ABSTRACT

Metabolic Health, Obesity, and Chronic Kidney Disease: Findings from the National Health and Nutrition Examination Surveys

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Rising rates of metabolic syndrome, obesity, and death from chronic kidney disease (CKD) have prompted further investigation into the association between metabolic syndrome and CKD. The purpose of this study was to report the frequency of metabolic phenotypes, constellations, and clusters as well as their relationship to renal function in a representative sample of individuals in the United States. We utilized a subsample from the 2013-2018 National Health and Nutrition Examination Surveys (NHANES) and complex survey sample weighting techniques to represent non-institutionalized US civilians. Four metabolic phenotypes were identified including metabolically healthy normal weight (MHN), metabolically healthy obese (MHO), metabolically unhealthy normal weight (MUN), and metabolically unhealthy obese (MUO). Renal function as measured by estimated glomerular filtration rate (eGFR) was compared among the phenotypes. Sixteen possible constellations of 3 or more risk factors were classified and four metabolic clusters, which represented MetS with hyperglycemia (Cluster I), MetS with hypertension (Cluster II), MetS with hyperglycemia and

hypertension (Cluster III) or MetS with normoglycemia and normotension (Cluster IV), were assessed for renal function and CKD status. The metabolically healthy normal (MUN) phenotype was most frequent in the subsample taken (38.40%). Renal function was lowest in this phenotype in the regression analysis (B= -9.60, p<0.001) and highest in the MHO (B= 2.50, p>0.05) and this persisted with more liberal definitions of metabolic syndrome. Systolic blood pressure had the strongest correlation with overall eGFR (r= -0.25, p<0.001) and individuals with low HDL had higher renal function compared to the overall sample. The constellation with the lowest renal function consisted of hypertension, high triglycerides, and large waist circumference (82.86 ml/min/1.73m^2). Cluster III had the highest odds of CKD (OR=2.57, 95%CL=1.79, 3.68) and Clusters II and III had the lowest renal function (87.82 and 87.28 ml/min/1.73m^2, respectively). In conclusion, the metabolically unhealthy phenotypes had the lowest renal function regardless of weight status. Metabolic constellations and clusters with hypertension as a risk factor had low renal function. HDL had a small negative correlation with renal function, indicating that more research should be done in this area.

Metabolic Health, Obesity, and Chronic Kidney Disease: Findings from the National Health and Nutrition Examination Surveys

by

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A Dissertation

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DEDICATION

To all who have encouraged me along my journey, especially my academic mentors, Dr. Rodney Bowden and Dr. Peter Brubaker, thank you.

CHAPTER ONE

Introduction

Precursors to Disease

Over the past three decades, the prevalence of obesity (1) and metabolic syndrome (2) has increased in the United States (US) and the across the world. These global pandemics have major implications for the rising rates of chronic kidney disease (3) (CKD). In the US specifically, obesity rates have consistently increased and now exceed 42% of the population (4). The rates of metabolic risk factors and chronic diseases are subsequent to the rising rates of widespread obesity that have plagued Americans over the past several decades. Obesity is attributable to a conglomeration of factors, including, but not limited to, changes in food systems and availability, environment, and genetics (5). Foods, for instance, have become more profitable when presented in forms that are processed, hyperpalatable (6), and mass-marketed. The mass-availability of processed foods is starkly juxtaposed with the inaccessibility of fresh produce and unprocessed foods, especially in impoverished regions of the country. Additionally, the developed world has moved towards convenience, productivity, and efficiency as motorized transportation and the widespread use of technology have resulted in increasing sedentary behavior (7). This has allowed for the relinquishing of physical activities in the occupational and household spheres and has further contributed to the obesity epidemic. While the etiology of obesity is not entirely clear, neither are its effects, making it an important area of scientific research and study.

Much of our understanding of the energy imbalance associated with obesity is limited to self-report questionnaires, and large discrepancies have been reported between objective and subjective measures of physical activity. A study of the 2005-2006 cycle of the National Health and Nutrition Examination Survey (NHANES) indicated that only 9.6% of Americans objectively met the guidelines for physical activity whereas 62% of the same sample reported meeting the guidelines in self-report questionnaires (8). This and other examples prove the difficulty in measuring lifestyle habits. Overreporting of physical activity and underreporting of caloric intake lead to a dissonance between questionnaire data and the available medical health data in the US, and therefore less reliability in tracking these items over time (8). While lifestyle habits play major roles in the pathway to obesity and chronic diseases, the measurement techniques utilized in lifestyle assessments have been found wanting. With rates of obesity, metabolic risk factors, and chronic diseases such as CKD increasing, it is crucial to identify simple objective measures that can be clinically assessed to prevent and ameliorate disease before permanent damage occurs.

The present study will outline the effects of obesity and the metabolic risk factors on renal outcomes. The kidneys are vascular organs that are directly affected by weight gain (9) and the risk factors associated with metabolic diseases (10). The process leading to CKD, and ultimately end-stage renal disease (ESRD), is characterized by the progressive and permanent scarring of the kidneys over time, known as glomerulosclerosis. The kidneys are essential organs, and the body has a limited survival period without their function (11), necessitating the implementation of a transplant organ

or mechanized blood filtration process, known as dialysis. This process is burdensome, costly, and associated with increased risk of mortality (12–15).

Obesity

Obesity is a global public health issue with prevalence increasing steadily over the past four decades (16). Current estimates in the United States (US) indicate that 42.4% of the population is obese, and future projections continue to suggest increasing rates of obesity (4). The habits of the developed world, which include overconsumption of processed, energy-dense foods and a predominantly sedentary lifestyle, are the major contributors to the obesity epidemic in the US. However, the etiology of obesity is complex and involves many considerations that extend beyond poor diet and physical inactivity. Underlying factors that contribute to obesity include the social determinants of health: economic stability, education access and quality, health care access and quality, neighborhood and built environment, and social and community context. Still, other factors can include genetic predisposition, changes in the epigenome, depression, anxiety, neurotransmitter activity, gut microbiota, infection, inflammation and metabolic changes triggered by nutrient intake (5). Due to the complex psychology, sociology, and pathophysiology of obesity, it has been posited that overeating and under-engaging in physical activities may be symptoms of disease, rather than the causes themselves (5). Adipose tissue, specifically that which is carried in excess in the central region of the body, is an inflammatory organ that actively produces inflammatory cytokines, or cell-signaling proteins. Obesity typically manifests over time and results in long-term inflammation, which is recognized by the immune system and causes proliferation of

lymphocytes and macrophages as well as the proliferation of blood vessels and connective tissues (17, 18). These processes are linked with systemic dysregulation of bodily functions, which often lead to morbidity and premature mortality.

Metabolic Syndrome

The numerous consequences associated with the obesity epidemic have resulted in a subsequent upsurge in metabolic risk factors. The various constellations of metabolic risk factors that develop concomitantly has been collectively termed the 'metabolic syndrome' (MetS), and was first described by Reaven *et al*. in 1988 (19). The components that make up MetS vary based on the defining entity, but all share the characteristics of abdominal obesity and insulin resistance (IR). The classification of abdominal obesity, in particular, can be measured a number of ways (e.g., body mass index, waist circumference, or body fat percentage) which may result in varying classifications and diagnoses. The best methods for classifying insulin resistance (e.g., fasting blood glucose, oral glucose tolerance test, blood insulin, hemoglobin A1C, or the homeostatic model assessment of insulin resistance) are also in need of further research (20). Additionally, those with prior diagnosis of atherosclerotic cardiovascular disease (ASCVD) or type 2 diabetes (T2D) may be excluded from analyses, depending on the defining criteria (20).

The most commonly used definition for classifying MetS is the 2005 revision of the National Cholesterol Education Program's (NCEP) Adult Treatment Panel (ATP) III (20). The criteria defined in the ATP III were established because they avoid emphasis on any single cause and are simple to use in a clinical setting (20). Using these criteria, MetS

is classified based on three or more of the following components: abdominal obesity determined by waist circumference measurement, dyslipidemia (including high triglyceride and/or low high-density lipoprotein), elevated blood pressure, and impaired fasting glucose. The values for each metabolic risk factor are listed in Table 1.1. The clinical diagnosis of MetS can be used as a tool to identify individuals at high risk of ASCVD and T2D. It is important to note, however, that MetS is not a discrete entity and has multiple causes as well as outcomes, given that it is a 'syndrome' classified by variable components (20). The prothrombotic and proinflammatory nature of abdominal obesity and IR are the predominant risk factors at play, but other factors such as race and ethnicity, physical inactivity, aging, and hormonal imbalance can increase the risk of MetS.

Table 1.1
Criteria for Metabolic Syndrome, Obesity, and Metabolic Phenotypes

Category	Classification	Values
Metabolic Risk Factors	Obesity	WC >101.6cm (M) or WC>88.9cm (F)
	Hyperglycemia	Fasting glucose ≥ 100 mg/dL or Rx
	Dyslipidemia (2^{nd} criteria)	TG ≥ 150 mg/dL or Rx HDL< 40 mg/dL (M), < 50 mg/dL (F); or Rx
	Hypertension	> 130 mmHg systolic or > 85 mmHg diastolic or Rx
Obesity	Obese BMI, non-Asian	BMI>30 kg/m^2
	Obese BMI, Asian	BMI>25 kg/m^2
	Obese WC, non-Asian	WC>101.6cm (M) or WC>88.9cm (F)
	Obese WC, Asian	WC>94cm (M) or WC>80cm (F)
Metabolic Phenotypes	MHN	Non-obese and <1 metabolic risk factor
	MHO	Obese and <1 metabolic risk factor
	MUN	Non-obese and >1 metabolic risk factor
	MUO	Obese and >1 metabolic risk factor

Metabolic syndrome is defined by the NCEP ATP III (2005 Revision) guidelines[20]. BMI is calculated as weight (kg) divided by height (m^2). WC, waist circumference; M, males; F, females; Rx, prescription medication; TG, triglycerides; HDL, high density lipoprotein. Metabolic syndrome and phenotype criteria adapted from Wildman et al.[91]

The prevalence of MetS has increased concurrently with rising obesity rates (Figure 1.1). Within the past three decades, the prevalence of MetS has increased from 25.3% to 34.2% in the US (2). The growing prevalence of both obesity and MetS is a major public health issue. Metabolic syndrome is a systemic disease manifested throughout the entire body, affecting multiple systems. The implications of this disease include greater risk of morbidity and mortality. The diagnosis of MetS alone brings with it a 2-fold increased risk for the development of cardiovascular disease (28) and stroke (29), and a 1.5-fold increased risk for all-cause mortality (30). While MetS is traditionally correlated with the development of ASCVD and T2D, it can also be used to predict and prevent a host of other chronic diseases.

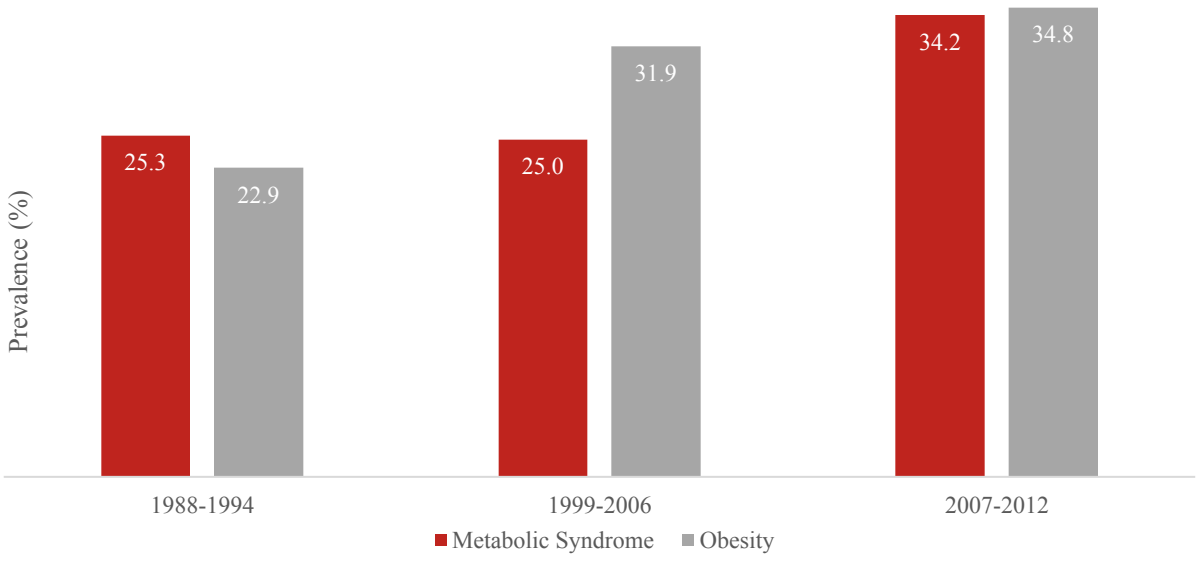

Figure 1.1. Prevalence of metabolic syndrome and obesity in the United States from 1988 to 2012. Metabolic syndrome data from Moore et al., 2017[2], n= 51,371, and obesity data from Fryar et al., 2016[1], n=50,209. All subjects were analyzed as part of the National Health and Nutrition Examination Surveys (NHANES) from 1988 to 2012.

Metabolic phenotypes consider the metabolic risk factors as well as obesity status. While the definition of metabolic phenotypes varies, there is consensus that the metabolically healthy obese (MHO) and the metabolically unhealthy normal weight (MUN) individuals are "intriguing" groups to study (31). Cardiovascular outcomes have been heavily studied in these groups, and burgeoning evidence suggests that researching metabolic phenotypes may also be beneficial in the study of CKD. However, metabolic phenotypes do not take into account the numerous iterations of metabolic risk factors that combine to form a 'syndrome'. Some risk factor combinations may be more synergistic than others in the pathophysiology to disease. Metabolic phenotypes are not specific enough to identify which metabolic risk factors contribute most to disease states, and which combinations are most detrimental. There is growing evidence for the study of metabolic constellations, which are groups of 3 or more metabolic risk factors (32, 33). The various iterations constituting the constellations sub-divide the diagnosis of MetS into its 16 possible combinations. Research relating the metabolic constellations to CKD is sparse to non-existent, and therefore provides a novel angle of assessing CKD risk. Furthermore, the study of metabolic clusters, which are groups of constellations based on a key metabolic risk factor, is scarcely studied. The metabolic clusters classify metabolic constellations using hyperglycemia and hypertension, which are the two main precursors to CKD in the developed world (34).

Chronic Kidney Disease

In the US, chronic kidney disease (CKD) is overwhelmingly associated with T2D and hypertension (HTN) (34), which are two of the five possible risk factors in MetS. In

many cases, T2D and HTN can be ameliorated, whereas the damage that occurs to the kidneys throughout the course of T2D, HTN, and/or CKD is largely permanent. In the milieu of metabolic dysregulation, the recurrent harm to the kidneys results in a very low glomerular filtration rate and eventual ESRD (see Table 1.2) which requires the use of an artificial kidney (hemodialyzer) or a kidney transplant to sustain life.

Table 1.2
Categories of Chronic Kidney Disease

GFR Category	Description	GFR (ml/min/1.73m2)
G1	Normal or high	≥ 90
G2	Mildly decreased	60-89
G3a	Mildly to moderately decreased	45-59
G3b	Moderately to severely decreased	30-44
G4	Severely decreased	15-29
G5	Kidney Failure (ESRD)	<15

This table was adapted from the 2012 Kidney Disease Improving Global Outcomes (KDIGO.
Abbreviations: GFR, glomerular filtration rate; ESRD, end-stage renal disease.

CKD Guidelines

End-stage renal disease is a burdensome health issue for both patient and provider, and it typically requires major lifestyle alterations such as organ transplant or a surgical fistula and lifelong hemodialysis treatments, which last approximately four hours and are performed three times per week. The economic and public health burdens are also high, with total Medicare spending on both CKD and ESRD patients estimated to be $120 billion in 2017 alone (35). Chronic kidney disease is in the top 10 leading causes of premature mortality in the US, and its prevalence has increased from 12% to 15% of the total population since 1988 (34–36). Additionally, the risks associated with CKD and subsequent ESRD include the development of cardiovascular disease (CVD), which is the primary cause of premature mortality in the US (37). Still, diagnoses and testing for CKD

remain low. Approximately 9 in 10 adults with CKD do not know they have it (34). In those with diagnosed T2D and HTN, the rate of urine albumin testing is 43.2%, and approximately one third of individuals diagnosed with ESRD receive little or no pre-ESRD nephrology care (38). The lack of recognition of CKD by both patient and provider is concerning, but this leaves room for better diagnostic techniques to develop.

Much of the former and current research in the areas of obesity and MetS focuses on the cardiovascular and metabolic (i.e., T2D) consequences of the diseases. While these focuses are of highest importance, they have also been proven to be reversible in many cases (39–42). The development of CKD and advancement to ESRD, however, is permanent and therefore should elicit an effort to generate research findings which will better predict, identify, and prevent kidney damage before it results in ESRD, CVD, or premature mortality.

Utilizing large, representative, population-based datasets, and predictive equations, it may be possible to predict CKD before its clinical manifestation utilizing known correlates of the disease. For instance, study authors have reported (9) that renal function may begin to decline when BMI increases above 30 kg/m^2, regardless of body fat percentage (9). There is also strong evidence that hyperinsulinemia and subsequent stimulation of insulin-like growth factor-1 (IGF-1) have vasodilatory effects that contribute to glomerular hypertension and hypertrophy over time (43, 44). Finally, metabolic phenotypes, metabolic clusters, and their correlations with renal decline are underdiagnosed and understudied, leaving room for research to improve upon clinical practice, especially in the preservation of kidney health and prevention of ESRD.

Purpose

The primary purpose of this study is to establish the renal function in strictly defined metabolic phenotypes. The secondary purpose of this study is to identify the metabolic clusters that are associated with CKD.

Significance of the Problem

During the process of CKD, cumulative damage occurs in the kidneys, which eventually results in permanent failure of the organs, known as ESRD. In the state of ESRD, individuals must be dialyzed or receive a kidney transplant in order to sustain life. It is important to study the risk factors involved in this process in order prevent, slow, and reverse CKD progression. The results of this study will add to the growing body of literature relating to CKD and will help to establish diagnostic tools that improve our understanding and recognition of CKD. The potential implications of this research include a better understanding of the metabolic phenotypes, the pathology of comorbidities in the development of CKD, and the integration of electronic health record (I) systems in the creation of "flagged" phenotypes, which will inform physicians and patients of potential risk of CKD prior to the development of CKD and/or ESRD.

This research study provides a novel approach which utilizes a strict definition of metabolic health, allowing for no risk factors in the classification of the "metabolically healthy" phenotypes in association with CKD. We will determine the most common metabolic risk factors, constellations, and clusters associated with CKD. Additionally, we will utilize data from the National Health and Nutrition Examination Surveys (NHANES) and complex survey sample weighting. While prior studies have utilized metabolic

phenotypes in the study of CKD and others have used the strict definition of metabolic health in the study of cardiovascular disease, no studies have assessed the strictly defined metabolic phenotypes or metabolic clusters in association with CKD. The proposed study will also utilize the NHANES survey sample weighting strategy in assessing the role of metabolic phenotypes and metabolic clusters with CKD.

Research Questions, Hypotheses, and Rationale

Research Question 1

Are the intriguing metabolic phenotypes, which include the metabolically healthy obese (MHO) and the metabolically unhealthy normal weight (MUN), associated with decreased renal function as compared to the metabolically healthy normal weight (MHN)?

Hypotheses

H_0: The MHO phenotype will have the same association with renal function as compared to the MHN phenotype.

