been associated with higher levels of OS, although it remains unclear whether increased OS is a cause or a consequence of hypertension [128]. Most of the evidence supporting the relationship between OS and hypertension comes from basic science and animal studies [39-41]. In humans, however, the results have not been entirely consistent and efficacy of anti-oxidant supplementation in reducing blood pressure has not been shown in large clinical trials [42, 43].

OS cannot be directly observed *in vivo,* due to the short lifespan of reactive oxygen and nitrogen species, however, it can be evaluated in humans using biomarkers [160]. While some biomarkers of OS are non-specific, others measure a particular biological or chemical aspect of the process [161, 162]. In humans, the results on the association between OS and hypertension

have mostly been driven by the type of OS biomarker and population being studied [35]. One of the goals of the current study was therefore to estimate the association between OS and hypertension in a racially and ethnically diverse population using four biomarkers of OS: F_2-isoprotanes (F_2-isoP)- a specific marker of lipid peroxidation [164], fluorescent oxidative products (FOP) – a non-specific marker that measures a mixture of analytes resulting from reactions of reactive oxygen species with lipids, proteins and or DNA [165], the number of copies of mitochondrial DNA (MtDNA) – a general marker of cumulative cellular damage [168, 170]; and γ-tocopherol (γ-Toc) – a marker of metabolic response to OS [173].

One potential reason for the inconsistencies in the relationship between hypertension and OS-related exposures (e.g. antioxidant intake) in humans may be the complexity of the processes through which diet, lifestyle, and other factors impact blood pressure. Previous studies have proposed oxidative balance score (OBS), a measure of the status of pro- and anti-oxidants, to be a more accurate representation of the overall OS-related exposures in an individual [156, 159, 175]. The current study therefore sought to build upon and expand on the existing literature. The specific objectives of the current study were to examine: 1) the relationship between hypertension and each of four biomarkers of OS and 2) the association between hypertension and OBS.

Materials and Methods

Study Population

We used cross-sectional data from a previously conducted Study on Race, Stress and Hypertension (SRSH). The study was designed to assess the differences in dietary, lifestyle, and psychosocial exposures in relation to blood pressure in a racially and ethnically diverse

population. The methods of the study has been described in detail elsewhere [175]. Briefly, the study included individuals aged 25-74 years who self-identified as Non-Hispanic Whites (NHW), African Americans (AA) or West African Immigrants (WAI) and who were residents of Georgia. NHW and AA subjects were selected from among 800 participants in a previously completed feasibility phase of the Georgia Cohort Study (GCS). The WAI subjects were recruited *de novo* using previously established ties with Atlanta churches that included large proportions of WAI. The sample of GCS participants was selected after the completion of the WAI recruitment and frequency matched to WAI participants on age and sex. There were 335 individuals who met the initial study inclusion criteria. Of this, 18 participants were excluded from the analyses; 7 had no value for hypertension and 11 were missing values for all four biomarkers of OS. All methods were reviewed and approved by the Institutional Review Boards of the Emory University and the Georgia State University.

Questionnaire data

The study-specific questionnaire provided data on demographic characteristics (age, sex, race/origin and education), medical history (hypertension and use of medications), and lifestyle (physical activity and smoking) for all participants. Blood pressure and anthropometric measures (height and weight) were also taken during data collection sessions. Self-administered questionnaires were returned during the data collection session. We used a previously validated tool for measuring physical activity [274]. The reported and measured BMI were highly correlated (r= 0.91).

Blood Samples

All participants provided blood samples that were drawn into five 10mL vacutainer tubes (2 sodium heparin tubes, 1 EDTA tube, and 2 red top tubes for serum collection) and immediately plunged into ice and protected from direct light. Plasma, serum, and buffy coat specimens were separated within 4-8 hours by centrifugation under refrigeration, aliquoted, frozen and stored at -80°C. The aliquots were then shipped overnight on dry ice for molecular analysis by the Molecular Epidemiology and Biomarker Research Laboratory (MEBRL) at the University of Minnesota, Minneapolis, MN.

Laboratory Analysis

Plasma lycopene, α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, α-tocopherol, and γ-tocopherol were measured by a high performance liquid chromatography (HPLC) assay using previously described methods [183-185]. Serum ferritin was measured by an antibody-based method using Roche 911 analyzer.

