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Integrated Clinical and Metabolic Phenotyping of Adults with Hidden Adiposity

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Integrated Clinical and Metabolic Phenotyping of Adults with Hidden Adiposity

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M.S., Georgia State University, 2015

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Abstract

Integrated Clinical and Metabolic Phenotyping of Adults with Hidden Adiposity

By Moriah P. Bellissimo

Obesity is a common medical condition characterized by excess body fat that leads to metabolic diseases including cardiovascular disease, type 2 diabetes, and some cancers. In addition to increased total body fat, visceral adipose tissue (VAT), fat stored intra-abdominally, is an independent contributor to metabolic disease risk. Clinical evaluation for obesity often uses body mass index (BMI), a ratio of weight to height. However, evidence indicates that metabolic health is heterogeneous and individuals with the same BMI can have vastly different comorbidities. As a result, use of BMI alone may inaccurately assess metabolic health of individuals. The purpose of this dissertation was to utilize body composition and fat distribution analysis to provide greater insight into metabolic health and obesity pathophysiology.

This dissertation included three cross-sectional studies with the following aims 1) leverage a large cohort of working adults (n=693) to investigate differences in diet quality scores and physical fitness levels between adults categorized as lean, as having normal weight obesity (NWO), or as having overweight-obesity; 2) in a subset of the cohort (n=179), compare the plasma metabolome between the body composition groups using high-resolution metabolomics (HRM); and 3) examine diet quality and body fat distribution between adults with CF (n=24) and age-matched healthy controls (n=25) and determine if these factors are related to clinical assessments.

Adults with NWO had lower physical fitness levels than lean adults and included more females than males. Reported diet quality was similar between NWO and lean adults, but higher diet quality was associated with lower body fat and VAT in all participants. HRM analyses detected metabolic perturbations that were not recognized by classic clinical laboratories. Adults with NWO had metabolomic profiles similar to adults with overweight-obesity, including upregulated linoleic acid metabolism and altered amino acid metabolism. Finally, in the clinical cohort, participants with CF reported lower diet quality scores compared to controls. One-third of adults with CF had NWO and participants with CF had more VAT than age-matched controls, which was related to fasting glucose levels and added sugar intake. Larger studies in longitudinal cohorts are needed to evaluate these relationships and confirm findings.

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CHAPTER 1

INTRODUCTION

More than one-third of adults in the United States have obesity, which is a leading risk factor for seven of the top 10 causes of death in the U.S. (1, 2). Treatment of obesity and obesity-related diseases, including type 2 diabetes, cardiovascular disease, hypertension, and some cancers, is costly and represents a major economic burden. It is estimated that $315.8 billion are spent every year treating obesity (3), which is projected to increase up to $66 billion annually by 2030 (4). Preventing and treating obesity is a top public health priority to reduce medical expenditures (5). Prevention and treatment efforts must be comprehensive as this disease has a complex etiology including environmental, genetic, and behavioral factors, and a multifaceted pathophysiology, which involves inflammation, oxidative stress, and metabolic impairment (6). Accurate assessment of individuals is critical for adequate treatment and prevention of obesity and its sequelae. However, classic tools have been insufficient at detecting adiposity and obesity-related complications in some individuals. Improved methodologies in body composition and fat distribution assessment, high-resolution metabolomics, and integrated clinical and functional outcomes, are allowing greater insight into the metabolic state of adiposity to design earlier screening and more effective treatment options.

A subtype of obesity that goes unrecognized ("hidden") by body mass index (BMI) measures alone are individuals with normal weight obesity (NWO). These individuals appear to be lean due to a normal weight according to BMI guidelines but have a higher than normal body fat percent, based on specific cut points (7). Despite a normal weight BMI, these individuals exhibit metabolic abnormalities and incur increased disease risk (8, 9). Individuals with NWO

may present with increased visceral adipose tissue (VAT), which is also not distinguished when using BMI. This adipose tissue is stored within the abdominal wall and close to organs such as the liver, pancreas, and intestines. VAT is a dysfunctional adipose tissue that promotes metabolic disease independently of total body fat (10-12).