H_1: The MHO phenotype will have decreased renal function as compared to the MHN phenotype.

H_0: The MUN phenotype will have the same association with renal function as compared to the MHN phenotype.

H_1: The MUN phenotype will have decreased renal function compared to the MHN phenotype.

Rationale

A recent meta-analysis by Alizadeh et al. (31) analyzed nine prospective cohort studies that compared CKD risk among metabolic phenotypes. This pooled study concluded that those who had metabolic abnormalities yet were of normal weight had an increased risk of CKD (OR = 1.58, 95% CI = 1.28, 1.96), and those who were metabolically healthy and obese had a similarly increased risk (OR = 1.55, 95% CI = 1.34, 1.79). Those at highest risk of CKD were the overweight and obese individuals, regardless of metabolic status, which refutes the notion that the overweight or obese states are benign conditions. The proposed analysis will be novel in comparison to the meta-analysis by Alizadeh et al. due to the "strict" definition of metabolic health (45–47), which allows for no metabolic risk factors.

Research Question 2

Individual metabolic risk factors pose risk of chronic diseases, and the various combinations that result in the diagnosis of MetS can work synergistically to pose even greater risk. Metabolic constellations are groups of three or more metabolic risk factors that result in the diagnosis of metabolic syndrome. The metabolic constellations can be grouped in to "clusters", which emphasize hyperglycemia (Cluster I), hypertension (Cluster II), hyperglycemia and hypertension (Cluster III), or normoglycemia and normotension (Cluster IV). Through the study of metabolic clusters, we aim to answer the following question: Which metabolic cluster will have the lowest renal function?

Hypotheses

H_0: The metabolic clusters will demonstrate the same renal function.

H_1: The metabolic clusters will demonstrate differing renal function.

Rationale

The individual risk factors associated with MetS pose a risk for CKD. In the US, the primary risk factor for the development of CKD is type 2 diabetes mellitus (T2D) and the second most common risk factor associated with CKD is hypertension (HTN) (34, 38). Obesity, specifically central obesity, is directly linked to insulin resistance (48) and T2D. MetS is also linked to CKD, but the diagnosis of MetS can present in 16 different constellations, making up four metabolic clusters, potentially with different etiologies and outcomes. Grouping the metabolic constellations into clusters (33) that account for the two most common risk factors associated with CKD, hyperglycemia and hypertension, will further elucidate the most detrimental metabolic combinations associated with CKD.

Delimitations, Limitations, and Assumptions

Delimitations

1) The proposed research study will be delimited to the years 2013-2018, which includes the four most recent survey cycles of the National Health and Nutrition Examination Surveys (NHANES).
2) Subjects who were recruited, consented, surveyed, and sampled by the NHANES research team will be included in the present analysis.

3) The analytic guidelines for the NHANES given by the Centers for Disease Control and Prevention (CDC) will be utilized to combine survey cycles and in complex survey sample weighting.
4) All included individuals must have complete information for metabolic risk factors, including, BMI, WC, fasting blood glucose, fasting triglycerides, high-density lipoprotein (HDL), blood pressure, and prescription medication information.
5) The information utilized to calculate glomerular filtration rate (eGFR) will be required for each subject- this includes serum creatinine (SCr), age, sex, and race.
6) Subjects will be required to be adults (18 and over) and under the age of 80. This age limit is determined because individuals 80 years and older in the NHANES dataset are top coded at 80 for subject deidentification, therefore this variable cannot be controlled for over 79 years of age.
7) Subjects who report having received dialysis in the year prior to the study will be excluded the analyses.
8) Subjects who were pregnant at the time of their participation in the NHANES study will be excluded from the present analysis.

Limitations

1) This study will be a cross-sectional analysis, limiting our scope of interpretation. We will demonstrate the association between CKD and metabolic risk factors but will not be able to determine the temporal sequence of events leading to CKD.

2) All study procedures were conducted by research personnel on the NHANES research team. Therefore, we do not have control over the survey methodology and sampling techniques used.
3) Glomerular filtration rate (GFR) will be estimated using an equation that utilizes serum creatinine, which can be affected by muscle mass, muscle breakdown, and hydration status.
4) Markers of kidney disease should be measured twice, separated by three months, to diagnose chronicity of the disease. However, we are limited to a single measure in the study subjects measured by NHANES.
5) The measure of C-reactive protein should be measured twice, approximately 2 weeks apart, to obtain an average measure of inflammation. However, we are limited to a single measure in the study subjects measured by NHANES.

Assumptions

1) We assume that the measures were taken according to the NHANES protocol in every case and reported accurately.
2) We assume that survey questions were answered and reported accurately by study participants.

CHAPTER TWO

Literature Review

Chronic Kidney Disease

Chronic kidney disease (CKD) is a condition that results in progressive decline in kidney function over time. Estimates demonstrate that 9 in 10 adults with CKD are unaware of having it (34), and the disease goes largely undiagnosed. Most individuals are not diagnosed with CKD until they develop ESRD or cardiovascular disease (CVD). The primary cause of CKD in the US is type 2 diabetes mellitus (T2D), and the second leading cause is hypertension (HTN) (34, 38). Type 2 diabetes mellitus accounts for approximately 38% of reported cases of ESRD and HTN accounts for an estimated 26% of reported cases of ESRD in the US (34, 38).Still, other risk factors, such as obesity, cardiovascular disease, family history, age, sex, race, and ethnicity play roles in the epidemiology of CKD (34).

The prevalence of CKD in the US has increased from 12 to 15% in the past decade (34), indicating that approximately 37 million adults in the US have a GFR lower than 60 ml/min/1.73m^2. The prevalence of CKD increases concomitantly with age. Approximately 7% of individuals age 18-44 years have CKD, 13% of individuals age 45-64 years have CKD, and 38% of individuals 65 years and older have CKD. Female sex is associated with lower GFR. Women (15%) are more likely to have CKD than men (12%) (34). Race and ethnicity also play roles in the epidemiology of CKD. Non-Hispanic

Blacks have a higher prevalence (16%) than non-Hispanic Whites (13%) or non-Hispanic Asians (12%) (34). Approximately 14% of Hispanics are reported to have CKD (34).

The pathophysiology underlining the progression from T2D to CKD begins with lipid accretion in the muscle and liver cells. Excess adipose tissue causes insulin resistance via the accumulation of intramyocellular fatty acyl CoA and diacylglycerol (DAG) in the cell, which induce protein kinase Cθ (PKCθ), leading to the phosphorylation of serine 302 of insulin receptor substrate-1 (IRS-1) (10). Phosphorylation of serine 302 increases resistance to tyrosine phosphorylation of IRS-1 by the activated insulin receptor, causing downstream effects that result in reduced translocation of glucose transporter type 4 (GLUT-4) to the plasma membrane and a subsequent reduction in glucose uptake, glycogen synthesis, and lipogenesis (10, 48–50) (see Figure 2.1). Once the cell becomes resistant to insulin, there is a decreased intracellular suppression of hormone sensitive lipase (HSL). When HSL activity is upregulated, triglycerides are hydrolyzed, resulting in a greater release of free fatty acids (FFAs) and very low-density lipoproteins (VLDL) by the liver into the bloodstream (51). High circulating FFAs cause a shift towards hepatic gluconeogenesis and reduced insulin-stimulated glucose transport into the muscle cells (48).This process also results in reduction of HDL due to increased activity of cholesteryl ester transfer protein (CETP) and the transfer of cholesteryl esters from HDL to TG-rich lipoproteins (51, 52). Reverse cholesterol transport from the arterial wall is suppressed and atherosclerotic plaques can form.

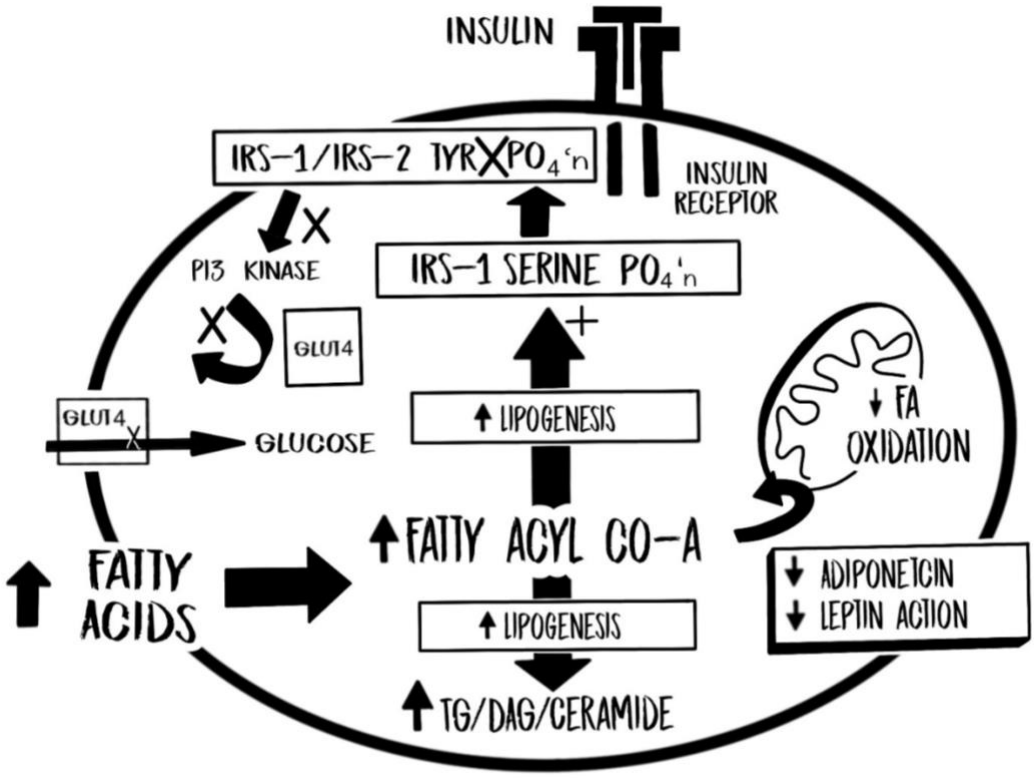

Figure 2.1. The process of insulin resistance in a skeletal muscle cell. An over-accumulation of triacylglycerols (TG) leads to a buildup of diacylglycerol (DAG), which activate protein kinase C (PKC) and phosphorylates insulin receptor substrate-1 (IRS-1) on the serine 302 residue. This prevents the auto-phosphorylation of tyrosine kinase and inhibits the recruitment of IRS-1 and 2. The downstream effects inhibit both the binding of phosphatidylinositol 3-kinases (PI3-kinase) to the IRS through its p85 subunit and the subsequent phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3). When the PIP3 concentrations do not increase, phosphoinositide-dependent kinase-1 (PDK1) and protein kinase B (PKB or AKT) are not recruited to the plasma membrane and AKT is not phosphorylated. Furthermore, the inhibition of the AKT substrate of 160 kDa (AS160) on glucose transporter type 4 (GLUT4) is not inactivated, and GLUT4 cannot undergo vesicular translocation to the cell membrane, rendering the cell incapable of glucose transport into the cell. This figure has been adapted, with permission, from Bagby et al., 2004[10] and the Journal of the American Society of Nephrology.

Insulin resistance within the cells results in elevated blood glucose, or hyperglycemia, which stimulates the beta cells of the pancreas to produce a compensatory amount of insulin, resulting in a state of hyperinsulinemia. When blood glucose levels are chronically elevated, excess blood glucose can be excreted by the kidneys via micturition. Chronic hyperglycemia can also cause advanced glycation end

products (AGEs), which result when proteins and/or lipids undergo non-enzymatic glycation and oxidation (51). One example of this is the case of hemoglobin, which is a blood protein that can become glycated. Hemoglobin A1C (HbA1C) is typically recycled every 120 days, and therefore can be used as a clinical marker to determine average levels of glycated hemoglobin over time, making it a proxy for chronic hyperglycemia. When 6.5% or more of hemoglobin becomes glycated, clinical diagnoses indicate insulin resistance.

The formation of AGEs cause cross-linking of collagen and elastin fibers that result in arterial stiffening (51, 53, 54). AGEs also promote inflammatory cytokines, upregulation of vascular endothelial growth factor (VEGF) (55), and dilation of the afferent arterioles in the kidney's glomeruli. At the same time, there is constriction of the efferent arteriole which is stimulated by endothelin-1 (ET-1) and the renin-angiotensin-aldosterone system (RAAS) (51). This high-pressure system in the Bowman's capsule causes glomerulosclerosis, or scarring of the small blood vessels of the kidney, known as glomeruli (56). In the early process of glomerulosclerosis, the kidneys will maintain filtration by shunting blood to working glomeruli, causing a state of hyperfiltration. Over time, hyperfiltration and inflammatory cytokines cause further scarring of the glomerular capillary wall, which consists of three interdependent components: 1) a layer of endothelial cells, 2) a glomerular basement membrane (GBM), and 3) epithelial cells called podocytes (57). High pressure in the glomeruli leads to increased GBM thickness and disrupts the macromolecular filtration, causing urinary albumin excretion (58). GBM thickening is followed by mesangial cell proliferation and expansion, which leads to declined renal function (59). The mesangial cell matrix invades the glomerular capillaries

and produces deposits known as Kimmelstiel-Wilson nodules (56, 60). Diabetic nephropathy is also characterized by podocyte detachment and widening of the slit membranes of the podocytes, which creates conduits for proteins to escape the glomerular circulation, and reduced endothelial fenestration, which decreases the glomerular hydraulic permeability (61). As these processes develop over time, damage occurs in the kidneys, eventually resulting in ESRD.

Hypertension is the second major contributor to CKD in the US (34, 35). Vascular disruption occurs in the form of vasoconstriction and systemic high blood pressure, manifesting as hypertension (HTN). Vascular disruption and HTN may precede insulin resistance and T2D but the downstream effects of insulin resistance are also contributors to endothelial dysfunction. During prolonged insulin resistance, systemic blood pressure rises due to multiple mechanisms acting simultaneously on the vascular endothelium. Insulin stimulates secretion of the vasoconstrictor endothelin-1 (ET-1) via the Ras-RAF-MAPK signaling pathway (62, 63). An early phenomenon of insulin resistance is elevation of ET-1 (64, 65), which has been found elevated in subjects with T2D (66–68). ET-1 may contribute to the development of endothelial dysfunction by chronic vasoconstriction and increased reactive oxygen species (ROS) circulating in the vasculature (69). In the kidneys, ET-1 is secreted by glomerular endothelial cells, epithelial cells, and mesangial cells, and causes activation of endothelin receptors, renal vasoconstriction, blunted sodium and water reabsorption, and increased glomerular proliferation (69). This results in a net increase in blood pressure due to resistance to blood flow and higher blood volume. Acutely, insulin signaling within the vascular endothelium stimulates activation of endothelial nitric oxide synthase (eNOS), which

produces the vasodilator nitric oxide (NO). However, in a prolonged insulin-resistant state, selective inhibition of phosphatidylinositol 3-kinase (PI3K) in endothelial cells blocks the effect of insulin on eNOS expression and increases the expression of adhesion molecules (70). Additionally, asymmetric dimethylarginine (ADMA), an inhibitor of NO (71), increases in the presence of native or oxidized LDL-cholesterol (72), and its clearance is blunted by an increase in oxidative stress. A decreased number of antioxidants and an increased number of ROS cause the oxidative stress that leads to decreased dimethylarginine dimethylaminohydrolase (DDAH) (73), thereby decreasing the capacity of DDAH to eliminate ADMA by conversion to L-citrulline (74). The blunting of ADMA conversion to L-citrulline by DDAH decreases the production of L-arginine, which is a substrate for the enzymatic reaction by which eNOS produces NO (71). The net resistance to blood flow is determined by the balance of vasodilator and vasoconstrictor effects on the vasculature. In the state of hyperinsulinemia, the scales tip towards vasodilation, and over time, manifest in the form of chronic hypertension.

Hypertension is also brought about by arteriosclerosis, or hardening of the arteries, which inhibits flexibility of the arteries through which blood flows and causes resistance to flow. At the level of the glomerulus, there is constriction of the afferent arteriole, which causes low blood pressure and renal ischemia. The low-pressure system causes release of renin from the adrenal gland and subsequent conversion of serum angiotensinogen to angiotensin I (ANGI). Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II (ANGII), which causes vasoconstriction as a method for increasing renal blood pressure. There is a concomitant increase in aldosterone, which causes retention of sodium, and therefore water, which further

increase blood pressure (56). This pathway results in a similarly destructive milieu within the kidneys, eventually causing renal damage, CKD, and ESRD.

In clinical settings, the categories of CKD (Table 1.1) are classified by calculating an estimated glomerular filtration rate (eGFR) using a blood or urine marker with an equation. The most commonly used marker for calculating eGFR is serum creatinine (SCr), as it is a waste product that is freely filtered across the glomerulus and is not reabsorbed in the tubules of the kidney, yet other markers such as cystatin C (CyC) and urine creatinine (uCR) are used as a standalone or in conjunction with SCr to estimate filtration rate. The two most commonly used equations in clinical practice are the 4-variable Modification of Diet in Renal Disease (MDRD) equation established by Levey et al. in 2006 (75) and the 4-variable Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation established by Levey et al. in 2009 (76). These equations both take into account SCr, age, race, and sex. The MDRD equation has been validated in numerous samples of individuals with and without CKD, and has demonstrated consistency in individuals with CKD, the elderly, and those who are African American (77–79). However, other reports indicate that the MDRD equation underestimates GFR in patients with a GFR between 90 and 60 ml/min/1.73m^2. Glomerular filtration rates are estimated using the MDRD study equation as follows:

$$\text{eGFR} = 175(SCr^{-1.154}) \times (age^{-0.203}) \times (0.742\ if\ female) \times\ (1.212\ if\ Black)$$

Where eGFR is the estimated glomerular filtration rate and SCr is serum creatinine.

The CKD-EPI equation is based on the same variables as the MDRD equation, but it uses different contributions for age, race, and sex, as well as a 2-slope spline to model the relationship between GFR and SCr. The CKD-EPI equation has been reported

to be more accurate than the MDRD equation in individuals with higher GFRs (76), resulting in reduced misclassification of CKD. The CKD-EPI equation is estimated as follows:

$$\text{eGFR} = 141 \times \min\left(\frac{\text{SCr}}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{SCr}{\kappa}, 1\right)^{-1.209} \times 0.993^{Age} \times 1.018\ (if\ female) \times 1.159\ (if\ Black)$$

Where eGFR is the estimated glomerular filtration rate, SCr is serum creatinine, κ is 0.7 if female or 0.9 if male, α is -0.329 if female or -0.411 if male, min is the minimum of $\frac{SCr}{\kappa}$ or 1, and max is the maximum of $\frac{SCr}{\kappa}$ or 1.

The eGFR is used to classify renal function in terms of percentage of function. Therefore, category 1 (or G1) indicates 90% or greater function of the kidneys. When kidney function drops below 60 ml/min/1.73m^2, mildly to moderately decreased renal function should be clinically diagnosed. This stage is associated with azotemia, which occurs when blood urea nitrogen (BUN) and SCr levels are noticeably increased in the blood. Reduced renal filtration may not be known before this point because of compensatory hyperfiltration in healthy nephrons. Therefore, reduced renal function may go undiagnosed until kidney function declines significantly. Category G5 is associated with uremia and ESRD, which is a life-threatening state. When kidney failure occurs, BUN and SCr levels can rise to toxic ranges and can result in death if not properly treated. Medical interventions which prolong or prevent death in patients with ESRD include dialysis and/or a kidney transplant. However, 5- and 10-year adjusted survival rates are extremely low, averaging 44.6% and 22.8%, respectively for those with ESRD (83).