Gas chromatography-mass spectrometry (GCMS) [161], a gold standard for the measurement of F_2-isoP, was used to measure plasma free F_2-isoP. The F_2-isoP were extracted from the plasma sample with deuterium (4)-labeled 8-iso-prostaglandin F_2 alpha as an internal standard.

The measurement of FOP was performed using a modified Shimasaki method [186], which has been previously described elsewhere [187]. A mixed solution was centrifuged for 10mins at 3000rpm, 1mL of supernatant was added to cuvettes for spectrofluorometric readings, and a relative fluorescence intensity unit per milliliter of plasma was estimated using the spectrofluorometer [187]. Calibration was performed using standard quinine diluted in 0.1 $NH_2SO_4.$

The copy number of MtDNA was determined using real-time quantitative PCR described by Shen et al [188]. Two primers were used, one for MtDNA and the other for nuclear DNA. The ratio of MtDNA and nuclear DNA was determined using serially diluted genomic sample DNA of a healthy referent [188].

Oxidative balance score

The OBS was estimated using *a priori* selected 13 pro- and anti-oxidants components according to our previous study [175] and those of others [189, 190] as listed in Table 1. The score combined plasma micronutrient measurements and lifestyle behaviors. The plasma level of pro- and anti-oxidants, were divided into sex and race/origin specific tertiles. The number of minutes of physical activity per week was also divided into tertiles. For anti-oxidants (α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, α–tocopherol) and physical activity, the first to third tertiles were assigned scores of 0-2. For pro-oxidants (ferritin), the first to third tertile were assigned a score of 2-0 respectively. To maintain scoring consistency, we assigned scores of 0-2 to the other categorical OBS components. We assigned a score of 0-2 for obese (BMI $\geq$30kg/m^2), overweight (BMI=25-29.99kg/m^2) and normal weight (BMI <25kg/m^2) respectively. For smoking or alcohol use: never-smokers or never-drinkers received a score of two; former smokers and former drinkers or those with missing information received a score of one; and current smokers and current drinkers received a score of zero. For NSAIDs and aspirin, zero points were assigned to participants who reported no regular use of these medications, one point to those who did not report usage or were missing information, and two points to those who reported regular use. Regular users for both aspirin and NSAID were defined as individuals who were taking these medications at least once every week. The points assigned to each component

were summed up to represent the overall OBS. OBS was categorized into three approximately

equal intervals; 3-10, 11-17 and 18-25 representing low, medium and high OBS, respectively.

OBS was also used in a separate analysis as a continuous variable.

Blood Pressure and Hypertension

Trained and certified staff took the blood pressure measures. After participants had rested

for about five minutes seated, three blood pressure measures were taken with at least a minute

interval using mercury sphygmomanometry and *appropriately sized arm cuffs. The mean of the*

three blood pressure measures was estimated and used in this study. Systolic and diastolic blood

pressure (SBP and DBP) measures were expressed as separate continuous variables. Individuals

were considered hypertensive if they met any of the following conditions; (a) ever been told by a

health care professional that s/he has hypertension, (b) self-reported antihypertensive medication

use, (c) had systolic blood pressure (SBP) equal or greater than 140mmHg (c) had diastolic blood

pressure (DBP) equal or greater than 90mmHg.

Statistical Analysis

The F_2-isoP, FOP, MtDNA and γ-Toc were each dichotomized into a 'low' and 'high'

using their respective sex and race/origin specific median as the cut-off. SBP and DBP were

modeled as continuous variables. Hypertension was dichotomized (hypertensive and

normotensive). OBS was used as both a continuous and a three level categorical variable.

The first series of statistical analyses examined the association between SBP, DBP and each of

the OS markers and OBS as continuous variables in linear regression models. In the second set

of analyses, we examined the association of hypertension with OBS and with each biomarker of

OS, using categorical definitions of the outcome. The odds ratios (OR) for the continuous OS variables in the logistic equation was each scaled to their respective one standard deviation. For oxidative stress markers, each linear and logistic regression model adjusted for race/origin, age, sex, education and BMI. For analyses involving OBS, we did not control for BMI because it was included in the score.

All analyses were performed using pairwise deletion method as the default (Method 1). To estimate the effect of missing data, sensitivity analyses were performed by imputing the missing values. We imputed in two different fashions: 1) using five times multiple imputation method available in SAS and 2) by replacing missing values with sex and race specific mean. All models were assessed for collinearity among independent variables and goodness of fit. All estimated measures of association were accompanied by 95% confidence intervals (CI). Statistical significance was determined at two sided p-value of <0.05. All analyses were performed in SAS statistical software version 9.3 [182].

Results

The study population included 100 (32.5%) WAI, 121 (39.3%) NHW, and 87 (28.3%) AA participants. Approximately 33% of the study participants were hypertensive. Hypertension was more common in AA (45.2%) than in NHW (33.1%) and WAI (24.0%) participants. Varying number of participants had a measure for each of the four biomarkers of OS; F_2-isoP (n=221), FOP (n=266), MtDNA (n=173), and γ-Toc (n=278).

Among the participants, most (60.3%) were females and more than a third had a college degree (41.9%, Table 2). About 32% were current alcohol users and 5% were current cigarette smokers. Individuals with hypertension were older and more likely to use aspirin and NSAID

regularly. As expected, individuals with hypertension had significantly higher BMI compared to their normotensive counterparts (Table 2). Inter-individual variability for OS markers was greatest for FOP (range 0.01-0.21 expressed as average standard reference adjusted) and F_2-isoP (range 14.5-280.1 pg/mL): these two biomarkers showed approximately 20-fold difference between the lowest and the highest values. Other OS biomarkers and OBS did not vary as much within the study population: the ranges for the values were 1.22-5.57 expressed as relative copy numbers for MtDNA, 0.06-0.56 mg/dL for γ-Toc, and 4.0-24.0 for OBS. OBS was inversely but not statistically significantly correlated with F_2-isoP (r= -0.18), MtDNA (r= -0.08) and γ-Toc (r= -0.04). By contrast correlation between OBS and FOP was positive (r= 0.30) and statistically significant (Table 3).

In the linear regression models evaluating the relationship between blood pressure and each of the OS markers and OBS, increasing levels of γ-Toc were associated with increasing levels of SBP (β=22.27, p=0.0150) and DBP (β=14.76, p=0.0120) in the crude analyses, but the results were attenuated and no longer statistically significant after adjusting for study covariates. MtDNA copy number was also inversely associated with DBP (β=-2.32, p=0.0123) in the crude but not in the adjusted model. The other OS markers and OBS were not associated with blood pressure in the crude or the adjusted models. The sensitivity analyses were consistent with the original results except for FOP and F_2-isoP that changed direction in some instances; however all before- and after-imputation results were statistically non-significant (Table 4).

In the logistic regression models that used hypertension as the binary outcome variable, the associations with the OS biomarkers were in the hypothesized direction but none of the results were statistically significant after controlling for covariates. Sensitivity analyses did not substantially affect the results.

There was a statistically significant association between OBS and hypertension after controlling for race/origin, age, sex and education. The adjusted OR for middle and higher categories of OBS vs. lower category (reference) were 0.30 (95% CI=0.13-0.72) and 0.17 (95% CI=0.03-0.95) respectively. For the continuous OBS, the adjusted OR was 0.87 (95% CI=0.79-0.96). In the sensitivity analyses, the results for the continuous OBS were similar to the original analyses but the associations with categorized OBS were substantially attenuated (Table 5).

Discussion

Hypertension is a major public health problem in most parts of the world. It is highly prevalent and considered a major risk factor for cardiovascular diseases. Endothelial dysfunction, defined as a shift in endothelium actions towards reduced vasodilation, pro-inflammatory and pro-thrombotic state has been associated with the pathophysiology of hypertension [133]. Although the underlying mechanism to endothelial dysfunction is complex and multifactorial, current evidence indicates that OS may be a key factor in this process [275]. An area of recent hypertension management research is in the therapeutic intervention that target OS [161, 275]. This approach requires an in-depth understanding of the complex role of OS in the pathogenesis of hypertension. In the present cross-sectional study, we examined the relationship between high blood pressure and OS and OBS, hypothesizing that higher level of OS and lower levels of OBS would be associated with high blood pressure/hypertension.