Chronic kidney disease poses major personal and societal burdens due to the necessary life-altering treatments and costly medical care. The initiation of dialysis requires a commitment of 4 hours per day, 3 days per week, where patients must undergo machine dialysis in order to avoid uremia and/or death. According to the US Renal Data System (USRDS), one year of hemodialysis costs $72,000, and ESRD alone accounted for $36 billion of Medicare spending in 2017 (84). These numbers are projected to increase in the coming years due to the rising levels of obesity, metabolic syndrome, and insulin resistance that have occurred over the past several decades.

When identified early, CKD may be ameliorated or slowed, and ESRD may be prevented altogether. Previous reviews suggesting nutrition (85) and exercise intervention strategies are promising, but there is also room for improvement in clinical diagnoses. In the age of big data science, it may be possible to identify and flag medical patients who present with risk factors and/or unique phenotypes that are known correlates of disease. In the case of CKD, this scientific innovation is crucial because the damage is permanent, especially for those in the later stages.

The Metabolic Syndrome

The metabolic syndrome (MetS) was first identified and defined by Reaven et al. in 1988 (19). The syndrome has had many names including Syndrome X and the Insulin Resistance Syndrome (20). While many definitions have been reported in describing MetS, there is consensus that central adiposity and insulin resistance are the main characteristics of the disease. These two risk factors, however, are not enough to classify as a syndrome, because they would typically incite a single diagnosis of IR and/or T2D.

Rather, the MetS includes a constellation of three or more risk factors that are associated with both atherosclerotic cardiovascular disease (ASCVD) and T2D (20). The most widely recognized risk factors include atherogenic dyslipidemia, elevated blood pressure (BP), and elevated blood glucose with concurrent increases in prothrombotic and proinflammatory markers. There are many other components that serve as risk factors for the development of MetS, including older age, physical inactivity, atherogenic diet (e.g., diets high in saturated fat and cholesterol), and hormonal imbalance. It is important to note that only salient risk factors should be considered so as to provide the most parsimonious definition of MetS. However, MetS likely has multiple causes and multiple outcomes, given that it is not a discrete entity, but rather a 'syndrome', which manifests itself in various ways (20).

The most widely used definition of MetS to date is that of the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III (ATP III) (20). In this scientific statement, Grundy et al. (20) updated previous guidelines and developed a definition of MetS that was consistent with the scientific literature. By this definition, an individual who develops three or more of the following risk factors has MetS: waist circumference (WC) >101.6 cm in males or WC >88.9 cm in females; fasting blood glucose ≥ 100 mg/dL or prescription medication for hyperglycemia; triglycerides ≥ 150 mg/dL or lipid lowering medication; high-density lipoprotein (HDL) <40 mg/dL in males, HDL <50 mg/dL in females, or prescription medication; and blood pressure (BP) >130 mmHg systolic, BP >85 mmHg diastolic, or blood pressure medication (see Table 1.1). The authors of the NCEP ATP III guidelines note that it is important to recognize that MetS is not caused by a single factor, but that there is considerable variation among

individuals in how the disease presents. Therefore, these guidelines were adopted because they are "simple to use in a clinical setting" and avoid emphasis on a single cause (20).

One of the major causes of MetS is attributable to the rising rates of obesity that are secondary to the overconsumption of processed foods and physical inactivity in the US Visceral adipose tissue (VAT), in particular, has been identified as a major contributor of IR (20). In contrast, higher levels of subcutaneous fat and adipose tissue carried in the lower body do not have the same deleterious effects on metabolic health (86). Upper body, or central, obesity tends to be more metabolically active and releases adipokines, which contribute to an inflammatory state, as well as non-esterified fatty acids (NEFAs), which contribute to the accumulation of lipid in muscle and liver cells (20, 87–89).

It is important to note that not all MetS is caused by central obesity, such as in the case of the metabolically healthy obese phenotype (MHO). While this phenotype is relatively rare (prevalence rates range from 5.5 to 10% of the population (46, 90–92)), there is strong evidence that individuals can be obese yet metabolically healthy. The MHO phenotype may provide grounds for clinical study. If the protective mechanisms associated with the MHO phenotype are identified, it may be possible to isolate a given gene, hormone, metabolite, or behavior to treat metabolic risk factors. One of the most common demarcations, however, of the MHO phenotype is a younger average age as compared to their metabolically unhealthy counterparts. Another hallmark of the MHO phenotype is its transient nature, which has been identified in many longitudinal studies (46). It is rare that individuals classified as MHO will stay metabolically healthy for more than 5 to 10 years.

The projected outcomes of MetS are primarily ASCVD and T2D, but there are many other chronic conditions associated with MetS that deserve attention as well: non-alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), cholesterol gallstones, lipodystrophies, sleep apnea, and CKD (20). Addressing the various components associated with MetS can have beneficial secondary effects such as reduction or prevention of chronic conditions and mitigation of the risk of CV events and premature mortality. There is evidence that improving clinical markers such as reduction of low-density lipoprotein (LDL), management of BP, and reversal of IR are important in addition to improving lifestyle factors such as smoking cessation, improving diet, and engaging in physical activity (20).

The prevalence of MetS is increasing within the US. Over the last 3 decades, rates have risen from 25% to 34% (2), and there are fewer than 20% of individuals in the US who do not have at least one metabolic risk factor (90, 93). With rates on the rise, it is important that researchers and physicians work together to further refine the risk factors for MetS as well as outcomes of the disease. Greater knowledge and understanding of the etiology of chronic diseases may help ameliorate the impending health and financial burdens looming over our country.

Obesity

It is known that obesity is a major contributor to the chronic disease conditions that plague developed countries, and it is one of the largest ongoing domestic health battles in the US today. According to the CDC, the most recent obesity rate the US was 42.4%, and overweight status accounts for another third of the population, leaving less

than a third of the country at or below normal weight (4, 94). While these numbers are alarming, their implications are even more extreme. The sequelae of the rising obesity rates are the related rises in IR, T2D, CVD, NAFLD, cancer, CKD, and neurodegeneration (95).

While the complete etiology of obesity is still unknown, there is a vast body of literature indicating that it is multifaceted and extends beyond the simple explanation of "calories in, calories out". The social determinants of health, including one's economic stability, education access and quality, health care access and quality, neighborhood and built environment, and social and community context play determinant roles in access to health-promoting resources and healthy food. The environments where individuals are born, live, learn, work, play, worship, and age affect a wide range of risk factors and outcomes specific to obesity (96). Other causes that have been identified include neurotransmitter activity, changes in the epigenome and gut microbiota, and metabolic changes triggered by specific nutrients (5). It is hypothesized that obesity, much like anorexia nervosa, is rooted in a psychological disease that causes individuals to overeat. By this theory, overconsumption may be more of a symptom of disease than it is a cause itself. Increasing rates of depression (97) in the US coupled with longer work hours in sedentary jobs and mass availability of processed, energy-dense foods provide the 'perfect storm' to create the environment that we see in the US today.

The presentation and distribution of adipose tissue in the body are of greater importance than obesity alone. The term "adiposopathy" was first described by Bays et al. (98) and refers to the "sick fat" that contributes to chronic diseases. For instance, higher levels of the hormone cortisol can alter fat distribution to shift from the

subcutaneous and gluteal regions to the central region of the body (10). When this occurs, adipose tissue is predominantly deposited into the visceral adipose tissue (VAT). The VAT is associated with metabolic risk factors such as IR (99) and T2D (48, 100), higher than normal rates of morbidity, and all-cause mortality.

Visceral adipose tissue is recognized as an endocrine organ and is associated with elevated free fatty acids, macrophage infiltration, and the dysregulation of hormones and cytokines (10, 17, 95). The cytokines, or cell-signaling proteins, that are secreted by adipose tissue are known as adipokines. Both hormones and cytokines can be dysregulated by the onset of greater amounts of body fat, specifically fat accumulation in the abdominal region of the body (10, 20). As adipose tissue increases in the visceral, intramuscular, and hepatic regions, it promotes resistance to insulin, which is a hormone that stimulates glucose uptake in these organs. Insulin resistance begins to occur when lipids accumulate in the muscle and liver cells (Figure 2.1). This may be a compensatory cytoprotective response by the cells in order to prevent glucose-derived lipogenesis and subsequent overaccumulation of intracellular lipids (10, 48, 95). Adiponectin is one of the major adipokines that is dysregulated in obesity. In a healthy state, adiponectin regulates glucose levels and fatty acid oxidation and it has positive effects on inflammation, atherosclerosis, T2D, and IR (17). However, adiponectin is reduced in the obese state. Leptin is an adipokine that inhibits hunger and is increased in those with higher amounts of subcutaneous fat but it decreases with higher levels of VAT (10). As obesity ensues, leptin resistance occurs, downregulating leptin's hunger-mediating effects and exacerbating one of the major causes of obesity.

When adipose tissue increases significantly, it becomes hypoxic due to lack of blood and oxygen to supply the growing tissue (95, 101). The hypoxia contributes to necrosis (or cell death), macrophage infiltration into the adipose tissue, angiogenesis (or the making of new blood vessels), and inflammation (17). The proinflammatory adipose tissue associated with adiposopathy also causes activation of the immune system, which recruits a greater number of proinflammatory macrophages (95). Macrophages are active participants in the inflammatory process, producing inflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) (102). Interleukin-6 causes an acute phase response in the liver, stimulating the liver hepatocytes to synthesize and secrete C-reactive protein (CRP), which is an indicator of systemic inflammation. C-reactive protein is considered elevated above 1.0 mg/L, and high above 3.0 mg/L. The inflammatory state, which is signaled by CRP levels, can be acute or chronic, and is influenced by medications such as hormone therapy, pregnancy, birth control pills, and arthritis. Alternatively, statin drugs and non-steroidal anti-inflammatory drugs may lower CRP levels (103).

Metabolic risk factors do not always coexist with obesity, such as in the case of the metabolically healthy obese (MHO), indicating the possibility of a "healthy" form of obesity. The MHO phenotype has been described as a "favorable fat phenotype" characterized by the ability to store fat in adipose tissue, with lower VAT, higher adiponectin levels, less inflammation, and lower macrophage infiltration in adipose tissue (5, 104). The favorable fat is typically distributed in the gluteofemoral region, and has been demonstrated to have a beneficial adipokine profile, less risk of coronary heart disease, and T2D (105).

Additionally, dyslipidemia and insulin resistance do occur in individuals who are of normal weight. The term associated with this condition is commonly referred to as the metabolically unhealthy normal weight (MUN) phenotype. This important distinction is a reminder that accumulation of intracellular lipids and their metabolites (19, 24) (e.g., DAG and PKC) are the driving forces of insulin resistance, regardless of perceivable obesity status (48). The MUN phenotype can demonstrate "normal weight" measured by BMI and/or WC, yet have an unfavorable metabolic profile which exposes them to higher risk of IR, T2D, dyslipidemia, NAFLD, and coronary heart failure (106). VAT alone is sufficient to generate metabolic syndrome (10).

The Dual Roles of Obesity and Metabolic Syndrome in Association with CKD

The early risk factors associated with obesity traditionally present as inflammatory processes (107) and metabolic risk factors. The inflammatory cytokines secreted by the adipose tissue, such as leptin, IL-6, and TNF-alpha, are involved in both metabolic dysregulation as well as renal impairment (108).

Obesity was first linked to proteinuria and glomerulomegaly (glomerular hypertrophy) in a study by Weisinger et al. in 1974 (109), and abdominal obesity has since been linked to microalbuminuria (10, 110). The cytokines and adipokines secreted in the weight gain process may further contribute to the pathogenesis of CKD (31). Overweight ($25 \leq BMI \leq 30$), and obese ($BMI \geq 30$) individuals have a 40% to 80% increased risk of CKD, respectively (111). The pathogenesis from weight gain to renal damage likely begins with hyperinsulinemia. In the early stages, normoglycemia is maintained but structural changes begin to take place in the kidney. During this time,

glomerular hypertrophy occurs as a result of persistent high insulin levels and the retention of sodium and water that raise systemic blood pressure (56). In persistent insulin resistance, hyperglycemia eventually ensues, and insulin levels slowly decline. Animal models have demonstrated that when a high-fat diet is introduced, detrimental structural changes occur in the kidney in as little as 7 to 9 weeks (112). These changes include glomerulomegaly, Bowman's capsule expansion, glomerular cell proliferation, mesangial cell expansion, and glomerular and tubular basement membrane thickening (10). This group of factors quickly results in damage of the kidney which presents as proteinuria, or the leaking of protein into the urine (10).

An elevated BMI due to increased muscle mass has also been shown to cause high excretory load (9). A small study conducted by Schwimmer et al. in 2003 analyzed the renal function of non-obese subjects with a high body mass index ($BMI \geq 30 kg/m^2$) due to high muscle mass. These individuals were found to be at risk of developing a secondary form of focal segmental glomerulosclerosis similar to obesity-induced glomerulopathy (9). Even though evidence demonstrates signs of renal damage early in the weight gain process, it is still unknown where in the course of obesity and metabolic syndrome renal injury begins and where interventions should take place to prevent the irreversible loss of nephron (10).

Metabolic Phenotypes

Metabolic phenotypes are used to describe the interaction between metabolic risk factors, obesity, and an outcome. The definitions used for metabolic risk factors vary, but the most widely used and trusted source for determining metabolic risk factors is the

2005 revision of the NCEP ATP III guidelines (20). The metabolic risk factors associated with the NCEP ATP III were outlined to diagnose MetS, are designed to be "simple to use in a clinical setting", and include five factors: obesity, hyperglycemia, dyslipidemia (2 criteria), and hypertension.

When determining metabolic phenotypes, obesity is considered a separate criterion used to classify the phenotype status as normal weight, overweight, and/or obese. Obesity can be defined using waist circumference (WC), a measure of central adiposity, or body mass index, a ratio of weight to height. While both measurements are flawed and do not take into account all factors involved in the weight gain process, they are extremely beneficial and have a historically strong positive correlation with disease states and mortality rates. While some researchers make the determination to use BMI or WC to classify metabolic phenotypes based on available data (46), others have analyzed the metabolic phenotypes using both and found similar results (90). Various cutoff definitions have been suggested for BMI and WC alike. The most widely used definition for BMI is that set by the World Health Organization (WHO); normal weight is $18.5 \leq BMI < 25 kg/m^2$, overweight is $25 \leq BMI < 30 kg/m^2$, and obesity is $BMI \geq 30 kg/m^2$. Asian countries have used a more conservative cutoff for obesity of a $BMI \geq 25 kg/m^2$, and recent studies of metabolic phenotypes have classified Asian subjects separately using this criteria (113–115). Waist circumference cutoffs also vary, but the NCEP ATP III criteria indicates obesity at >88.9cm for females or >101.6cm for males. More conservative definitions have been encouraged (99, 116) for classifying central obesity at >80cm and >90-94cm for females and males, respectively, specifically in Asian populations.

Metabolically healthy or unhealthy status is determined by the four remaining metabolic risk factors: hyperglycemia, the two dyslipidemia criteria, hypertension, or prescription medication for treatment of any of these factors. Prior research in the area of metabolic phenotyping has heavily debated the number of risk factors that should be used to classify "metabolically unhealthy" status, with the most conservative definition being one or more (46, 90, 117, 118) risk factors, and the more liberal ranging from two or more (91, 115) to three or more (113, 114, 119, 120) risk factors. The discrepancies among the definitions of metabolic health have hindered comparability in prior studies, therefore, Lavie et al. (45) recently proposed a harmonized definition that classified the metabolically healthy phenotype as having zero of the four metabolic risk factors. This rationale is based on the notion that individuals with hyperglycemia, dyslipidemia, and/or hypertension cannot be considered "healthy" and therefore should not be classified as such (45). Several large studies (46, 90, 117, 118, 121) have recently used this definition, confirming its efficacy in classifying metabolic phenotypes.

When classifying obesity and metabolic health as dichotomous (yes/no) variables, these parameters result in four distinct categories or metabolic phenotypes: metabolically healthy normal weight (MHN), metabolically healthy obese (MHO), metabolically unhealthy normal weight (MUN) and metabolically unhealthy obese (MUO). While some of the literature (31) separates body size into three categories (normal weight, overweight, and obese), this is a less common distinction, and can result in a loss of parsimony in analyses. When too many subgroups are classified, the results may be more confusing or take on less meaning when extrapolating to a clinical setting.

The "strict" definition given for classifying metabolic health gives a clear distinction for what is meant by "health". By utilizing the most conservative criteria for health, researchers are able to determine the impact of a single metabolic risk factor on the outcome variable. This is especially important in classifying the MHO phenotype, as it represents an individual who is in a state of benign obesity. Prior efforts have been made to elucidate the differences between the "transient" and the "persistent" MHO phenotypes, but more work needs to be done. In identifying the factors that contribute to the transient state of the MHO phenotype, we may be able to elucidate the causative factors that undergird chronic diseases. Furthermore, the persistent MHO phenotype may help us uncover lifestyle and medical strategies for long-term prevention of disease.

The outcomes typically associated with the diagnosis of MetS are T2D and CVD, and much of the literature surrounding the metabolic phenotypes aims to determine long-term CVD risk (46, 90, 118). However, many disease outcomes can be better understood, correlated, and predicted when considering the metabolic phenotypes. For instance, a recent meta-analysis analyzed studies that used metabolic phenotypes to determine risk of CKD (31), but the definition of metabolic health varied, and the six studies included in the meta-analysis utilized a definition of metabolic health that allowed up to two metabolic risk factors to be considered "healthy". Nonetheless, there is a broad spectrum of medical outcomes that can be studied using metabolic phenotypes as a diagnostic and predictive tool. Clinical diagnoses may be more accurate and timelier when large-scale data analytics and routine clinical tests are married in an electronic health record system.

The frequency of the strict MHN phenotype is remarkably low in the US according to data collected within the past decade. Less than one in eight (93), or

approximately 12.3% (85) of the US population is free of obesity, hyperglycemia, dyslipidemia, hypertension, and is not on medication to treat a cardiometabolic risk factor. The strict MHO phenotype is the least prevalent in prior literature and accounts for approximately 5.5% (46, 90) of the population. However, less strict definitions of metabolic health estimate the MHO phenotype to range from 3% (122) to 11% (92, 119) or higher based on the defining parameters. The low prevalence of metabolically healthy phenotypes leaves a large gap that is filled by the metabolically unhealthy phenotypes, which account for approximately 77.8% of the population, with the largest group being the MUN phenotype, which was prevalent in 44.3% of the US population in the 2015-2016 NHANES sample (90). While obesity makes up nearly 40% of the population, 85.8% of those individuals have at least one metabolic risk factor, resulting in a prevalence rate of 33.5% of the population being classified as MUO (90).

"Intriguing" Metabolic Phenotypes

The recent meta-analysis by Alizadeh et al. identified the MHO phenotype as one of the most intriguing phenotypes due to its paradoxical nature. The seemingly counterintuitive phenotypes, MHO and MUN, account for the smallest and the largest phenotype groups in the US, respectively, when metabolic health is defined conservatively (90). These phenotypes are unique in that they do not fit into expected categories. Factors contributing to the intriguing metabolic phenotypes may include genetic predisposition, but more prominently, lifestyle factors may play major roles in how genes are expressed.