We found increasing levels of γ-Toc to be associated with higher SBP/DBP and higher odds of being hypertensive; although the association was less evident in the multivariable analyses. Cooney and colleagues [173] have characterized γ-Toc as an antioxidant defense indicator whose level may increase to reflect the metabolic response to OS. Consistent with this

characterization, we also found a statistically significant positive correlation between γ-Toc and F$_2$-isoP, a validated marker of OS [163] . Jiang and colleagues have also associated γ-Toc and its metabolites with anti-inflammatory function and found their levels to rise in response to inflammatory signals [276, 277]. γ-Toc enhances cellular immune response by protecting cells against damaging effects of endogenous nitric oxide (NO) generation [278] while enhancing cellular NO synthesis [279]. These data indicate the important physiologic functions of γ-Toc, particularly, in relation to OS [173].

The findings from the final models did not support our hypothesis that increasing levels of OS markers would be associated with high blood pressure, although, we found the adjusted associations to be in the hypothesized direction. This null finding is consistent with other clinical studies that reported no significant difference in OS levels among individuals with and without hypertension [280, 281].

The observed association between OBS and high blood pressure/hypertension supported our second hypothesis that higher OBS levels would be inversely related to high blood pressure. This finding is consistent with other studies that also noted an inverse relationship between OBS and poor health including all-cause mortality [156] and colorectal adenoma [159]. Several previous studies found an inverse association between some of the OBS components and blood pressure [129, 282-284]. For example Li and Xu recently concluded from a meta-analysis that lycopene supplementation reduces systolic blood pressure [285]. Chen and colleagues also found lower levels of both α- and β-carotenes in persons with hypertension [286].

The use of dietary anti-oxidants to reduce blood pressure is plausible because these compounds have been shown to reduce the bioavailability of reactive oxygen species, increase production of nitric oxide (NO), down-regulate nicotinamide adenine dinucleotide phosphate (NADPH)

oxidase and up-regulate endothelial NO synthase (eNOS) [36, 287]. Higher production and bioavailability of NO in the endothelial cells is important for vascular relaxation [135, 136, 139]. Despite compelling evidence from experimental biology studies, the findings from clinical studies of the effect of anti-oxidant supplementation for blood pressure reductions have not produced desired results [43, 288]. The possible reasons for the discrepancy in the use of anti-oxidants to treat conditions related to oxidative stress have been articulated in a number of reviews [154, 155, 289]. With respect to hypertension, Montezano and Touyz identified three reasons for the discrepancy between anti-oxidants supplementation and reduction in blood pressure: the type of antioxidants used; patient cohorts; and the trial design [36]. One other possible reason is that anti-oxidants in diets are mixed and work as continuous chain while supplementations are usually a couple of specific anti-oxidants and therefore, may lack this anti-oxidants chain. Also, it has been found that if an antioxidant is not restored by the next in the chain after scavenging ROS, it begins to act as a pro-oxidant [153]. The evidence therefore suggests that biochemical interactions exist among antioxidants which may be lacking in supplements due to the use of one or two individual antioxidants [154, 155].

Given the inconsistent relationship between OS markers and high blood pressure/hypertension and the inconclusive association between individual anti-oxidants and high blood pressure/hypertension from previous studies, the use of OBS seems promising, as it represents the overall patterns of pro- and anti-oxidant exposures.

An important methodological feature of the present study is the use of a racially and ethnically diverse population. This allowed for assessing multiple biomarkers of OS and their relation to each other and to hypertension in US born whites and blacks and in West Africans. In addition, the use of plasma levels of micro-nutrients in this study may accurately represent

current intake and availability of pro- and anti-oxidants compared to food frequency questionnaire-derived measures [290]. The major limitation of this study is the missing information on several variables used in the analyses. Although sensitivity analyses did not affect the overall conclusions, some of the results changed following imputation of missing data.

Conclusion

The presented results suggest that higher OBS may be inversely associated with hypertension, a finding that is consistent with several previous studies. By contrast after controlling for confounders, markers of OS were not associated with blood pressure or hypertension. The discrepancy between relatively consistent associations observed for pro- anti-oxidant exposures and largely null results for markers of OS indicate that OS-related lifestyle and dietary factors may act through other mechanisms. The observed results need to be confirmed in independently conducted, preferably longitudinal, studies. If these findings are indeed confirmed, the mechanisms by which OBS may influence the risk of hypertension need to be explored further.

Acknowledgement

Conflict of interest

Authors declare no conflict of interest.

Table 4.1. OBS Assignment

Component	Score Assignment
Plasma Zeaxanthin	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma Cryptoxanthin	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma Lycopene	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma α-carotene	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma β-carotene	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Plasma α-tocopherol	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Serum Ferritin	2=low (1st tertile), 1=medium (2nd tertile), 0=high (3rd tertile)
Physical Activity	0=low (1st tertile), 1=medium (2nd tertile), 2=high (3rd tertile)
Alcohol use	0=current drinker, 1=former drinker/missing, 2=never drinker
Smoking	0=current smoker, 1=former smoker/missing, 2=never smoked
Aspirin use	0=no regular user, 1=unknown/missing, 2=regular user
NSAID use	0=no regular user, 1=unknown/missing, 2=regular user
Obesity	0=obese, 1=overweight, 2=normal weight

NSAID = Non-steroidal anti-inflammatory drugs. Normal weight=BMI<25kg/m^2, overweight=BMI between 25.0-29.9kg/m^2, Obese=BMI ≥30kg/m^2.

Table 4.2. Distribution of main study variables

Demographic Characteristics	Overall (n=317)	Hypertensive and Normotensive Compared		
		Hypertensive (n=106)	Normotensive (n=211)	p-value
Age	47.1 (11.9)	54.2 (10.6)	43.5 (10.9)	<0.001
Female (%)	60.3	64.2	58.3	0.3206
Race				
WAI	32.5	23.1	37.3	0.0064
NHW	39.3	38.5	39.7	
AA	28.3	38.5	23.0	
Education				
Less than HS	6.8	3.9	8.3	0.0495
HS Grad	16.1	23.1	12.6	
Some College	35.2	36.5	34.5	
College Grad	41.9	36.5	44.7	
OBS Measures				
Current Alcohol User (%)	32.2	29.3	33.7	0.2065
Current Smoking (%)	5.0	5.1	5.0	0.0856
Obesity (%)	44.3	68.9	31.7	<0.001
Regular Aspirin User (%)	23.3	45.6	12.9	<0.001
Regular NSAID User (%)	26.6	32.5	23.8	0.1545
Plasma Zeaxanthin, ug/dL	20.9 (10.5)	22.6 (11.4)	20.1 (10.0)	0.0572
Plasma Cryptoxanthin, ug/dL	7.7 (9.1)	8.5 (14.4)	7.4 (4.8)	0.3164
Plasma Lycopene, ug/dL	45.3 (24.9)	38.7 (18.6	48.4 (27.0)	0.0021
Plasma α-carotene, ug/dL	11 (15.1)	8.6 (13.2)	12.2 (15.9)	0.0604
Plasma β-carotene, ug/dL	22.5 (23)	19.2 (18.9)	24.1 (24.6)	0.0901
Plasma α-tocopherol, ug/dL	0.96 (0.28)	1.0 (0.3)	0.9 (0.3)	0.0012
Serum Ferritin, ug/dL	128.1 (226.5)	141.0 (135.2)	121.8 (259.9)	0.5009
OBS Score	12.2 (3.8)	12.0 (4.2)	12.4 (3.6)	0.4407
Biomarkers				
γ-Toc (mg/dL)	0.2 (0.09)	0.22 (0.09)	0.19 (0.09)	0.0206
F_2-isoP (pg/mL)	56.6 (34.87)	63.5 (43.8)	53.1 (29.0)	0.037
FOP (Av Std Ref Adj)	0.04 (0.02)	0.05 (0.03)	0.04 (0.02)	0.2015
MtDNA (rel copy number)	3.2 (0.83)	3.15 (0.73)	3.19 (0.88)	0.7746
Blood Pressure				
SBP (mmHg)	124.0 (14)	132.7 (14.1)	119.7 (11.7)	<0.0001
DBP (mmHg)	76.3 (9.2)	78.9 (9.6)	75.1 (8.7)	0.0004
BMI (kg/m^2)	29.8 (6.6)	33.0 (6.7)	28.4 (5.9)	<0.0001

For continuous variables, t-test was used to test the difference between hypertensive and normotensives while chi-square test was used for categorical variables. Av Std Ref Adj= Average standard reference adjusted.