The MHO phenotype, specifically, is of special interest in recent literature because it presents a state of obesity, which is typically associated with inflammation and disease, in an individual who has no other conceivable risk factors or disease pathologies. While this phenotype has been established as somewhat transient in nature (46), there is potential for further study into what makes it transient. Even more intriguing, there is potential to study the persistent MHO phenotype, in which an individual remains in a consistent state of obesity over time without any associated metabolic consequences. Both the detrimental and the protective mechanisms associated with this phenotype could help inform future lifestyle, drug, and gene therapy to develop mechanisms which favor the persistent MHO state. Specific to chronic disease, a perpetual state of MHO which thwarts cardiovascular and renal damage could provide insight into how to protect some of the most vital organ systems in the body.

The MUN phenotype, originally established by Ruderman et al. in 1981 (123) as the "metabolically obese normal weight" is hypothesized to be characterized by hyperinsulinemia and central adiposity, despite meeting the standards for normal weight according to standard measures. This phenotype is also worthy of study in that it may be one of the greatest "trojan horses" in modern medicine (92, 120). This phenotype is often overlooked in clinical practice because individuals who present with the MUN phenotype have the relative appearance of physical health and therefore are not perceived as high risk. This metabolic phenotype, when persistent over time, may be just as detrimental as a chronic illness and may lead to unforeseen outcomes such as CV events, CKD, and premature mortality. In fact, a study by Aung et al. published in 2014 demonstrated that in unadjusted results, the MUN phenotype was at the highest risk of CVD as compared to

all other categories. Adjustment for age, sex, ethnic origin, and smoking attenuated these results slightly, but this phenotype is still among the highest risk in terms of CV events, comparable to the MHO and MUO phenotypes (46, 90).

Metabolic Clusters

An alternative view of the impact of metabolic risk factors on CKD is the assessment of various risk factors and combinations thereof which constitute MetS. Individual risk factors of MetS, including obesity, hyperglycemia, hypertension, and dyslipidemia pose a threat to health and increase proclivity for chronic diseases. The presence of at least one metabolic risk factor is associated with a greater risk of developing MetS over a 5 year period compared to those with zero risk factors at baseline (32). Approximately three-quarters of those with MetS at baseline have persistent MetS over a 5-year period and the remaining one-quarter go on to develop 4 or 5 risk factors after a 5-year follow-up (32).

The criteria for diagnosing MetS require the concurrent diagnoses of at least 3 of the 5 metabolic risk factors. These criteria result in 10 possible constellations of 3 risk factors, 5 possible constellations of 4 risk factors, and 1 possible constellation of 5 risk factors, representing 16 possible metabolic constellations. However, dividing MetS into 16 subcategories, or constellations, can drastically decrease the sample size and statistical power of the study. Therefore, metabolic clusters, which classify the constellations into 4 groups, can serve as a useful tool for understanding the most detrimental clustering phenotypes. This analysis avoids the issue of small sample sizes by combining the 16 metabolic constellations into the following four clusters (33): cluster I: subjects with at

least 3 metabolic risk factors, including hyperglycemia, cluster II: subjects with at least 3 metabolic risk factors, including hypertension, cluster III: subjects with at least 3 metabolic risk factors, including hyperglycemia and hypertension, and cluster IV: subjects with at least 3 metabolic risk factors, excluding hyperglycemia and hypertension. The metabolic clusters are associated with variations in cardiovascular and mortality risk, represented in the recent study by Khosravi et al. This original research investigation found that the cluster associated with stroke was cluster III, whereas the cluster associated with ischemic heart disease and CVD was cluster II (33). To the best of our knowledge, the four cluster categories outlined in the study by Khosravi et al. (33) have never been analyzed for association with CKD. This unique view of kidney and metabolic health may further elucidate the synergistic effects of metabolic risk factors, constellations, and clusters and their association with CKD.

The National Health and Nutrition Examination Survey

The Center for Disease Control and Prevention's (CDC) National Center for Health Statistics (NCHS) has many programs which are designed to produce health statistics for the US. The National Health and Nutrition Examination Survey (NHANES) (124) is a unique program within the NCHS because it combines interviews and physical examinations in order to provide information on diet, health habits, disease rates, and disease risk. The NHANES data, when properly analyzed, is designed to be representative of the US population. This survey system utilizes at-home visits and Mobile Examination Centers (MEC) to assess the health and nutritional status of approximately 5,000 adults and children each year. Participants in the NHANES study

are chosen from various counties within the US, and 15 counties are visited each year totaling 30 counties per survey cycle. The NHANES research team consists of a physician, a dentist, medical and health technicians, and dietary and health interviewers.

NHANES data is reported in 2-year cycles, with approximately 10,000 participants per cycle. Information from the interviews include demographic, socioeconomic, dietary, and health-related data whereas the physical examinations consist of medical and dental information, anthropometric measures, and laboratory tests (blood and urine). All data collected is immediately and automatically uploaded to a computer network, which lessens reporting error and speeds the process involved in disseminating the data.

CHAPTER THREE

Methodology

Complex Survey Sample Analysis

The design of the NHANES study includes a complex, four-stage, probability cluster. NHANES samples are taken every year in the US and reported on the CDC website in two-year cycles. The first of four stages of sampling begin with the county, which is the primary sampling unit (PSU). Samples are taken from 15 different counties within the US each year, totaling 30 counties per NHANES cycle. The second stage includes segments by which the counties are divided, which are determined by census blocks. The third stage further divides the census blocks into households followed by the fourth stage, which takes into account the individual survey subjects. The sample taken is limited to civilian, non-institutionalized individuals who live within the US. In order to increase the reliability and precision of estimates for underrepresented populations, oversampling of individuals 60 and over, African Americans, Asians, and Hispanics is routinely conducted.

Sample weights can be assigned to each individual in a sample in order to extrapolate the results to a represent all US non-institutionalized civilians. Sample weighting procedures are outlined by the NCHS Estimating and Weighting Procedures documents (125, 126). Weighting takes into account the known probability of selection, non-responders, and the differences between the sample and the US population as a whole. The sample weighting is conducted in three steps. The first accounts for the

oversampling of minority groups, the second adjusts for non-responders, and the third is a post-stratification that matches the sample to the known civilian, non-institutionalized population US which is determined by information from the US Census Bureau (124).

Merging Data

Single-year and single-cycle datasets can be unstable due to large variance and underrepresentation of subgroups. Therefore, for the following analysis, three two-year survey cycles (2013-2014, 2015-2016, and 2017-2018) have been combined to include data that spans the years from 2013 to 2018. Each individual cycle consists of multiple datasets which will be merged to form a single dataset. The present analyses include the following datasets from each survey cycle: demographic variables and sample weights (DEMO.XPT), dietary interview – individual foods, first and second days (DR1IFF.XPT and DR2IFF.XPT), dietary interview – total nutrient intakes, first and second days (DR1TOT.XPT and DR2TOT.XPT), blood pressure (BPX.XPT), body measures (BMX.XPT), albumin and creatinine – urine (ALB_CR.XPT), apolipoprotein B (APOB.XPT), cholesterol – high-density lipoprotein (HDL.XPT), cholesterol – low-density lipoprotein and triglycerides (TRIGLY.XPT), cholesterol – total (TCHOL.XPT), high-sensitivity C-reactive protein (HSCRP.XPT) in cases where applicable, insulin (INS.XPT), plasma fasting glucose (GLU.XPT), pregnancy test – urine (UCPREG.XPT), standard biochemistry profile (BIOPRO.XPT), alcohol use (ALQ.XPT), income questionnaire (INQ.XPT), kidney conditions – urology questionnaire (KIQ.XPT), medical conditions questionnaire (MCQ.XPT), physical activity questionnaire (PAQ.XPT), prescription medications questionnaire (RXQ.XPT), prescription

medications – drug information (RXQ_DRUG.XPT), smoking – cigarette use (SMQ.XPT), and smoking – recent tobacco use (SMQRTU.XPT).

Individual survey cycle datasets will be merged using a one-to-one merge. Data is first sorted using the PROC SORT function to sort the data by the survey participant identification number (variable name: SEQN). The data will be subsequently merged by the SEQN variable using a DATA step with MERGE option in SAS. The four cycles will be concatenated using the SET statement in a DATA step to produce aggregated estimates.

For the following project, complete case analysis will be conducted. The data files in each cycle do not contain the same number of records for each individual, such as in the case where an individual completes the questionnaire but not the examination. In this case, and in cases where important study variables are missing, the subject will be excluded from our analysis.

Study Sample

The inclusion criteria set for the present study include complete subject information for BMI, fasting blood glucose, fasting blood triglyceride, high-density lipoprotein-cholesterol (HDL-C), blood pressure (BP), serum creatinine (SCr), and race. Additionally, subjects are required to be adults (18 and over) and under the age of 80 at the time the sample was taken. The upper age limit was chosen because individuals 80 years and older in the NHANES dataset are top coded at 80 for subject deidentification, therefore age cannot be controlled for over 79 years.

The combined 2013-2018 samples span four NHANES survey cycles and six years of data collection. Collectively, there were 29,400 individuals selected for NHANES from 90 different study locations. Of those selected, 6,610 will be assessed for the metabolic phenotype analysis and 2,767 will be assessed in the analysis of constellations and clusters. The complex survey sample weighting will be used to extrapolate these values, which will be representative of the US population.

Definition of Metabolic Phenotypes

In the proposed study, obesity will be defined in two ways: using body mass index (BMI) or waist circumference (WC). In the case of BMI, obesity will be defined as a BMI $\geq$ 30 kg/m^2 for all non-Asian individuals and a BMI $\geq$ 25 kg/m^2 for all individuals identified as Asian. In the case of WC, obesity will be defined as a WC > 101.6 cm in males or WC > 88.9 cm in females for all non-Asian individuals and WC > 94 cm in males or WC > 80 cm in females for all individuals identified as Asian (99, 116).

Metabolic health will be defined as the absence of all metabolic risk factors in Table 1.1 as defined by the NCEP ATP III (20), excluding the measure of obesity. Therefore, the four metabolic phenotypes were defined as follows: metabolically healthy normal weight (MHN) which requires the absence of all metabolic risk factors and absence of obesity, metabolically healthy obese (MHO) which requires the absence of all metabolic risk factors and presence of obesity, metabolically unhealthy normal weight (MUN) which requires the presence of one or more metabolic risk factors and absence of obesity, and metabolically unhealthy obese (MUO) which required the presence of one or more metabolic risk factors and presence of obesity.

Definition of Metabolic Clusters

The metabolic clusters will be defined by all possible combinations of three or more metabolic risk factors using the NCEP ATP III criteria. This includes 10 possible combinations of 3 risk factors, 5 possible combinations of 4 risk factors, and 1 possible combination of 5 risk factors (see Table 3.1). The constellations will be grouped into four metabolic clusters (33) and will be subsequently analyzed (see Table 3.2): Cluster I, subjects with at least 3 metabolic risk factors, including hyperglycemia. This cluster consists of 4 subgroups; Cluster II, subjects with at least 3 metabolic risk factors, including hypertension. This cluster consists of 4 subgroups; Cluster III, subjects with at least 3 metabolic risk factors, including hyperglycemia and hypertension. This cluster consists of 7 subgroups; and Cluster IV, subjects with at least 3 metabolic risk factors, excluding hyperglycemia and hypertension. This cluster consists of 1 subgroup. See Table 3.3 for the definitions of metabolic phrases.

Table 3.1
Criteria for Metabolic Constellations

3 risk factors	4 risk factors	5 risk factors
WC, FG, BP	WC, FG, BP, TG	WC, FG, BP, TG, HDL
WC, FG, TG	FG, BP, TG, HDL	
WC, FG, HDL	BP, TG, HDL, WC	
WC, BP, TG	TG, HDL, WC, FG	
WC, BP, HDL	HDL, WC, FG, BP	
WC, TG, HDL		
FG, BP, TG		
FG, BP, HDL		
FG, TG, HDL		
BP, TG, HDL		

Cutoff values for all metabolic risk factors are outlined in Table 1. WC, high waist circumference; FG, high fasting glucose or hyperglycemia; BP, high blood pressure or hypertension; TG, triglycerides or dyslipidemia; HDL low, high-density lipoprotein or dyslipidemia, second separate criteria.

Table 3.2
Criteria for Metabolic Clusters

Cluster I	Cluster II	Cluster III	Cluster IV
FG, TG, HDL	HTN, TG, HDL	FG, HTN, TG	HDL, WC, TG
FG, TG, WC	HTN, TG, WC	FG, HTN, HDL	
FG, HDL, WC	HTN, HDL, WC	FG, HTN, WC	
FG, HDL, WC, TG	HTN, HDL, WC, TG	FG, HTN, HDL, TG	
		FG, HTN, WC, HDL	
		FG, HTN, WC, TG	
		FG, HTN, WC, TG, HDL	

Cutoff values for all metabolic risk factors are outlined in Table 1. WC, high waist circumference; FG, high fasting glucose or hyperglycemia; BP, high blood pressure or hypertension; TG, triglycerides or dyslipidemia; HDL, low high-density lipoprotein or dyslipidemia, second separate criteria. There is an emphasis of hyperglycemia in Cluster I, hypertension in Cluster II, hyperglycemia and hypertension in Cluster III, and normoglycemia and normotension in Cluster IV.

Table 3.3
Metabolic Phrases

Metabolic Phrase	Definition
Metabolic Risk Factors	The 5 cutoff values associated with the clinical diagnosis of metabolic syndrome as defined by the NCEP ATP III (2005 Revision) guidelines[20]. These include obesity, hyperglycemia, dyslipidemia (2 criteria) and hypertension.
Metabolic Syndrome	A medical diagnosis determined by at least 3 of the 5 metabolic risk factors.
Metabolic Health	The absence of all metabolic risk factors (hyperglycemia, dyslipidemia, and hypertension), with the exception of obesity.
Metabolic Phenotypes	The interaction of metabolic health and obesity status, resulting in 4 categories: metabolically healthy normal-weight, metabolically healthy obese, metabolically unhealthy normal-weight, and metabolically unhealthy obese.
Metabolic Constellations	All possible combinations of 3 or more metabolic risk factors that could be used to diagnose metabolic syndrome, resulting in 16 possible constellations.
Metabolic Clusters	Groups of constellations categorized into 4 groups, with an emphasis on hyperglycemia (Cluster I), hypertension (Cluster II), hyperglycemia and hypertension (Cluster III) or no hyperglycemia or hypertension (Cluster IV).

Definition of Renal Function and Chronic Kidney Disease

For demographic purposes, renal function will be measured using both the Modification of Diet in Renal Diseases (MDRD) study equation (76) and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (76), but all published reports will utilize CKD-EPI estimates only. CKD will be identified using the CKD-EPI

equation and is classified as an eGFR<60 ml/min/1.73m^2 (categories G3 to G5) or an albumin to creatinine ratio ≥30 mg/g (127). End-stage renal disease is defined as category G5 or an eGFR<15 ml/min/1.73m^2. All individuals who self-reported dialysis use within the year prior to the study will be excluded from the analyses.

Questionnaire and Demographics Data

The NHANES interview-style questionnaires include demographic, socioeconomic, dietary, health history and lifestyle information. For the present analysis, we will utilize information for age, sex, race and ethnicity, socioeconomic status (SES), total dietary intake, physical activity (PA), smoking, dialysis history, and prescription medications. Subjects who fall at or below 100% of the poverty level defined by the US federal government will be considered low SES. NHANES uses this cutoff, which is a common criterion for determining eligibility in federal assistance programs (124). A composite variable will be created to average the two-day dietary intake for each individual. Physical activity, reported in minutes per day and number of days per week, will be classified using the guidelines from the Physical Activity Guidelines Advisory Committee Report (128). Individuals will be considered physically active if they took part in ≥150 minutes of moderate-intensity physical activity or ≥75 minutes of vigorous-intensity physical activity per week, or an equivalent combination of the two (128, 129). Subjects are considered smokers if they have smoked at least 100 cigarettes in their lifetime or if they report having smoked in the past 5 days. All others will be considered "non-smokers". The percentage of glycated hemoglobin (HbA1c) will not be reported in the present study because its value was determined by questionnaire rather than a blood

panel. Alcohol intake will not be analyzed because the reporting method changed during the 2017-2018 cycle and could not be compared to prior surveys.

Examination and Laboratory Data

The NHANES examination includes anthropometric measures, blood pressure, blood panels and urinalysis. The BMI was calculated using height, which is measured in meters (m) on a calibrated stadiometer, and weight, which is measured on a calibrated digital weight scale or a portable scale. The waist circumference (WC) was taken at the level of the uppermost lateral border of the iliac crest and reported in centimeters (cm) for each subject. Three consecutive measures of blood pressure (BP) are taken after a 5-minute seated rest period. In cases where the BP measurement was interrupted or incomplete, a fourth measure was taken and reported. The present analysis will report the mean blood pressure for each subject by averaging the three available systolic and diastolic blood pressures. Fasting blood samples were taken and reported for blood lipids and blood glucose. The lipid sample was analyzed using the Roche/Hitachi Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN) and the serum low-density lipoprotein (LDL), expressed in milligrams per deciliter (mg/dL) was calculated utilizing the Friedewald calculation (130):

$$LDL = total\ cholesteol - HDL - \frac{Triglycerides}{5}$$

Fasting serum insulin was analyzed using the Tosoh AIA system analyzer and the fasting plasma glucose was analyzed using the Roche Cobas C311 system. The homeostatic model assessment of insulin resistance (HOMA-IR), which is a method utilized to

quantify insulin resistance and beta-cell function, will be calculated using fasting glucose (mmol/L) and fasting insulin (microU/L) in the following equation (131):

$$HOMA - IR = \frac{fasting\ glucose\ \times fasting\ insulin}{22.5}$$

Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured beginning in the 2015-2016 cycle of NHANES. TheBeckman UniCel DxC 600 and 600i Synchron chemistry analyzers were used to measure hs-CRP in the 2015-2016 cycle and the Roche cobas 6000 was used in the 2017-2018 cycles. Serum creatinine (SCr) was analyzed as part of the standard biochemistry profile using the DxC 800 chemistry analyzer. The estimated glomerular filtration (eGFR) rate will be calculated using the following MDRD (75) and CKD-EPI (76) equations:

MDRD:

$$\text{eGFR} = 175(SCr^{-1.154}) \times (age^{-0.203}) \times (0.742\ if\ female) \times\ (1.212\ if\ Black)$$

Where eGFR is the estimated glomerular filtration rate and SCr is serum creatinine.

CKD-EPI:

$$\text{eGFR} = 141 \times \min\left(\frac{\text{SCr}}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{SCr}{\kappa}, 1\right)^{-1.209} \times 0.993^{Age} \times 1.018\ (if\ female) \times 1.159\ (if\ Black)$$

Where eGFR is the estimated glomerular filtration rate, SCr is serum creatinine, κ is 0.7 if female or 0.9 if male, α is -0.329 if female or -0.411 if male, min is the minimum of $\frac{SCr}{\kappa}$ or 1, and max is the maximum of $\frac{SCr}{\kappa}$ or 1.

MDRD and CKD-EPI equations will be calculated, and but the CKD-EPI equation will be the sole value reported in the demographic tables. The CKD-EPI equation will be used to determine CKD (eGFR<60 ml/min/1.73m^2) and as the outcome

variable for major regression analyses. The CKD-EPI equation has been reported to be more accurate than the MDRD equation in individuals with higher GFRs (76).

Statistical Analysis

Statistical analyses will be conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Listwise deletion will be used for cases not meeting the study inclusion criteria. Masked variance pseudo-primary sampling unit (PSU), masked variance pseudo-stratum, and fasting subsample 2-year MEC weights from NHANES will be used for sample weighting. Unweighted demographic information will be described for the total sample with continuous variables reported as mean and standard deviation (SD) using the PROC MEANS procedure. Unweighted categorical variables will be reported as frequency and percentage (n, %) using the PROC FREQ procedure. Weighted data will be reported for the total sample, metabolic phenotypes, and metabolic cluster categories. Weighted continuous variables are reported as mean and standard error of the mean (SE) using the PROC SURVEYMEANS procedure and categorical variables will be reported as a percentage (%) and the standard error of percent (SE) using the PROC SURVEYFREQ procedure. The PROC CORR function will be used to determine Pearson's product moment correlation coefficients (r) in the case of two continuous variables. The PROC CORR function will be used to conduct point-biserial and point-polyserial correlations in the cases of one continuous and one dichotomous variable or one continuous and one polychotomous variable, respectively. Simple regression analyses of weighted data (PROC SURVEYREG) will be used to identify statistical differences between continuous variables and χ^2 tests using weighted data (PROC

SURVEYFREQ) will be used to identify statistical differences between categorical variables in the demographic output.

Logistic regression models (132) using weighted data (PROC SURVEYLOGISTIC) will be used to determine the odds of CKD in the four metabolic clusters (see equation below).

$$P(Y = 1) = \frac{1}{1 + e^{-(b_0 + b_1 x_1 + b_2 x_2 \ldots + b_n x_n)}}$$

Where $P(Y)$ is the probability of chronic kidney disease (CKD) occurring or not occurring using the values of the predictor variables. The value of Y is 1 if CKD is present and 0 otherwise, and x_1, x_2, …., x_n are the predictor variables. The constant β_0, determines the rightward or leftward shift of the curve, and the slope is determined by β_1, β_2, …, β_n. The natural log is taken, which allows the equation to be reported in terms of log-odds (logit), a linear function of the predictors. The slope coefficients (β_1, β_2, …, β_n) are the amount of logit change for every one-unit change in the predictor variables (x_1, x_2, …., x_n). The e term represents error.

Similarly, three linear regression models (132) using weighted data (PROC SURVEYREG) will be used to determine the influence of metabolic phenotypes and metabolic clusters on eGFR (see equation below).

$$Y = \beta_0 + \beta_1(x_1) + \beta_2(x_2) \ldots + \beta_n\ (x_n) + e_i$$

Where Y is the dependent variable, eGFR, β_0 is the intercept, β_1, β_2, …, β_n are the slope coefficients that give weight to the independent variables, x_1, x_2, …, x_n, according to their relative contributions in predicting the outcome variable, eGFR (132). The e_i term represents error.

CHAPTER FOUR

Metabolic Health, Obesity, and Renal Function: 2013-2018 National Health and Nutrition Examination Surveys

Abstract

Background: Rising rates of metabolic syndrome, obesity, and mortality from chronic kidney disease (CKD) have prompted further investigation into the association between metabolic phenotypes and CKD. Purpose: To report the frequency of strictly defined metabolic phenotypes, renal function within each phenotype, and individual risk factors associated with reduced renal function. Methods: We utilized the 2013-2018 National Health and Nutrition Examination Surveys (NHANES) and complex survey sample weighting techniques to represent 220 million non-institutionalized US civilians. Metabolic health was defined as having zero of the risk factors defined by the National Cholesterol Education Program with the exception of obesity, which was defined as BMI $\geq$ 30 kg/m^2 in non-Asians and BMI $\geq$ 25 kg/m^2 in Asians. Results: The metabolically healthy normal (MUN) phenotype comprised the highest proportion of the population (38.40%) whereas the metabolically healthy obese (MHO) was the smallest (5.59%). Compared to the MHN reference group, renal function was lowest in the strictly defined MUN (B= -9.60, p<0.001) and highest in the MHO (B= 2.50, p>0.05) and this persisted when increased number of risk factors were used to define metabolic syndrome. Systolic blood pressure had the strongest correlation with overall eGFR (r= -0.25, p<0.001) and individuals with low HDL had higher renal function compared to the overall sample. Conclusions: The MUN phenotype had the greatest association with poor renal function.

While the MHO had higher renal function, this may be due to a transient state caused by renal hyperfiltration. Further research should be done to investigate the association between dyslipidemia and CKD.

Keywords: Chronic Kidney Disease, CKD, Metabolic Phenotypes, Obesity, Metabolic Risk Factors

Introduction

In the past three decades the incidence of end-stage renal disease (ESRD) has increased by approximately 93% (1) and chronic kidney disease (CKD) is the 3rd fastest growing cause of premature mortality (2). CKD is a costly (3) and burdensome health issue which more often results in premature mortality than in ESRD (4). Steady increases in rates of metabolic syndrome and obesity are occurring in the US, with both conditions recently exceeding previous levels at 34.2% (5) and 42.6% (6) of the US population, respectively. Approximately 15% of US adults are estimated to have CKD (7), and it is likely that the prevalence will increase given the associations of CKD with metabolic risk factors such as type 2 diabetes mellitus (T2D), hypertension (HTN) (7), and obesity (7–9).

Metabolic phenotypes, which take into account metabolic risk factors and obesity, have been utilized to assess the risk of various outcomes such as cardiovascular disease (CVD), mortality, and CKD. A recent meta-analysis by Alizadeh et al. (10) analyzed nine prospective cohort studies that compared CKD risk among metabolic phenotypes and found that the metabolically healthy obese (MHO) and the metabolically unhealthy

normal weight (MUN), termed the "intriguing" phenotypes, shared similarly elevated risk of developing CKD with pooled relative risks (RR) of 1.55 and 1.58, respectively. This meta-analysis included studies with primarily Asian populations, limiting generalizability, and the definitions of the metabolic phenotypes varied, hindering the comparability between studies.

Prior research in the area of metabolic phenotyping has reported equivocal findings regarding the number of risk factors used to define the "metabolically unhealthy" status, with the most strict definitions determining that one or more (11–14) risk factors should be considered unhealthy, and the more liberal ranging from two or more (15, 16) to three or more (17–20) risk factors. A recent publication by Lavie et al. (21) proposed a harmonized definition that classifies the metabolically healthy phenotype as having zero of the four metabolic risk factors. This rationale is based on the notion that individuals with hyperglycemia, dyslipidemia, and/or hypertension cannot be considered "healthy" and therefore should not be classified as such (21). Several large studies (13, 14, 22) have previously used this definition, and several more (11, 12, 23) have adopted it since it was first proposed by Lavie *et al*.

In this study our primary purpose is to report the prevalence of the strict metabolic phenotypes in the US population utilizing NHANES data and complex survey sample weighting. Additionally, we will report the association between renal function and the metabolic phenotypes utilizing the three most common definitions of metabolic health. Lastly, we will identify and report the individual risk factors associated with reduced renal function.

Methods

The institutional review board at Baylor University determined the present study exempt from review [IRB ID# 1505514-1]. The project was classified as non-human subjects research because the data are deidentified and widely available for use via the CDC. Survey sample weighting, which includes a complex, four-stage, probability cluster, was utilized for the present analyses. Sample weighting procedures are outlined by the National Center for Health Statistics Estimating and Weighting Procedures documents (24, 25).

Study Sample

The National Health and Nutrition Examination Surveys (NHANES) are studies conducted in 2-year cycles by the Centers for Disease Control and Prevention (CDC). The NHANES design utilizes complex survey sample weighting procedures to produce nationally representative health statistics for the US. The population sampled by NHANES was limited to civilian, non-institutionalized individuals who lived within the US at the time of sampling. In order to increase the reliability and precision of weighted estimates for underrepresented populations, oversampling of individuals 60 and over, African Americans, Asians, and Hispanics was routinely conducted. Sample weights were assigned to each individual in a sample in order to extrapolate the results to a represent all US non-institutionalized civilians.

Three cycles of NHANES data, including the 2013-14, 2015-16, and 2017-18 cycles, were merged for 29,400 subjects. The data for the present study were a subset using criteria that required subjects to be adults (18 and over) and under the age of 80 at

the time the sample was taken (n=12,594 excluded). The upper age limit was chosen because individuals 80 years and older in the NHANES dataset are top coded at 80 for subject deidentification, therefore age cannot be controlled for over 79 years. Subjects who self-reported pregnancy and/or tested positive on a pregnancy test were excluded from analysis (n=190 excluded). Subjects who did not have complete information to classify metabolic phenotype, including fasting glucose, fasting triglycerides, high-density lipoprotein (HDL), blood pressure, and body mass index (BMI), were excluded from analysis (n=9,988 excluded). Lastly, those who reported use of dialysis in the 12 months prior to the study were excluded (n=18 excluded), resulting in a final sample of 6,610 study subjects.

Definition of Metabolic Phenotypes

Metabolic risk factors were defined using criteria from the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III (ATP III) (26), with the exception of obesity, which was defined as a BMI $\geq$ 30 kg/m^2 for all non-Asian individuals and a BMI $\geq$ 25 kg/m^2 for all individuals identified as Asian (16, 18, 20). Metabolically healthy or unhealthy status was determined by the four remaining metabolic risk factors: hyperglycemia, which was defined as a fasting glucose $\geq$ 100 mg/dL or prescription medication for hyperglycemia; the two dyslipidemia criteria, which were defined as a fasting triglyceride $\geq$ 150 mg/dL, a high-density lipoprotein level < 40 mg/dL for males, < 50 mg/dL for females, or a prescription medication for dyslipidemia; and hypertension was defined as a resting systolic blood pressure > 130 mmHg, a resting diastolic blood pressure > 85 mmHg or prescription medication for

hypertension (Table 4.1). In the primary analyses, metabolic health was defined as the absence of all metabolic risk factors in Table 4.1 excluding the measure of obesity. Therefore, the metabolically healthy normal weight (MHN) phenotype was defined as the absence of all metabolic risk factors and absence of obesity; metabolically healthy obese (MHO) required the absence of all metabolic risk factors and presence of obesity; metabolically unhealthy normal weight (MUN) required the presence of one or more metabolic risk factors and absence of obesity; and the metabolically unhealthy obese (MUO) required the presence of one or more metabolic risk factors and presence of obesity.

Table 4.1.
Criteria for Metabolic Risk Factors and Metabolic Phenotypes

Category	Classification	Values
Metabolic Risk Factor	Obesity	Non-Asian BMI ≥ 30 kg/m^2, Asian BMI ≥ 25 kg/m^2
	Hyperglycemia	Fasting glucose ≥ 100 mg/dL or Rx
	Dyslipidemia (2^{nd} criteria)	TG ≥ 150 mg/dL or Rx HDL < 40 mg/dL (M), < 50 mg/dL (F); or Rx
	Hypertension	> 130 mmHg systolic or > 85 mmHg diastolic or Rx
Metabolic Phenotype	MHN	Non-obese and < 1 metabolic risk factor
	MHO	Obese and < 1 metabolic risk factor
	MUN	Non-obese and > 1 metabolic risk factor
	MUO	Obese and > 1 metabolic risk factor

Metabolic syndrome is defined by the NCEP ATP III (2005 Revision) guidelines (26). BMI is calculated as weight (kg) divided by height (m^2). Rx, prescription medication for given risk factor; TG, triglycerides; HDL, high density lipoprotein; M, males; F, females; MHN, metabolically healthy normal weight; MHO, metabolically healthy obese; MUN, metabolically unhealthy normal weight; MUO, metabolically unhealthy obese.

Renal Outcome Measures

Renal function was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (27):

$$\text{eGFR} = 141 \times \min\left(\frac{\text{SCr}}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{SCr}{\kappa}, 1\right)^{-1.209} \times 0.993^{Age} \times 1.018\ (if\ female) \times 1.159\ (if\ Black)$$

Where eGFR is the estimated glomerular filtration rate, SCr is serum creatinine collected as part of the standard biochemistry profile using the DxC 800 chemistry analyzer, κ is 0.7 if female or 0.9 if male, α is -0.329 if female or -0.411 if male, min is the minimum of $\frac{SCr}{\kappa}$ or 1, and max is the maximum of $\frac{SCr}{\kappa}$ or 1. The CKD-EPI equation has been reported to be more accurate than the MDRD equation in individuals with higher GFRs (27). CKD was defined as an eGFR<60 ml/min/1.73m^2 (categories G3 to G5) and/or an albumin to creatinine ratio ≥30 mg/g (28). All individuals who reported use of dialysis in the 12 months prior to the study were excluded from the analyses.

Questionnaires, Examinations and Laboratory Data

The NHANES interview-style questionnaires include demographic, socioeconomic, dietary, health history, and lifestyle information. Age, binary sex, and race/ethnicity were determined by questionnaires that were asked in the home by trained interviewers using the Computer-Assisted Personal Interview (CAPI) system. Total caloric intake was determined using two 24-hour dietary interviews and a composite variable was created to average dietary intake for two-day samples. Dietary intakes were assessed on all days of the week with the 2 measurements typically separated by 3 days. Eighteen percent of the dietary intake information was missing in the present sample. Subsample weights (WTDR2D sample weigh variable) were utilized to marginally adjust for race and Hispanic origin, age group, sex, weekday-weekend categories, and day two

non-responders. SES was determined by dividing family (or individual) income by the poverty guidelines defined by the US federal government. Subjects who fell at or below 100% of the poverty level for the given year, which is a common criterion for determining eligibility in federal assistance programs (29), were considered low SES. Physical activity (PA), reported in minutes per day and number of days per week, was classified using the guidelines from the PA Guidelines Advisory Committee Report (30). Individuals were considered physically active if they took part in ≥150 minutes of moderate-intensity recreational PA per week, ≥75 minutes of vigorous-intensity recreational PA per week, or an equivalent combination of the two (30, 31). Implausible PA values were reported in this sample, therefore, values ≥4 hours per day of recreational PA were top-coded at 4 hours. There was 49% missingness in the PA variable. Subjects were considered smokers if they have smoked at least 100 cigarettes in their lifetime or if they report having smoked in the past 5 days. All others were considered "non-smokers". International Classification of Diseases, Tenth Revision (ICD-10) codes were used to determine prescription medication (Rx) information for hyperglycemia (R73, E11, E11.2, E11.2P, E11.4, and E11.P), hypercholesterolemia (E78.0, E78.0P, and E78.1), and hypertension (I10 and I10.P).

The NHANES examination includes anthropometric measures, blood pressure, blood panels and urinalysis. BMI was calculated using height, which is measured in meters (m) on a calibrated stadiometer, and weight, which is measured on a calibrated digital weight scale or a portable scale. The waist circumference (WC) was taken at the level of the uppermost lateral border of the iliac crest and reported in centimeters (cm) for each subject. Three consecutive measures of blood pressure (BP) are taken after a 5-

minute seated rest period. In cases where the BP measurement was interrupted or incomplete, a fourth measure was taken and reported. The present analysis reported the mean blood pressure for each subject by averaging the three available systolic and diastolic blood pressures. Fasting blood samples were taken and reported for blood lipids and blood glucose. The lipid sample was analyzed using the Roche/Hitachi Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN, USA) and the serum low-density lipoprotein (LDL), expressed in milligrams per deciliter (mg/dL) was calculated utilizing the Friedewald calculation (32). Fasting plasma glucose was analyzed using the Roche Cobas C311 system. Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured beginning in the 2015-2016 cycle of NHANES, therefore 36.7% of the sample has missing values for this variable since it was not collected in the 2013-2014 cycle. The Beckman UniCel® DxC 600 and 600i Synchron chemistry analyzers (Beckman Coulter, Brea, CA, USA) were used to measure hs-CRP in the 2015-2016 cycle and the Roche Cobas 6000 was used in the 2017-2018 cycles. The homeostatic model assessment of insulin resistance (HOMA-IR), which is a method utilized to quantify insulin resistance and beta-cell function, was calculated using the following equation (33): fasting glucose (mmol/L) × fasting insulin (microU/mL)/22.5. The albumin to creatinine ratio was reported in mg/g utilizing the fluorescein immunoassay by Sequoia-Turner Digital Fluorometer, Model 450 (Sequoia-Turner Corporation, Mountain View, CA, USA) to determine urinary albumin and the Roche Cobas 6000 Analyzer was used to measure urinary creatinine.

The percentage of glycated hemoglobin (HbA1c) was not reported in the present study because its value was determined by questionnaire rather than a blood panel.

Alcohol intake was not analyzed because the reporting method changed during the 2017-2018 cycle and could not be compared to prior surveys.

Statistical Analysis

Statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). A DOMAIN statement was used to analyze the subpopulation meeting study inclusion criteria. Masked variance pseudo-primary sampling unit (PSU), masked variance pseudo-stratum, and fasting subsample 2-year mobile examination center (MEC) weights from NHANES were used for sample weighting. Unweighted demographic information was represented using means and standard deviations (SD) for continuous variables or frequencies and percentages (n, %) for categorical variables. Weighted demographic data was reported for the total sample and metabolic phenotypes using a weighted mean and standard error of the mean (SE) for continuous variables or a percentage (%) and the standard error of percent (SE) for categorical variables. Simple regression analyses of weighted data were used to identify statistical differences between continuous demographic variables. Chi square (χ^2) tests were used to identify statistical differences between categorical demographic variables. Pearson's product moment correlation coefficients (r) were used to identify correlations between two continuous variables. Linear regression models with complex survey sample weighting were used to determine the influence of metabolic phenotype on renal function. In model 1, we considered one metabolic risk factor to be unhealthy, in Model 2, we considered 2 risk factors to be unhealthy, and in Model 3, we considered 3 risk factors to be unhealthy. For all analyses, the level of significance was set *a priori* at α=0.05.

Results

The weighted sample population of 6,610 subjects who met the study inclusion criteria represented 220,388,819 non-institutionalized US civilians. The weighted and unweighted demographic data are represented in Table 4.2. The prevalence of obesity was 42.49% with an average BMI of 29.4 (SE=0.18). The prevalence of individuals with at least one metabolic risk factor (excluding obesity) was 75.30%, and only 19.11% of the sample was metabolically health and non-obese. The most frequent metabolic phenotype was the MUN phenotype (38.40%) followed by the MUO (36.90%), and the phenotype that represented the smallest proportion of the sample was the MHO (5.59%). The metabolically unhealthy phenotypes were more likely to be male, older age, current or former smokers, have metabolic risk factors, and have poor renal function, whereas the metabolically healthy individuals tended to have higher HDL-cholesterol and reported that they engaged in greater amounts of recreational physical activity. The obese phenotypes were more likely to be female, non-Hispanic (NH) Black Americans, and have higher levels of hs-CRP, whereas the normal weight individuals were more likely to be NH White or NH Asian, despite more conservative obesity cutoff values for NH Asians. There was no statistically significant difference between phenotypes for daily caloric intake or frequency of individuals with low-SES.

Table 4.2.