Table 4.3. Correlations between OS markers and OBS; Spearman are above diagonal and Pearson are below the diagonal

	FOP	F$_2$-isoP	MtDNA	γ-Toc	OBS
FOP	1	-0.32**	-0.02	-0.15**	0.37**
F$_2$-isoP	-0.17**	1	-0.15	0.40**	-0.11
MtDNA	-0.01	-0.14	1	-0.12	-0.10
γ-Toc	-0.15**	0.36**	-0.15	1	-0.02
OBS	0.31**	-0.16	-0.09	-0.01	1

FOP = florescent oxidation products; F$_2$-isoP = F$_2$-isoprostanes; MtDNA = mitochondrial DNA copy number; γ-Toc = gamma tocopherol; OBS = oxidative balance score.
** $p < 0.05$

Table 4.4. Association between Blood Pressure and FOP, F2-isoP, MtDNA and G-Toc

	Method 1 (OR, 95% CI)		Method 2 (OR, 95% CI)		Method 3 (OR, 95% CI)	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
SBP						
FOP	76.12 (0.0901)	73.5 (0.0829)	70.33 (0.1142)	59.60 (0.1560)	76.32 (0.0865)	71.79 (0.0881)
F_2-isoP	0.02 (0.4146)	-0.04 (0.2145)	0.02 (0.5611)	-0.02 (0.4366)	-0.01 (0.9350)	-0.04 (0.0752)
MtDNA	-2.39 (0.0802)	-1.94 (0.1627)	-1.26 (0.2973)	-0.79 (0.4601)	-2.65 (0.0355)	-1.66 (0.1857)
γ-Toc	22.27 (0.0150)	6.72 (0.4786)	24.19 (0.0099)	7.53 (0.4367)	22.6 (0.0151)	6.02 (0.5231)
OBS	0.40 (0.1353)	0.12 (0.6284)	0.47 (0.0619)	0.21 (0.3950)	0.61 (0.0083)	0.37 (0.0981)
DBP						
FOP	-12.94 (0.650)	23.98 (0.3962)	-5.93 (0.8385)	22.08 (0.4116)	-16.00 (0.5844)	22.41 (0.4330)
F_2-isoP	0.02 (0.2544)	-0.02 (0.1601)	0.01 (0.4485)	-0.01 (0.6329)	0.01 (0.5110)	-0.02 (0.2408)
MtDNA	-2.32 (0.0123)	-0.44 (0.6356)	-1.14 (0.1561)	-0.28 (0.7099)	-2.26 (0.0062)	-0.39 (0.6498)
γ-Toc	14.76 (0.0120)	2.73 (0.6690)	18.25 (0.0028)	4.47 (0.4826)	14.44 (0.0180)	1.04 (0.8707)
OBS	0.25 (0.1342)	0.13 (0.4100)	0.12 (0.5509)	0.05 (0.8022)	0.21 (0.1750)	0.13 (0.3660)

Method 1= Original data used pairwise deletion, Method 2= Used multiple imputation to handle missing data, Method 3= Replace missing information with race and sex specific mean. FOP=fluorescent oxidative products; F_2-isoP = F_2-isoprostanes; MtDNA=mitochondrial DNA copy number; γ-Toc= γ-tocopherol; Crude =OR without controlling for any covariate. Adjusted = OR after adjusting for age, sex, race/origin, education and BMI (when predictor was not OBS). Each biomarker was dichotomized based on sex and race/origin specific median.

Table 4.5. The association between hypertension and FOP, F$_2$-isoP, MtDNA, γ-Toc and OBS