Demographic Information for Subsample from the 2013-2018 National Health and Nutrition Examination Survey

	Unweighted Total (n = 6,610)	Weighted Total (n = 220,388,819)	MHN (19.11%)	MHO (5.59%)	MUN (38.40%)	MUO (36.90%)	*p*-value
Demographic Variable	mean (SD)	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)	
Age (years)	47.03 (17.04)	45.61 (0.37)	35.72 (0.58)	36.22 (0.86)	49.31 (0.57)	48.31 (0.52)	<0.001
BMI (kg/m^2)	29.4 (7.33)	29.40 (0.18)	23.42 (0.13)	33.48 (0.28)	25.33 (0.09)	36.10 (0.24)	<0.001
Waist Circumference (cm)	99.35 (17.15)	99.83 (0.43)	83.75 (0.40)	105.93 (0.84)	92.25 (0.30)	115.19 (0.50)	<0.001
Caloric intake (Kcal/day)	2048 (853)	2087 (17)	2083 (43)	2032 (56)	2123 (25)	2058 (27)	0.207
Fasting Glucose (mg/dL)	110.71 (37.50)	107.74 (0.49)	91.54 (0.26)	92.41 (0.38)	108.35 (0.65)	117.83 (0.83)	<0.001
Triglycerides (mg/dL)	115.59 (112.38)	114.16 (1.70)	66.21 (1.21)	75.35 (1.81)	117.73 (2.03)	141.14 (3.20)	<0.001
HDL (mg/dL)	53.75 (16.11)	54.29 (0.36)	64.82 (0.65)	59.06 (1.07)	54.49 (0.53)	47.90 (0.37)	<0.001
LDL (mg/dL)	111.22 (35.56)	111.38 (0.72)	100.91 (1.32)	109.55 (1.79)	113.90 (1.13)	114.55 (1.01)	<0.001
Systolic BP (mmHg)	123.31 (18.00)	121.41 (0.29)	110.05 (0.40)	113.55 (0.49)	122.64 (0.44)	127.19 (0.37)	<0.001
Diastolic BP (mmHg)	70.13 (12.28)	70.30 (0.29)	65.32 (0.32)	68.11 (0.62)	70.49 (0.42)	73.01 (0.33)	<0.001
eGFR (ml/min/1.73m^2)	97.7 (22.17)	97.16 (0.50)	103.93 (0.91)	106.44 (1.25)	94.34 (0.64)	95.19 (0.61)	<0.001
hs-CRP (mg/L)	4.15 (8.25)	3.80 (0.18)	1.39 (0.07)	4.49 (0.52)	2.92 (0.26)	5.68 (0.28)	<0.001
ACR (mg/g)	41.66 (291.46)	29.14 (2.78)	16.70 (2.33)	10.49 (3.07)	23.34 (2.93)	44.45 (6.20)	<0.001
HOMA-IR	4.22 (8.52)	3.77 (0.10)	1.35 (0.03)	2.51 (0.10)	2.63 (0.07)	6.43 (0.22)	<0.001
	n (%)	% (SE)	% (SE)	% (SE)	% (SE)	% (SE)	P-value
Male Sex	3205 (48.49)	49.39 (0.67)	40.14 (2.28)	36.76 (3.24)	56.72 (1.48)	47.01 (1.44)	<0.001
Race/Ethnicity							
Mexican American	1041 (15.75)	9.49 (1.12)	8.02 (1.09)	8.97 (2.29)	9.06 (1.10)	10.78 (1.30)	<0.001
Other Hispanic	731 (11.06)	6.49 (0.79)	6.87 (1.34)	7.29 (1.75)	6.99 (0.85)	5.66 (0.65)	
NH White	2353 (35.60)	63.36 (1.98)	68.02 (2.66)	48.17 (4.57)	67.21 (1.89)	59.23 (2.44)	
NH Black	1376 (20.82)	11.29 (1.11)	6.77 (0.98)	30.32 (3.53)	3.54 (0.52)	18.81 (1.89)	
NH Asian	849 (12.84)	5.55 (0.52)	7.45 (0.76)	2.15 (0.63)	8.97 (0.97)	1.52 (0.16)	
Other/Multi-Racial	260 (3.93)	3.83 (0.40)	2.88 (0.58)	3.10 (1.01)	4.24 (0.59)	4.00 (0.61)	
Low SES	1355 (22.69)	15.43 (1.05)	13.10 (1.46)	16.56 (1.99)	15.22 (1.23)	16.67 (1.47)	0.143
CKD	966 (14.61)	12.07 (0.52)	6.31 (1.05)	3.60 (1.05)	11.73 (0.77)	16.70 (0.83)	<0.001
Physically Active	2317 (69.98)	69.38 (1.08)	77.93 (1.83)	76.24 (3.30)	67.79 (1.98)	62.88 (2.03)	<0.001
Smoker	2981 (45.10)	46.28 (1.26)	37.38 (2.46)	40.78 (3.55)	49.76 (1.57)	48.09 (1.43)	<0.001
Glucose Medication	797 (12.06)	9.14 (0.53)	0	0	8.57 (0.76)	15.86 (1.00)	<0.001
Cholesterol Medication	1206 (18.25)	17.27 (0.67)	0	0	21.64 (1.20)	24.29 (1.31)	0.158
Hypertension Medication	1678 (25.39)	22.09 (0.88)	0	0	23.23 (1.47)	35.69 (1.51)	<0.001

Metabolically healthy status is defined as having 0 risk factors, with the exception of obesity. P-values indicate a significant difference between the four metabolic phenotypes for the given variable. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BP, blood pressure; eGFR, estimated glomerular filtration rate using the CKDEPI equation; hs-CRP, high-sensitivity C-reactive protein; ACR, albumin to creatinine ratio; HOMA-IR, homeostatic model assessment for insulin resistance; NH, Non-Hispanic; SES, socioeconomic status; CKD, chronic kidney disease, determined by eGFR<60 and/or ACR ≥30. There was 18% missingness in the Caloric Intake variable, 49% in the Physically Active variable, and 36% missingness in the hs-CRP variable.

The linear regression analyses in Table 4.3 utilized three consecutive models to demonstrate eGFR in the metabolic phenotypes ranging from a strict definition of metabolic health to the conventional definition outlined by the NCEP ATP III. The most conservative definition defined metabolic health as 0 risk factors with the exception of obesity, where the frequency of MHN was 19.11%, MHO was 5.59%, MUN was 38.40%, and MUO was 36.90%. When metabolic health was defined as 1 or fewer metabolic risk factors, the frequency of each phenotype shifted towards metabolically healthy: MHN accounted for 36.67% of the population, MHO was 15.56%, MUN was 20.85%, and MUO was 26.92%. Further shifts towards the metabolically healthy phenotypes were demonstrated when metabolic health was defined as 2 or fewer metabolic risk factors: MHN accounted for 48.64% of the population, MHO was 27.36%, MUN was 8.88%, and MUO was 15.12%. Across models, the eGFR in the MHO phenotype was slightly higher than that of the reference although this association was not found to be significantly different. The MUN and MUO phenotypes had significantly lower eGFR than the reference group (MHN). Across all three models, the MUN phenotype consistently demonstrated the lowest average eGFR compared to all other phenotypes. This finding is consistent with the demographic information represented in Table 4.2.

Table 4.3.
Linear Regression Analyses of Metabolic Phenotypes

	Model 1[a]		**Model 2[b]**		**Model 3[c]**	
Coefficient	*B*	*SE B*	*B*	*SE B*	*B*	*SE B*
Intercept (MHN)	*103.93*	*0.91*	*101.98*	*0.79*	*99.43*	*0.73*
MHO	2.50	1.42	2.03	1.15	1.54	0.80
MUN	**-9.60****	0.80	**-12.30****	1.09	**-12.33****	1.20
MUO	**-8.74****	0.96	**-9.55****	1.01	**-10.53****	0.96
R^2	0.042		0.077		0.059	

* $p < 0.05$, ** $p < 0.001$. [a]Metabolic health defined as 0 metabolic abnormalities (except for obesity) and 1 risk factor considered unhealthy. [b]Metabolic health defined as 1 metabolic abnormality (except for obesity) and 2 risk factors considered unhealthy. [c]Metabolic health defined as 2 metabolic abnormalities (except for obesity) and 3 risk factors considered unhealthy.

Correlates of eGFR are demonstrated in Table 4.4. The risk factors found to be most closely associated with low overall eGFR were systolic blood pressure and waist circumference (r= -0.250, p<0.01 and r= -0.175, p<0.01, respectively). The measure of eGFR in the MUN phenotype demonstrated similar relationships with waist circumference and systolic blood pressure (r= -0.282, p<0.01 and r= -0.269, p<0.01, respectively). In the MHO phenotype, which included individuals with no risk factors, fasting triglyceride levels were the only risk factor and it had a modest negative relationship with eGFR (r= -0.159, p<0.05). In this group, obesity and waist circumference measurements did not have significant relationships with eGFR.

Table 4.4.
Correlates of eGFR

Risk Factor		Overall eGFR	MHO eGFR	MUN eGFR
FG, *r*		-0.119**	0.015	-0.069**
	n	6610	367	2537
TG, *r*		-0.083**	-0.159*	-0.044*
	n	6610	367	2537
HDL, *r*		-0.002	-0.065	-0.088**
	n	6610	367	2537
SBP, *r*		-0.25**	0.008	-0.269**
	n	6610	367	2537
DPB, *r*		-0.023	-0.084	0.000
	n	6610	367	2537
BMI, *r*		-0.056**	0.049	-0.124**
	n	6610	367	2537
WC, *r*		-0.175**	-0.033	-0.282**
	n	6445	358	2481

* $p < 0.05$, ** $p < 0.001$; r, Pearson's Correlation Coefficient; n, number of observations; eGFR, estimated glomerular filtration rate; FG, fasting glucose; TG, triglycerides; HDL, high-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference.

Figure 4.1 demonstrates the average eGFR in individuals with 1, 2, or 3 risk factors. This figure represents the impact of each metabolic risk factor, including obesity, on eGFR. The reference point was an individual with 0 risk factors (eGFR=103.93 ml/min/1.73m^2). Regardless of the number of risk factors an individual had, those with hypertension consistently had the lowest eGFR, and the eGFR in those with hypertension decreased as the number of risk factors increased. Dyslipidemia in the form of high fasting triglycerides was the second most detrimental risk factor associated with eGFR. Individuals with low HDL as defined by the NCEP ATP III criteria consistently demonstrated the highest eGFR, despite this being a metabolic risk factor.

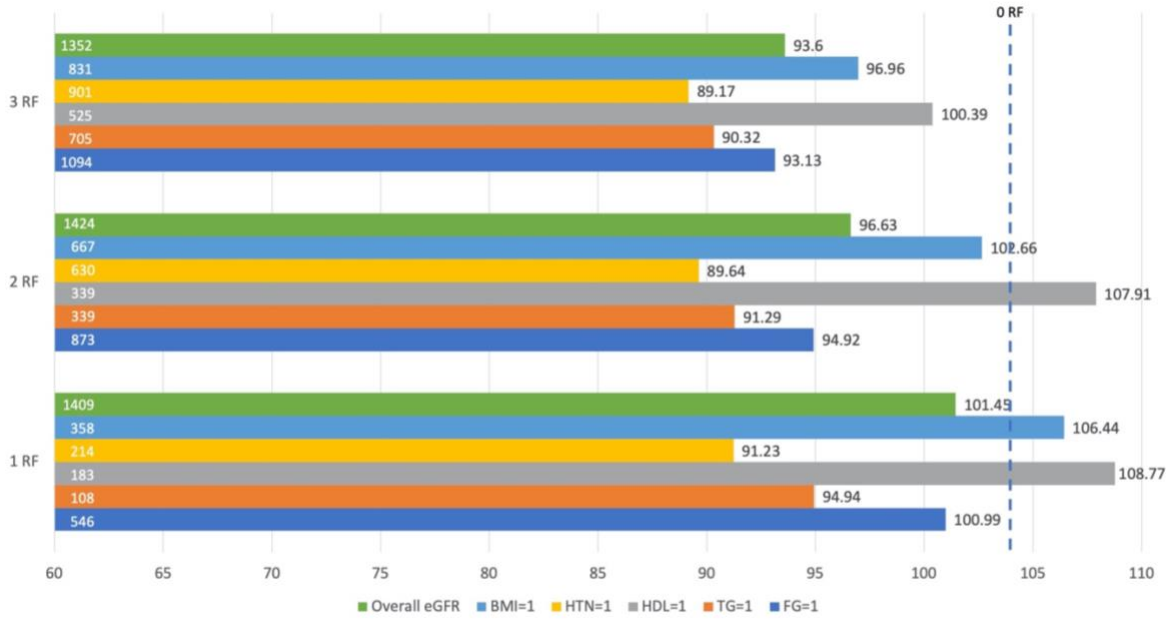

Figure 4.1. eGFR and Metabolic Risk Factors. This figure represents the average estimated glomerular filtration rate (eGFR) in individuals with 1, 2, and 3 risk factors, including obesity. By highlighting each individual risk factor, we demonstrate what eGFR would be if an individual had a particular risk factor either independently or in conjunction with other risk factors. The numbers on the left side of the horizontal bars indicate the sample size whereas the numbers to the right of each horizontal bar represent the eGFR for that condition. Overall eGFR is the average eGFR for individuals with 1, 2, or 3 risk factors; BMI=1 indicates presence of obesity; HTN=1 indicates hypertension; HDL=1 indicates dyslipidemia as determined by the high-density lipoprotein variable; TG=1 indicates dyslipidemia as determined by fasting triglycerides; and FG=1 indicates high fasting glucose. The eGFR for the reference group (0 risk factors) is 103.93 ml/min/1.73m^2.

Discussion

The purpose of the present study was to report the prevalence of the strict metabolic phenotypes, renal function in each phenotype, and the risk factors associated with renal function. Our primary outcomes indicate that the strictly defined MUN phenotype accounted for the largest proportion of the US population whereas the MHO phenotype accounted for the smallest. In previous studies using the same strict definition of metabolic health, the MUN phenotype varied from 35-45% of the population and the MHO phenotype ranged from 2.5-5.5% of the population, on average (11, 12, 23, 34). In

the present study, the proportions of the "intriguing" phenotypes fall within the purviews of prior research. Similar overall results can be seen in previous studies (12, 34), although the MHN and MUN populations can vary widely depending on the population measured. Kouvari et al. reported a large percentage of the MHN phenotype (36.30%) in the relatively homogenous Greek population assessed in the ATTICA cohort study, (11) which is almost double the frequency of the MHN in the present study. Our prior research has identified a large percentage of the MUO phenotype (57.79%) in a federally qualified health center in the Southern US (23), which is 1.5 times the proportion that we have established here. The sample used in the present study is representative of the entire US population, and therefore consists of greater racial and ethnic diversity than the study by Kouvai *et al*, as well as greater socioeconomic and geographic diversity than our prior study.

In the study sample, renal function was lowest in the MUN phenotype. However, it is important to note that CKD was more prevalent in the MUO phenotype due to the definition of CKD and the high ACR ($\bar{x}$=44.45 mg/g, SE= 6.20) in the MUO phenotype. These findings persisted across multiple definitions of metabolic health ranging from the strict definition to the standard definition of MetS, demonstrating that one metabolic risk factor may be similarly indicative of renal dysfunction as two or three risk factors, but that CKD status was more highly dependent upon ACR than eGFR. The MUN phenotype, while not typically perceived as high risk (10), has been correlated with adverse health outcomes such as poor renal function (23), type 2 diabetes, cardiovascular events, and mortality (35). In our study as well as previously reported findings (17, 23), the MUN phenotype was correlated with older age. CKD has also been reported to be

more common in individuals of older age (7), though this finding may be due to the prolonged presence of metabolic risk factors rather than age itself. A recent pilot study by Valdez et al. demonstrated that renal health was independent of age in individuals with no metabolic risk factors (36). Still, more research is warranted to assess the renal risk in individuals with one or more metabolic risk factors and normal weight, given that this constitutes a majority of the US population.

Overweight ($25 \leq BMI \leq 30$), and obese ($BMI \geq 30$) individuals have a 40% to 80% increased risk of CKD, respectively (37). However, in the present study the MHO phenotype presented with renal function that was comparable to the reference group (MHN). Similar to previous findings (38), the MHO phenotype was younger in age, indicating that the findings could be attributed to the short amount of time that these individuals have been in the obese state. In the early stages of obesity, the kidneys engage in compensatory vasodilation and hyperfiltration in an attempt to maintain sodium balance despite increased tubular sodium reabsorption (39). Over time, the high-pressure system caused by hyperfiltration causes glomerulosclerosis, which may not be detectable via changes in serum creatinine values until renal function has decreased by approximately 50% (40). The higher eGFR demonstrated in the MHO phenotype presents a phenomenon which may be explained by the transient state of "healthy obesity" wherein the detrimental metabolic effects of the obese state have not yet had time to manifest (11). This finding demonstrates the inadequacy of BMI as a proxy measure for body composition, warranting future research on the relationship between body composition and renal function.

Long-term studies have demonstrated higher risk of CVD and mortality in the MHO phenotype (41, 42). Additionally, a longitudinal study by Kouvari et al. demonstrated that 52% of individuals classified as MHO transitioned to the MUO status within a 10-year timeframe (11). While we cannot determine chronicity of disease in the present cross-sectional sample, we did observe possible indicators of future disease. A high hs-CRP level was detected in the obese phenotypes, which is indicative of systemic inflammation likely due to excess adipose tissue (43, 44). Additionally, the lipid profile of the MHO phenotype was within normal range yet inferior to that of the reference group. On average, triglycerides and LDL were 10 points higher than the MHN phenotype and HDL was 5 points lower, increasing the risk of future CKD (45, 46). Although individuals classified as MHO have healthy metabolic and renal markers in cross-sectional analyses (47), it is likely that the inflammatory process of persistent obesity will be followed by metabolic risk factors and eventual declines in renal health. Further research is warranted to investigate the specific conditions necessary to maintain metabolic health in the presence of obesity.

In the overall sample we found HTN, a high WC, and high fasting glucose to be negatively correlated with eGFR, which is intuitive given that hypertension and hyperglycemia are the two main precursors of CKD in the developed world (7). In the MUN phenotype, eGFR had the largest correlations with HTN and WC. While these individuals were not obese as classified by BMI cutoffs, they did demonstrate a WC that was approximately 10 cm greater than that of the MHN, indicating that they carry more of their weight in the central region of their body. Central adiposity in the form of visceral adipose tissue (VAT) has been identified as a major contributor of insulin

resistance (26) and is more metabolically active than subcutaneous fat or adipose tissue carried in the lower limbs (48). The metabolically active VAT is possibly a major contributor to the metabolically unhealthy status and reduced renal function observed in this phenotype.

Unique to our study, HDL had a small negative association with eGFR, indicating that lower levels of HDL were correlated with a higher eGFR. This was demonstrated in Figure 4.1 where individuals with low HDL as one of their risk factors had a higher average eGFR than individuals with any other risk factor. While these results contradict many previous research findings, (38, 45, 46, 49) there have also been studies that have confirmed greater risk of mortality associated with high HDL levels (50). It is possible that the weak negative correlation demonstrated in our study could be explained by outliers with rare genetic variations in HDL receptors (51) or high levels of inflammation (52). In future investigations, HDL function may prove to be more important than quantity. Still, further research should be done to understand these findings.

Strengths and Limitations

This study is the first to utilize a strict definition of metabolic health in the assessment of CKD while also utilizing NHANES complex survey sample weighting techniques. Much of the research in metabolic phenotypes and renal function is conducted in Asian populations whereas our sample was taken from a racially and ethnically diverse population in the US. The large sample size and use of the complex survey sample weighting techniques allowed us to report unique findings that are representative of the US population. This study was limited by the cross-sectional nature

of the data, which prevents us from making inferences about the temporal sequence of events leading to declines in renal function. NHANES sampling techniques and measures are widely accepted, yet selection bias may still occur. For example, 18 individuals who met the inclusion criteria of the present study reported dialysis in the past year. Given the voluntary nature of research, it is likely that few of the ill and/or infirmed individuals selected for this study chose to participate. To marginally correct for this, the sample weighting procedures adjust for nonresponse to reduce potential bias. The sample sizes of the four phenotypes varied widely and the MHO phenotype was very small (5.59% of the population), lowering the statistical power in comparisons made using this phenotype. Additionally, the amount of variance explained by each of the regression models, demonstrated by the R^2 values, was very low. A larger amount of variance could be explained by including variables such as age, sex, and race/ethnicity, but these values were considered in the equation estimating GFR, and therefore were not added to the regression models. Metabolic risk factors, drug information, and BMI were considered in the metabolic phenotypes and therefore were not added to the regression equation. SES and caloric intake were not statistically different among the four phenotypes, and there was a large percentage of missingness in the PA, smoking, and hs-CRP variables, excluding these variables from the regression analyses. Therefore, the regression models are presented with the unadjusted results, which explain a small percentage of the variance in renal function yet demonstrate significant differences between the phenotypes. Glomerular filtration rate was estimated using an equation that utilizes serum creatinine, which can be affected by muscle mass, muscle breakdown, exercise, nutrition, medications, and hydration status. We were limited to one-time measures of eGFR and

hs-CRP due to the cross-sectional nature of the study. To diagnose CKD, measures of SCr should be taken twice, approximately 3 months apart. Measures of hs-CRP should also be taken twice, approximately 2 weeks apart, to obtain an average measure of inflammation.

Conclusions

In the present study, we utilized a complex survey sample weighting technique to identify a sizable frequency of individuals with metabolic risk factors and/or obesity in the US population. We observed higher proportions of males and individuals of older age in the metabolically unhealthy phenotypes whereas in the obese phenotypes we observed higher proportions of non-Hispanic Black individuals and greater levels of inflammation represented by hs-CRP values above 3.0 mg/L. Using a strict definition of metabolic health, we found that renal function was lowest in the MUN phenotype. These findings persisted when using more lenient definitions of metabolic health. The renal health of the MHO phenotype was not statistically different from the reference group; however, these findings are likely transient given previous reports from longitudinal studies. Hypertension, waist circumference, and HDL were negatively correlated with renal function, implicating future research in the area of dyslipidemia and renal function.

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CHAPTER FIVE

Metabolic Constellations, Clusters, and Renal Function: Findings from the 2013-2018 National Health and Nutrition Examination Surveys

Abstract

Background: Metabolic syndrome (MetS) has been associated with decreased renal function and chronic kidney disease (CKD), but to date no research has been discovered regarding the 16 possible constellations that result in the diagnosis of MetS. Purpose: The purpose of this study is to report renal function in 16 metabolic constellations grouped into 4 metabolic clusters. Methods: Individuals (n=2,767; N=86,652,073) from the 2013-2018 National Health and Nutrition Examination Surveys who met the criteria for MetS were included. Sixteen possible constellations of 3 or more risk factors were analyzed for renal function. Four metabolic clusters, which represented MetS with hyperglycemia (Cluster I), MetS with hypertension (Cluster II), MetS with hyperglycemia and hypertension (Cluster III) or MetS with normoglycemia and normotension (Cluster IV), were assessed for renal function and CKD status. Results: Cluster III had the highest odds of CKD (OR=2.57, 95%CL=1.79, 3.68). Clusters II and III had the lowest renal function and were not found to be different from one another (87.82 and 87.28 ml/min/1.73m^2, p=0.71). The constellation with the lowest renal function consisted of hypertension, high triglycerides, and large waist circumference (82.86 ml/min/1.73m^2) whereas the constellation with the highest renal function consisted of hyperglycemia, low HDL, and large waist circumference (107.46 ml/min/1.73m^2). Conclusions: The 16 constellations of MetS do not

have the same effects on renal function. More research is needed to understand the relationship between the various iterations of MetS and renal function.

Keywords: Metabolic Syndrome, Metabolic Constellations, Metabolic Clusters, Chronic Kidney Disease, Renal Function

Introduction

Metabolic syndrome (MetS) is a clustering of three or more interrelated metabolic risk factors including abdominal obesity, two dyslipidemia criteria including high triglycerides and low high-density lipoprotein (HDL) cholesterol, elevated blood pressure, and impaired fasting glucose (1, 2). Within the past three decades, the prevalence of individuals with MetS has increased from 25.3% to 34.2% in the United States (US) (3), trending with obesity, which has increased from 31.9% (4) to 42.6% (5) of the US population in the same time period. The diagnosis of MetS is predictive of cardiovascular disease (CVD) (6) and type 2 diabetes mellitus (T2D) (1, 2), but it has also been associated with stroke (7), all-cause mortality (8), and chronic kidney disease (CKD) (9, 10).

The criteria established by the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III (ATP III) require the concurrent presence of at least three of the five metabolic risk factors in order to formally diagnose MetS. The combinations of three or more risk factors can result in 16 possible iterations of MetS, known as metabolic constellations. While each unique combination results in a diagnosis of MetS, it is possible that the various iterations have different etiologies and increase the risk of

different outcomes, with some being more synergistic (6) than others in promoting disease outcomes. A study by Khosravi et al. (11) categorized the 16 metabolic constellations into four metabolic clusters, which were designed to emphasize hyperglycemia, hypertension (HTN), hyperglycemia and HTN, or normoglycemia and normotension (see Table 5.1). Khosravi et al. found that the cluster emphasizing HTN (cluster II) was highly associated with ischemic heart disease and CVD whereas the cluster that included both hyperglycemia and HTN (cluster III) was most closely associated with stroke (11).

Metabolic syndrome and the individual risk factors associated with it pose risks to renal function (9, 10). Chronic kidney disease has been established as the 3rd fastest growing cause of premature mortality (12), and its strong associations with MetS indicate that mortality from CKD will continue to increase. In the US, the primary risk factor for the development of CKD is type 2 diabetes mellitus (T2D) and the second most common risk factor associated with CKD is HTN (13, 14). In many cases, the effects of CVD and T2D have been reported to be reversible, (15–18) whereas glomerulosclerosis that occurs during the progression of CKD is non-reversible.

To the best of our knowledge, the four metabolic cluster categories outlined in the study by Khosravi et al. (11) have never been analyzed for association with renal function. Given that hyperglycemia and HTN are the primary causes of CKD in the developed world, this unique analysis may assist in further elucidating the synergistic (6) effects of the metabolic risk factors and their associations with renal function. The purpose of the following study is to analyze and report renal function in each of the metabolic constellations and clusters in a representative sample of US adults. We

hypothesize that the metabolic clusters will not be equally associated with renal function, and that those with HTN will have the lowest renal function.

Methods

Datasets were merged from the 2013-14, 2015-16, and 2017-18 cycles of NHANES. Data were acquired from the Center for Disease Control and Prevention (CDC) website to be used in the present analyses. Survey sample weighting was conducted using a complex, four-stage, probability cluster from the National Center for Health Statistics Estimating and Weighting Procedures documents (19, 20). The NHANES study routinely oversamples underrepresented populations, including those 60 and over, African Americans, Asians, and Hispanics. Sample weights are assigned to each subject, allowing researchers to extrapolate the results to be representative of all non-institutionalized US civilians. This study was considered exempt by the sponsoring university.

Study Sample

The sample population consisted of 29,400 non-institutionalized civilians living in the US. An inclusion variable was created to identify a subsample that met the study criteria. Subjects with missing information pertaining to MetS were not retained (21,462 excluded). Additional inclusion criteria required that subjects have full information for CKD (0 excluded), had not been on dialysis in the 12 months prior to the examination (13 excluded), were not pregnant (54 excluded), and were between 18 and 79 years of age (1,416 excluded). The upper age limit was set because age is top coded at 80 years of age

by NHANES for subject deidentification. Finally, individuals who did not have MetS were not included (3,688), creating a final subsample of 2,767 subjects.

Definition of Metabolic Risk Factors

The metabolic risk factors were defined using the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III (ATP III) (2). Individuals were considered obese if they had a waist circumference (WC) >101.6cm for non-Asian males, >88.9cm for non-Asian females, >94cm for Asian males, or >80cm for Asian females. Prescription medication information was classified using the International Classification of Diseases, Tenth Revision (ICD-10) codes. Medications prescribed for hyperglycemia (R73, E11, E11.2, E11.2P, E11.4, and E11.P), hypercholesterolemia (E78.0, E78.0P, and E78.1), and HTN (I10 and I10.P) were taken into account in the present study. Hyperglycemia was classified as a fasting blood glucose ≥ 100 mg/dL or a prescription for glucose-lowering medication. Dyslipidemia was classified based on two criteria; the first of which was a fasting triglyceride level ≥ 150 mg/dL or prescription medication, and the second was a high-density lipoprotein-cholesterol (HDL) measurement <40 mg/dL for males or <50 mg/dL for females or prescription medication for dyslipidemia. Hypertension was classified as a resting systolic blood pressure >130 mmHg or a resting diastolic blood pressure >85 mmHg or a prescription medication for HTN.

Definition of Metabolic Constellations and Clusters

Metabolic constellations were defined as all possible combinations of 3 or more metabolic risk factors that could be used to diagnose MetS, resulting in 16 possible

constellations. Metabolic clusters were modeled after the study by Khosravi et al. (11) where the constellations were categorized into 4 distinct groups, with an emphasis on hyperglycemia (Cluster I), HTN (Cluster II), hyperglycemia and HTN (Cluster III) or normoglycemia and normotension (Cluster IV). Cluster I included 4 subgroups of subjects with at least 3 metabolic risk factors, including hyperglycemia. Cluster II included 4 subgroups of subjects with at least 3 metabolic risk factors, including HTN. Cluster III included 7 subgroups of subjects with at least 3 metabolic risk factors, including hyperglycemia and HTN. Cluster IV included 1 subgroup of subjects with at least 3 metabolic risk factors, excluding hyperglycemia and HTN (see Table 5.1).

Table 5.1.
Criteria for Metabolic Clusters

	Cluster I	Cluster II	Cluster III	Cluster IV
Constellations	FG, TG, HDL	HTN, TG, HDL	FG, HTN, TG	HDL, WC, TG
	FG, TG, WC	HTN, TG, WC	FG, HTN, HDL	
	FG, HDL, WC	HTN, HDL, WC	FG, HTN, WC	
	FG, HDL, WC, TG	HTN, HDL, WC, TG	FG, HTN, HDL, TG	
			FG, HTN, WC, HDL	
			FG, HTN, WC, TG	
			FG, HTN, WC, TG, HDL	

Cutoff values for all metabolic risk factors are outlined by the NCEP ATP III 2005 Revision[2]. Cluster groups were adapted from Khosravi et al[11]. WC, large waist circumference; FG, high fasting glucose or hyperglycemia; HTN, high blood pressure or hypertension; TG, triglycerides or dyslipidemia; HDL, low high-density lipoprotein or dyslipidemia, second separate criteria. Cluster 1: MetS with hyperglycemia, Cluster 2: MetS with hypertension, Cluster 3: MetS with hyperglycemia and hypertension, Cluster 4: MetS with normoglycemia and normotension.

Outcome Measures

Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, (21) which has been demonstrated to be more accurate in predicting eGFR in individuals with higher values than the Modification of Diet in Renal Disease (MDRD) equation (21). Chronic kidney disease was defined as an eGFR<60 ml/min/1.73m^2 and/or an albumin to creatinine ratio (ACR) ≥30 mg/g (22).

Demographic and Biochemical Information

Interview-style questionnaires were conducted by trained interviewers using the Computer-Assisted Personal Interview (CAPI) system to determine demographic, socioeconomic, and lifestyle information for NHANES participants. The poverty index indicated the ratio of family income to poverty as defined by the Department of Health and Human Services (HHS). This index is commonly used as a criterion for determining eligibility in federal assistance programs (23). A value of 1.0 indicates that an individual is at 100% of the poverty level, whereas a value of 2.0 indicates that an individual is at 200% of the poverty level. Physical activity (PA) was measured by NHANES interviewers who recorded exercise intensity, average number of minutes per day, and average number of days per week that subjects participated in PA. The exercise guidelines from the PA Guidelines Advisory Committee Report (24) were used to determine if subjects participated in ≥150 minutes of moderate-intensity recreational PA per week, ≥75 minutes of vigorous-intensity recreational PA per week, or an equivalent combination of the two. Individuals who met these criteria were considered physically

active (24, 25). The smoking variable indicated if individuals had smoked at least 100 cigarettes in their lifetime or if they have used cigarettes in the past 5 days (26). Individuals not meeting these thresholds were considered non-smokers. The prescription medication information used to classify metabolic risk factors was determined using the International Classification of Diseases, Tenth Revision (ICD-10) codes. The ICD-10 codes identified for this study were indicated for treatment of hyperglycemia (R73, E11, E11.2, E11.2P, E11.4, and E11.P), hypercholesterolemia (E78.0, E78.0P, and E78.1), and HTN (I10 and I10.P).

NHANES examinations were conducted in a mobile examination center (MEC) and included anthropometric measures, blood pressure, blood panels and urinalysis. Body mass index (BMI) was calculated using height in meters (m) measured on a calibrated stadiometer, and weight in kilograms (kg) measured on a portable scale. Waist circumference (WC) in centimeters (cm) was measured at the uppermost border of the iliac crest. After a 5-minute rest, blood pressure was taken three consecutive times, and a fourth time for individuals with interrupted or incomplete values. The three available blood pressures were averaged for the present analyses. A fasting lipid panel was analyzed using the Roche/Hitachi Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN, USA). Fasting blood glucose was measured using the Roche Cobas C311 system. The albumin to creatinine ratio (ACR) was analyzed using the fluorescein immunoassay by Sequoia-Turner Digital Fluorometer, Model 450 (Sequoia-Turner Corporation, Mountain View, CA, USA) for urinary albumin and the Roche Cobas 6000 Analyzer for urinary creatinine.

Statistical Analysis

All statistical analyses were conducted in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). A DOMAIN statement was used in all analyses to utilize sampling weights for the analytic sample. Sample weights were assigned using a cluster variable known as the primary sampling unit (PSU), a stratification variable, and a subsample weight. Unweighted continuous variables were presented as mean and standard deviation (SD) and unweighted categorical data was presented as frequency and percentage (%). Weighted continuous variables were presented as mean and standard error of the mean (SE) and weighted categorical variables were presented as percentage and standard error of percent (SE). Simple regression was used to determine the difference between weighted cluster values in the case of continuous variables. Chi square (χ^2) tests were used to determine differences between cluster variables in the case of categorical variables. A linear regression analysis was conducted to determine the difference in eGFR between clusters and a logistic regression analysis was used to determine odds of CKD in each of the clusters. The assumptions of multiple regression were found tenable using histograms and Q-Q plots. The level of significance was set *a priori* at $\alpha=0.05$.

Results

A subsample of 2,767 individuals representing 86,652,073 non-institutionalized US civilians with MetS was analyzed in the present study. The demographic data for the unweighted and weighted samples are in Table 5.2. Due to oversampling of individuals 60 and over, African Americans, Asians, and Hispanics, the unweighted sample is slightly older in age and more racially and ethnically diverse than the weighted sample.

The four metabolic clusters represent individuals with MetS who have hyperglycemia without HTN (Cluster I), HTN without hyperglycemia (Cluster II), hyperglycemia and HTN (Cluster III) and normoglycemia and normotension (Cluster IV). The cluster with the greatest weighted frequency was Cluster III (62.08%) followed by Cluster I (24.71%). Cluster IV (3.66%) was least frequent in this population, followed by Cluster II (9.55%).

Cluster I had the highest frequency of Mexican Americans (16.13%) and a high average WC (110.44 cm), though all clusters demonstrated a WC that would qualify as a metabolic risk factor (110.70 cm). Cluster I also demonstrated a poor lipid profile with the second-highest TG value (182.31 mg/dL) and second-lowest HDL value (42.21 mg/dL) in the study sample. Still, this group demonstrated the second highest eGFR (97.67 ml/min/1.73m^2). Cluster II was predominantly female (57.4% female), had the highest frequency of NH Blacks (17.46%), and demonstrated the second lowest eGFR (87.82 ml/min/1.73m^2). Individuals in cluster III were more likely to be male (54.25% male) and were 20 years older, on average, than those in cluster IV. Individuals in Cluster III had the lowest socioeconomic status at 209% of the poverty level, which equates to an approximate income of $27,000/year for an individual or $55,385/year for a family of four. This cluster also demonstrated the highest WC (111.60 cm), ACR (64.78), and lowest eGFR (87.28 ml/min/1.73m^2) in the sample. Cluster IV was predominately female (56.18% female), demonstrated the poorest lipid profile with a fasting TG level of 228.30 mg/dL and a low HDL level at 37.16 mg/dL. However, this cluster demonstrated the best renal function represented by a high eGFR (106.44 ml/min/1.73m^2) and a low ACR (15.35).

Table 5.2.
Demographic Information for the Subsample and Metabolic Clusters

	Total		Cluster				*p*-value
Demographic Variable	Unweighted Total (n=2,767)	Weighted Total (n=86,652,073)	Cluster I (24.71%)	Cluster II (9.55%)	Cluster III (62.08%)	Cluster IV (3.66%)	
Male Sex	1364 (49.3)	51.23 (1.30)	48.07 (3.16)	42.60 (4.30)	54.25 (1.78)	43.82 (6.03)	0.033
Age (years)	54.81 (14.62)	53.16 (0.52)	45.78 (0.91)	52.82 (1.00)	57.08 (0.50)	37.40 (1.45)	<0.001
Race/Ethnicity							
Mexican American	460 (16.62)	9.53 (1.08)	16.13 (1.79)	5.80 (1.59)	7.35 (1.10)	11.67 (3.11)	<0.001
Other Hispanic	320 (11.56)	5.54 (0.73)	6.44 (1.08)	3.23 (0.98)	5.35 (0.78)	8.64 (2.49)	
NH White	1020 (36.86)	65.77 (1.88)	65.29 (2.82)	67.14 (3.70)	66.02 (2.11)	61.10 (6.15)	
NH Black	603 (21.79)	11.10 (1.18)	4.59 (0.97)	17.46 (2.59)	12.78 (1.34)	9.98 (3.10)	
NH Asian	260 (9.40)	4.09 (0.42)	3.10 (0.57)	0	4.59 (0.51)	3.22 (1.57)	
Other/Multi-Racial	104 (3.76)	3.98 (0.49)	4.43 (0.91)	0	3.91 (0.75)	5.39 (2.95)	
Meets PA Rec**	720 (62.28)	60.34 (1.86)	58.28 (5.58)	65.46 (5.43)	60.37 (2.35)	62.42 (9.11)	0.849
Current Smoker	1388 (50.16)	52.08 (1.53)	51.85 (2.69)	48.09 (4.49)	53.06 (1.79)	47.38 (5.45)	0.577
Poverty Index	2.41 (1.59)	2.89 (0.06)	2.75 (0.12)	2.81 (0.16)	3.02 (0.08)	2.09 (0.16)	<0.001
BMI (kg/m^2)	32.78 (7.03)	33.11 (0.27)	33.35 (0.45)	31.90 (0.51)	33.23 (0.26)	32.47 (0.72)	0.04
Fasting Glucose (mg/dL)	125.57 (46.83)	121.83 (0.95)	119.44 (1.76)	93.20 (0.39)	128.83 (1.42)	93.80 (0.60)	<0.001
SBP/DBP	131/72	129/73	117/69	133/76	135/74	114/70	<0.001
WC (cm)	109.25 (15.14)	110.70 (0.55)	110.44 (0.91)	106.75 (1.36)	111.60 (0.53)	107.41 (1.63)	<0.001
Fasting TG (mg/dL)	156.85 (153.88)	159.07 (3.62)	182.31 (7.85)	162.38 (8.10)	145.23 (3.26)	228.30 (13.01)	<0.001
HDL (mg/dL)	47.56 (14.56)	47.36 (0.42)	42.21 (0.67)	47.13 (1.07)	50.05 (0.54)	37.16 (0.65)	<0.001
eGFR $ml/min/1.73m^2$	90.21 (21.97)	90.60 (0.72)	97.67 (1.37)	87.82 (1.33)	87.28 (0.76)	106.44 (2.27)	<0.001
ACR	69.28 (416.42)	48.96 (6.53)	23.97 (5.55)	23.18 (7.72)	64.78 (10.82)	15.35 (5.20)	0.003

Unweighted categorical data presented as n (%), continuous as mean (SD). Weighted categorical data presented as % (SE), continuous as mean (SEM). Cluster I: MetS with hyperglycemia, Cluster II: MetS with HTN, Cluster III: MetS with hyperglycemia and HTN, Cluster IV: MetS with normoglycemia and normotension. Abbreviations: HTN, hypertension; NH, non-Hispanic; PA, physical activity; BMI, body mass index; SBP/DPB, systolic blood pressure/ diastolic blood pressure; WC, waist circumference; TG, triglycerides; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio; MetS, metabolic syndrome; SD, Standard deviation; SE, standard error; SEM, standard error of the mean. The *p*-value indicates the probability that there is a difference between the clusters for each variable. **There was 41.78% missingness in the physical activity variable.