	Method 1 (OR, 95% CI)		Method 2 (OR, 95% CI)		Method 3 (OR, 95% CI)	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
FOP						
Low	Ref	Ref	Ref	Ref	Ref	Ref
High	1.29 (0.78-2.15)	1.23 (0.66-2.29)	1.16 (0.89-1.51)	1.09 (0.81-1.49)	1.23(0.76-1.97)	0.09 (0.60-1.96)
FOP (Cont, 1-SD)	1.17 (0.91-1.51)	1.23 (0.85-1.78)	1.16 (0.91-1.47)	1.12 (0.86-1.63)	1.17 (0.91-1.51)	1.22 (0.85-1.75)
F$_2$-isoP						
Low	Ref	Ref	Ref	Ref	Ref	Ref
High	1.28 (0.73-2.23)	1.00 (0.50-2.00)	1.20 (0.74-1.94)	1.02 (0.74-1.40)	1.03 (0.64-1.65)	0.95 (0.54-1.69)
F$_2$-isoP (Cont, 1-SD)	1.33 (1.07-1.76)	0.97 (0.64-1.47)	1.01 (1.00-1.01)	1.00 (0.99-1.01)	1.20 (0.93-1.54)	0.97 (0.65-1.45)
MtDNA						
Low	Ref	Ref	Ref	Ref	Ref	Ref
High	1.32 (0.70-2.47)	1.33 (0.61-2.91)	1.16 (0.90-1.49)	1.18 (0.87-1.61)	1.41 (0.86-2.33)	1.35 (0.68-2.70)
MtDNA (Cont, 1-SD	0.95 (0.69-1.31)	0.96 (0.64-1.45)	1.04 (0.71-1.54)	1.09 (0.73-1.62)	0.96 (0.71-1.31)	1.00 (0.67-1.50)
γ-Toc						
Low	Ref	Ref	Ref	Ref	Ref	Ref
High	1.54 (0.94-2.55)	0.78 (0.41-1.47)	1.23 (0.96-1.58)	0.91 (0.67-1.25)	1.45 (0.91-2.32)	0.83 (0.45-1.50)
γ Toc (Cont, 1-SD)	1.33 (1.04-1.69)	0.99 (0.71-1.36)	1.32 (1.04-1.66)	1.03 (0.03-33.43)	1.33 (1.04-1.69)	0.99 (0.72-1.36)
OBS						
Low (4-10)	Ref	Ref	Ref	Ref	Ref	Ref
Medium (11-17)	0.61 (0.31-1.19)	0.30 (0.13-0.72)	0.92 (0.59-1.43)	0.89 (0.53-1.49)	0.70 (0.42-1.17)	0.58 (0.31-1.08)
High (18-25)	0.50 (0.13-1.97)	0.17 (0.03-0.95)	0.82 (0.39-1.71)	0.56 (0.22-1.43)	0.85 (0.29-2.48)	0.46 (0.12-1.73)
Continuous	0.95 (0.87-1.03)	0.87 (0.79-0.96)	0.96 (0.90-1.03)	0.91 (0.84-0.99)	0.98 (0.91-1.04)	0.93 (0.86-1.00)

Method 1= Original data used pairwise deletion, Method 2= Used multiple imputation to handle missing data, Method 3= Replaced missing information with race and sex specific mean. FOP=fluorescent oxidative products; F$_2$-isoP = F$_2$-isoprostanes; MtDNA=mitochondrial DNA copy number; γ-Toc= γ-tocopherol; Crude =OR without controlling for any covariate. Adjusted = OR after adjusting for age, sex, race/origin, education and BMI (when predictor was not OBS). Each biomarker was dichotomized based on sex and race/origin specific median. Con, 1-SD= Continuous variable scaled to 1 standard deviation.

CHAPTER 5. DISCUSSIONS AND FUTURE DIRECTIONS

Overview of findings

The goal of this dissertation was to investigate the associations between (1) psychosocial stress and glycemic control, (2) psychosocial stress and changes in renal function over time, and (3) oxidative stress (OS) and hypertension among adults.

In the first study, a longitudinal data from Kaiser Permanente Georgia (KPGA) on Health and Healthy behaviors was used to examine the relationship between baseline psychosocial stress at the work environment and glycemic control. I applied both cross-sectional and longitudinal data analytic approach to examine this relation. Contrary to our expectation, we did not find a significant association between work-related psychosocial stress at baseline and glycemic control either at baseline or over time after controlling for study covariates.

In the second study, the same longitudinal data from KPGA as was in the first study was used to examine the association between general measures of psychosocial stress and changes in estimated glomerular filtration rate (eGFR) over a period of four years. The results did not support our hypothesis that higher psychosocial stress would be associated with changes in eGFR over time after controlling for study covariates. However, age, race, insulin use and blood pressure were found to be associated with renal decline among our study population.