The results of the regression analyses are presented in Table 5.3. In the linear regression analysis, renal function in each of the clusters was assessed. Clusters I and IV demonstrated the highest renal function whereas clusters II and III, which both included HTN, demonstrated the lowest renal function. In post-hoc testing, clusters 2 and 3 were not found to be statistically different from each other (p=0.71). All other comparisons were significantly different at the p<0.01 level of significance. The logistic regression analysis reports the odds of CKD for each of the clusters, with Cluster I as the reference. CKD was defined as an eGFR<60 ml/min/1.73m^2 and/or an albumin to creatinine ratio (ACR) ≥30 mg/g (22). Cluster III was the only cluster that was significantly different from the referent group, and individuals in this group had the highest odds of CKD (OR=2.57, 95% CL= 1.79, 3.68, p<0.001).

Table 5.3.
Regression Analyses of Renal Function in Metabolic Clusters

	Linear Regression			Logistic Regression		
Coefficient	*b*	SE_b	*p*-value	OR	95% CL	*p*-value
Cluster I (Reference)	97.67	1.37	<0.001	-	-	-
Cluster II	-9.84	1.47	<0.001	1.66	(0.92, 2.99)	0.092
Cluster III	-10.39	1.32	<0.001	2.57	(1.79, 3.68)	<0.001
Cluster IV	8.77	2.56	0.001	0.46	(0.19, 1.15)	0.096
	Sum of weights = 86,652,073			Sum of weights = 86,652,073		
	F Value= 49.17, R^2=0.067			F Value = 27.59		

The linear regression represents the eGFR in each of the metabolic clusters. This model is presented as an intercept (Reference) and beta values (b) reported in mL/min/1.73m^2; SE_b is the standard error of beta; The logistic regression represents the odds of CKD for each metabolic cluster. This model is presented as odds ratios (OR) and 95% confidence limits (95% CL) where Cluster I is the referent group, and the reference value is 1.0. P-values were considered significant at α=0.05. The sum of weighted observations in this subsample was 86,652,073.

The frequency of CKD in each of the metabolic clusters is reported in Table 5.4. There were 19.22% that met the criteria for CKD, the majority of which were in cluster III (76.76%). Cluster III was the largest subgroup, approximately one quarter of which had CKD (23.76%). Cluster II was significantly smaller than Cluster III, representing only 8.31% of CKD cases, but it demonstrated a higher prevalence of individuals with CKD (16.73%) than the national average (13). Cluster I was the second largest group, accounting for 13.91% of all CKD cases. However, this cluster demonstrated a lower proportion of CKD in the cluster (10.82%) than clusters II and III. The smallest cluster, cluster IV demonstrated the lowest frequency of CKD (5.30%).

Table 5.4.
Frequency of CKD in Metabolic Clusters

Cluster	CKD Cases Weighted Frequency	Total Weighted Frequency	CKD cases in cluster/total CKD cases (%, SE)	CKD cases in cluster/total cluster sample (%, SE)	*p*-value
I	2,317,090	21,412,722	13.91 (1.79)	10.82 (1.43)	<0.001
II	1,384,483	8,276,247	8.31 (1.89)	16.73 (3.70)	
III	12,782,719	53,790,449	76.76 (2.42)	23.76 (1.34)	
IV	168,245	3,172,655	1.01 (0.40)	5.30 (1.94)	

The frequency of chronic kidney disease is reported for each metabolic cluster. CKD was defined as an eGFR<60 ml/min/1.73m2 and/or an albumin to creatinine ratio (ACR) ≥30 mg/g (22). The CKD cases in cluster/total CKD cases column represents the proportion of individuals with CKD divided by the total number of individuals with CKD across all clusters (n=16,652,538). The CKD cases in cluster/total cluster sample column represents the number of individuals with CKD in a given cluster divided by the total number of individuals in the cluster. Abbreviations: CKD, chronic kidney disease; SE, standard error. The sum of weighted observations in this subsample was 86,652,073. The p-value indicates the probability that there is a difference in number of CKD cases between any of the four clusters.

The renal function for each metabolic constellation, grouped by cluster, is represented in Figure 5.1. The constellations with the lowest renal function were HTN + TG + WC (82.86 ml/min/1.73m^2, n=111), FG + HTN + TG (83.80 ml/min/1.73m^2, n=180), and FG + HTN + WC + TG (84.24 ml/min/1.73m^2, n=469). These constellations shared HTN and low TG as risk factors. The constellations with the highest renal function included FG +

HDL + WC (107.46 ml/min/1.73m^2, n=195), HDL + WC + TG (106.44 ml/min/1.73m^2, n=91), and FG + TG + HDL (99.00 ml/min/1.73m^2, n=64). These constellations shared HDL as a risk factor.

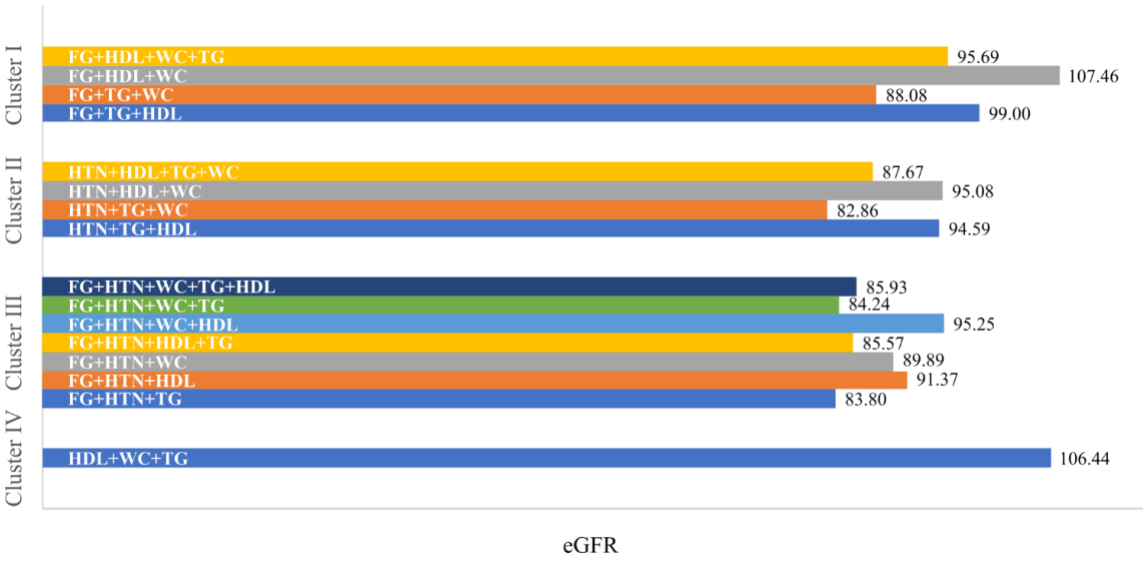

Figure 5.1. Renal function in the metabolic constellations, categorized by cluster**.** Data presented as eGFR (ml/min/1.73m^2) for each of the metabolic constellations. Cluster 1: MetS with hyperglycemia, Cluster 2: MetS with hypertension, Cluster 3: MetS with hyperglycemia and hypertension, Cluster 4: MetS with normoglycemia and normotension. Abbreviations: FG, high fasting glucose; TG, high fasting triglycerides; HDL, low high-density lipoprotein; WC, high waist circumference; HTN, hypertension. The sum of weighted observations in this subsample was 86,652,073.

Discussion

In the present research study, we analyzed the renal function in individuals with metabolic syndrome to determine if there were differences in the various constellations and clusters of the disease. We found that the constellations with HTN and high triglycerides trended towards low eGFR whereas those with low HDL trended towards higher eGFR. The clusters associated with lowest renal function were Clusters II and III. Renal function was not found to be statistically different between these two clusters. The

findings confirmed our hypotheses that there was a statistical difference in renal function between the four clusters and that HTN had a negative effect on renal function.

The study by Khosravi *et al*, which we used to model the metabolic constellations and clusters, was similar in sample size and age compared to our study, but the distribution of metabolic clusters was different. Our findings indicated that a majority of the individuals in the US who have MetS were in Cluster III (hyperglycemia and HTN), whereas the study by Khosravi et al. demonstrated a majority of their sample was classified into Cluster II (HTN with normoglycemia). The large variation in sample distribution across the four clusters could be due in part to the locations from which the samples were taken. Compared to the US population, the Iranian population in the Khosravi et al. study has a lower obesity rate (42.6% (5) and 22.7% (27), respectively) which may explain the lower proportion of individuals with hyperglycemia, since insulin resistance is frequently prompted by obesity (28).

We categorized metabolic constellations into four metabolic clusters which emphasized hyperglycemia, HTN, both hyperglycemia and HTN, or normoglycemia and normotension. In the study by Khosravi *et al*, Clusters II and III were found to be at highest risk of ischemic heart disease (IHD), CVD, and stroke. Similarly, our linear regression model demonstrated the lowest renal function in Clusters II and III, indicating a similar pattern of metabolic dysregulation. Our logistic regression model demonstrated the highest odds of CKD in Cluster III, which agrees with both the physiological (28) and epidemiological (13, 14) research that indicates T2D and HTN as the main risk factors associated with CKD.

When looking specifically at the constellations associated with disease, Khosravi et al. demonstrated that the constellations most highly associated with IHD, CVD, and stroke were FG + HTN + TG, FG + HTN + HDL, and FG + HTN + HDL + TG, respectively. We found that renal function was lowest in the HTN + TG + WC constellation, classified in Cluster II. In our sample, the common denominators in the 3 constellations with lowest eGFR were HTN and high TG, whereas the common denominator in the 3 constellations with highest eGFR was low HDL. Similarly, high TG was found to be associated with renal dysfunction in the Atherosclerotic Risk in Communities (ARIC) study (29). However, the ARIC study (29) and others (30, 31) have found low HDL to be associated with renal decline, which is not consistent with our findings. It is possible that this phenomenon could be explained by the unique sample chosen, which only includes individuals with MetS. Low HDL may be the least predictive of renal dysfunction. Previous findings have indicated that individuals with a rare impairment of the scavenger receptor BI may have high levels of HDL, yet increased risk of coronary heart disease (32). Additionally, individuals with extremely high levels of HDL have been shown to be at greater risk for all-cause mortality (33). Further research should be done to better understand these, and other paradoxical findings related to high HDL levels.

The subsample of the US population that was analyzed in this study only included individuals with MetS, which is known to have a bidirectional relationship with CKD (9, 10). The proportion of CKD in this population was 19.22%, which is slightly higher than the 15% (13) reported for the US population. The higher rates of CKD were only seen in Clusters II and III, whereas clusters I and IV demonstrated lower than average

proportions of CKD. Despite having MetS, individuals in Cluster IV had a higher eGFR and a lower proportion of CKD cases (5.30%) compared to the average population. Since this study is the first of its kind, this may be an indicator that certain configurations of MetS are less detrimental to renal health than others. Specifically, the constellation with low HDL, high WC, and high TG may not play as crucial of a role in the pathophysiology of CKD. However, this condition may be transient given the younger age (37.40 years) of individuals in Cluster IV and the pattern of abdominal fat over time, which leads to dysregulation of hormones and cytokines and the development of insulin resistance (2, 28).

One of the significant challenges with CKD epidemiology and treatment is that it goes largely undiagnosed (13). There is an absence of signs and symptoms for the disease and many individuals are not tested or diagnosed as part of routine medical care due to the misperception that renal function declines due to older age. Nine out of every 10 adults with CKD are unaware that they have it, and one in two adults with very low kidney function do not know they have CKD (13). One way to improve the early identification and prevention of CKD is to identify risk at earlier ages. A better understanding the etiology of the disease and the risk factors associated with renal decline are needed. Identifying the unique metabolic constellations that are most closely associated with CKD may aid in establishing screening procedures for medical reporting systems, which will enable physicians and medical staff to begin prophylactic treatment for declining renal function before it progresses to a critical stage.

The predominant characteristics of MetS are insulin resistance and abdominal obesity (2), and it has been demonstrated that MetS is directly linked with atherosclerotic

CVD and T2D (1, 2). MetS has also been linked to CKD (9, 10), but we have established that there may be specific amalgamations of MetS that are more harmful to the kidneys than others. We saw a 24.6 ml/min/1.73m^2 range in eGFR between the highest and lowest renal function among the 16 constellations that were analyzed. We also established a wide variation in frequency of CKD, with 23.76% of Cluster III demonstrating the criteria for CKD and only 5.30% of Cluster IV demonstrating the criteria for CKD. Many iterations of MetS have been assessed in cross-sectional and longitudinal studies, resulting in a wide range of disease outcomes and risk associations (26, 34–36). The present study supports previous research findings of high variability of outcomes reported with MetS, indicating that not all constellations or clusters are equally detrimental to renal function. While this study was limited to a cross-sectional sample of non-institutionalized US citizens, it typifies new means for predicting and preventing CKD. Future research efforts should focus on the connection between metabolic constellations, clusters, and renal health.

Strengths and Limitations

No studies have evaluated the relationship of metabolic constellations or clusters and CKD. To the best of our knowledge, this is the first study to analyze metabolic constellations and clusters with reference to renal function. The use of NHANES complex survey sample weighting techniques allowed us to extrapolate the results to the greater US population. Prior research in this area has been primarily in an Iranian population. The large datasets provided by NHANES were representative of a racially and ethnically diverse population. The study is limited by its cross-sectional nature,

which precluded us from making causal inferences regarding the development of renal dysfunction. The sample sizes of the four clusters varied widely which lowered the statistical power of the study and made comparisons difficult between constellations and clusters. The primary measure of renal function was calculated using an equation for estimating glomerular filtration rate. This measure is highly influenced by serum creatinine values, which can be affected by hydration status, muscle mass, nutrition, exercise, medication, and muscle breakdown. Additionally, diagnosis of CKD should occur by utilizing two measurers of eGFR, separated by three months. Due to the cross-sectional nature of the study, we were unable to identify chronicity of disease.

Conclusions

The present study demonstrated that the diagnosis of MetS varied widely in prevalence and renal outcomes based on the clustering of risk factors. A majority of the US citizens in this subsample had both hyperglycemia and HTN (Cluster III), and this cluster demonstrated the lowest renal function with the highest odds of CKD. The cluster with HTN and normoglycemia (Cluster II) also had low renal function, statistically equivalent to Cluster III. There was a 24.6 ml/min/1.73m^2 range in eGFR between the highest and lowest renal function among the 16 constellations that were analyzed. The three constellations with the lowest renal function shared HTN and high triglycerides as metabolic risk factors, whereas the three constellations with the highest renal function shared low HDL as a risk factor. These findings indicate that the 16 possible constellations which lead to the diagnosis of metabolic syndrome may play varying roles in the pathophysiology leading to renal decline. Clinicians can utilize the findings in this

study to support patient health by identifying individuals at greatest risk for renal decline and CKD. Future research studies should aim to better understand the complex relationship between the metabolic constellations, clusters, and renal function. Additionally, longitudinal assessments are warranted to determine how metabolic constellations change over time and how their chronicity may affect renal function.

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CHAPTER SIX

Summary of Conclusions

The purpose of this study was to test the following hypotheses:

H_1: The MHO phenotype will have decreased renal function as compared to the MHN phenotype.

In our analysis of the metabolically healthy obese (MHO) phenotype, we found that renal function was not statistically different from the metabolically healthy normal (MHN) referent group (p=0.09). The eGFR in the MHO phenotype was 106.44 ml/min/1.73m^2 whereas the eGFR in the MHN was 103.93 ml/min/1.73m^2. Similar to previous findings (133), the MHO phenotype was younger in age, indicating that the findings could be attributed to the short amount of time that these individuals have been in the obese state. Although individuals classified as MHO have healthy metabolic and renal markers in cross-sectional analyses (134), it is likely that the inflammatory process of persistent obesity will be followed by metabolic risk factors and eventual declines in renal health. Further research is warranted to investigate the specific conditions necessary to maintain metabolic health in the presence of obesity.

H_2: The MUN phenotype will have decreased renal function compared to the MHN phenotype.

In the study sample, renal function was lowest in the MUN phenotype. This finding persisted across multiple definitions of metabolic health ranging from the strict definition to the standard definition of MetS, demonstrating that one metabolic risk factor

may be similarly indicative of renal dysfunction as two or three risk factors. The MUN phenotype, while not typically perceived as high risk (31), has been correlated with adverse health outcomes such as poor renal function (135), type 2 diabetes, cardiovascular events, and mortality (92). In our study as well as previously reported findings (119, 135), the MUN phenotype was correlated with older age. CKD has also been reported to be more common in individuals of older age (34), though this finding may be due to the prolonged presence of metabolic risk factors rather than age itself. A recent pilot study by Valdez et al. demonstrated that renal health was independent of age in individuals with no metabolic risk factors (136). Still, more research is warranted to assess the renal risk in individuals with one or more metabolic risk factors and normal weight, given that this constitutes a majority of the US population (38.40%).

H_3: The metabolic clusters will demonstrate differing renal function.

The linear regression model in manuscript 2 demonstrated the lowest renal function in Clusters II and III, whereas Clusters I and IV had higher renal function. Renal function in Clusters II and III was not statistically different. The highest odds of CKD were in Cluster III, which is congruent with both the physiological (10) and epidemiological (34, 38) research which indicates T2D and HTN as the main risk factors associated with CKD. There was a 24.6 ml/min/1.73m^2 range in eGFR between the highest and lowest renal function among the 16 constellations that made up the four clusters. The constellations and clusters with hypertension trended towards lower renal function whereas those with low HDL as a risk factor had higher renal function.

Conclusions

In conclusion, this is the first study to utilize a representative sample of individuals from the United States to assess metabolic phenotypes, constellations, and clusters in relation to renal function and chronic kidney disease. We found that the metabolic phenotypes most closely associated with renal dysfunction were the metabolically unhealthy phenotypes. This finding was consistent across all definitions of metabolic health including phenotypes with 1, 2, or 3 risk factors considered as unhealthy. The metabolically healthy obese phenotype had renal function that was comparable to the metabolically healthy normal phenotype, but this finding may be transient given the cross-sectional nature of our study and the results from previously published longitudinal studies. Our assessment of the metabolic constellations and clusters demonstrated that the various iterations of metabolic syndrome do not appear to have the same effects on renal health. Individuals with hypertension tended to have poorer renal function, which is congruent with prior literature that has identified hypertension as one of two main causes of chronic kidney disease in the developed world. Additionally, we found a negative correlation between HDL and renal function. While there is scant research in this area, a few studies have demonstrated greater risk for cardiovascular diseases and mortality in individuals with extremely high HDL. Further research should be done to investigate and understand the unique findings demonstrated in this study.

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