In the third study, using data from the Study on Race, Stress and Hypertension, I examined the association between hypertension and: 1) OS markers (F_2-Isoprostanes, Flourescent oxidative products, Copy number of mitochondrial DNA and γ-tocopherol); and 2) oxidative balance score

(OBS). As expected, OBS was inversely associated with hypertension but no significant association was observed between OS and hypertension.

The results from the first two studies did not support our hypothesized association between psychosocial stress and the glycemic control and renal decline. Although, the results from both studies were consistent with the unique studies conducted to date on each topic, the null finding may partly be due to the measure of the psychosocial stress variable. The psychosocial stress concept is broad and encompasses stress from multiple sources. The use of psychosocial stress from only two major sources might have underestimated the extent of stress in the study population to observe an effect on DM management or decline in renal function among the study population. Studies that comprehensively assess psychosocial stress from diverse sources and further examine their relationship with a health outcome is limited. Due to the complex nature of the psychosocial stress concept and the multiple sources from which it could originate, future studies need to consider including stress from other sources including major life event, other existing chronic conditions, poverty and family circumstances.

In the third study, the first hypothesis that OS would be positively associated with hypertension was not supported but the second hypothesis that OBS would be inversely associated with OBS was supported. If the OBS and hypertension association is confirmed by future studies then the mechanism through which OBS impact hypertension need to be explored further. Also, although, the association between OS markers and hypertension was not significant in the final model after controlling for study covariates, each of the relationship was in the hypothesized direction. We observed low correlations between the markers of OS, although, these markers are assumed to be measuring the same concept, confirming the earlier suggestions that different OS markers may be explaining different aspects of the OS process and each may be independent from each other.

Implications for Future Research

The assessment of a comprehensive psychosocial stress is difficult. This is due to the broad nature of the concept of psychosocial stress. In this study, we used psychosocial stress from two major sources, which was identified as a limitation, because it might not be comprehensive enough. Future studies need to have a comprehensive assessment of psychosocial stress by including stress from multiple and diverse sources. Secondly, the study noted that psychosocial stress is personal, and response to stress may be impacted by several other factors such as culture, religion, race/ethnicity, the number of times experienced the stress, and the source of stress such as poverty, major life events, abuse or trauma [65-68, 291]. In the light of the individual variations in perception and response to stress, we propose that the assessment of psychosocial stress in future studies give consideration to the following: (1) assess past life experiences which have been identified to feed into stress perception and response [64]; (2) assess resiliency which can be used to adjust for the perception piece, a factor considered to mediate the relationship between stressful situation and health; and (3) control for contextual factors such as culture, religion, and race/ethnicity, which have been identified to play a significant role in response to psychosocial stress.

Our proposal for future studies to assess and include psychosocial stress from multiple sources would also mean the need to apply the appropriate statistical procedures and tools that would accurately combine psychosocial stress from those multiple sources. It was obvious from the literature that stress from different sources assesses different aspects of the psychosocial stress process, therefore, the use of statistical approach that will combine the different sources while controlling for measurement errors would be critical in future psychosocial stress research. Such statistical approach would include but not limited to the use of confirmatory factor analysis and

latent class analysis in combination with structural equation modeling. Another fact observed in the literature was that psychosocial stress from different sources might have varying impact on health. The implication of this finding is that future studies that combine psychosocial stress from multiple sources should also consider assigning weight to stress from different sources to reflect their impact on health based on the literature.

In the third study, the observed low correlations between the markers of OS, albeit these markers assessing the same process, might suggest that the markers are rather formative of the process of OS rather than a reflective, which is what has always been assumed to be the case. Future studies, particularly, those that will combine multiple OS markers as a latent factor should do so in the formative framework. The finding of an association between OBS and hypertension was important as most research that have utilized OBS as a way of comprehensively assessing the overall oxidative burden did so in relation to cancer. To our knowledge, this is the first study to examine the association between OBS and hypertension. More future studies, particularly, longitudinal studies, need to further examine the OBS and hypertension association. If this association is confirmed, then the mechanism through which OBS may relate to hypertension would need to be examined as well.

Finally, given what is known about γ –tocopherol level and the relationship with hypertension observed in this study, future studies need to examine the role of this compound in the pathophysiology of hypertension.

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