Whereas undernutrition has predominantly been a major concern for clinical populations, improved medical treatments and extended life expectancy have created a shift away from malnutrition prevention and towards a more obesogenic landscape (13, 14). Importantly, in many clinical populations, obesity and/or excess adiposity may exacerbate disease progression. In cystic fibrosis, for example, where patients are living much longer due to advancements in therapy, increased adiposity may be detrimental to lung function (15). However, heavy reliance on BMI in clinical settings may prevent detection of increased fat mass and related clinical and metabolic changes in these patients (16, 17).

The goal of this dissertation was to utilize sensitive body composition, plasma metabolomics, and lifestyle determinants to phenotype individuals with NWO and CF. The **overarching hypothesis** was that the use of detailed body composition assessments would enable more sensitive detection of adiposity, which would be related to low diet quality and physical fitness, altered metabolic profiles, and negative clinical outcomes. To address this hypothesis, we conducted the following Specific Aims:

Specific Aims and Hypotheses

Specific Aim 1 (*Chapter 4):* Examine differences in lifestyle and classic clinical biomarkers among adults categorized into three body composition subtypes (lean, NWO, and overweight-obese).

We hypothesized that individuals with NWO would have similar diet quality scores, physical fitness levels, and clinical biomarkers as individuals with overweight-obesity and lower diet quality scores and physical fitness levels than lean participants.

Specific Aim 2 *(Chapter 5):* Perform untargeted high-resolution metabolomics to investigate differences in the plasma metabolome of adults categorized as lean, as having normal weight obesity (NWO), or as having overweight-obesity.

We hypothesized that individuals with NWO would have metabolomic profiles that are similar to subjects with overweight-obesity and distinct from subjects who are lean.

Specific Aim 3 *(Chapter 6)*: Examine differences in body composition, body fat distribution, and diet quality between adults with CF and healthy controls, as well as their relationships with CF clinical outcomes (lung function and fasting blood glucose concentrations).

We hypothesized that adults with CF would have increased VAT, which would be associated with poor diet quality and negative clinical outcomes in this clinical population.

These aims and hypotheses were addressed in three research studies, each represented by a separate chapter within this dissertation. The first two studies were conducted in a general ambulatory cohort of working adults. The third study assessed the inter-relationships between diet, body composition, and clinical outcomes in a population with CF. Chapter 2 of this dissertation provides an overview of the pathophysiology of obesity and outlines the classic and novel assessment tools used in this dissertation to examine factors related to adiposity. Chapter 3 provides details about the parent studies utilized in each research project and the methods of

assessment used in each study. Chapters 4-6 detail the research conducted to answer the respective specific aim listed above. Chapter 7 of this dissertation includes a discussion of the overall research, clinical applications, and future directions for proceeding in this research area.

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CHAPTER 2

BACKGROUND

Obesity Pathophysiology

When energy intake exceeds energy expenditure, either through excess energy intake or low energy expenditure, body fat mass increases. When this state of positive energy balance is sustained chronically, individuals develop obesity (1). Overtime, accretion of lipids and expansion in the volume of adipose tissue is paralleled by increases in the volume of skeletal muscle and the liver, as well as other organs (2). This results in persons with obesity having greater fat and lean mass as well as a higher resting energy expenditure, cardiac output, blood pressure, and pancreatic ß-cell mass compared to individuals with normal weight (2, 3). These alterations at the tissue and organ level in obesity are also accompanied by cellular changes outlined below.

In adipose tissue of persons with obesity, there is increased infiltration of macrophages and other immune cells. These immune cells release proinflammatory cytokines (2) such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Inflammation-induced by local cytokines causes adipose tissue to shift from storing fatty acids to releasing fatty acids (3). Adipocytes secrete cell-signaling proteins (adipokines) at a rate that is influenced by adipose tissue amount and location (2). In persons with obesity, there is altered release of proinflammatory adipokines and oxidant production, contributing to chronic, low-grade inflammation and oxidative stress that leads to metabolic dysregulation (3-5). In addition to elevated inflammation and oxidative stress, increased hydrolysis of triglycerides and elevated free fatty acids and lipid intermediates contribute to the development of insulin resistance, which

frequently occurs in persons with obesity (2). Location of fat storage is also recognized as an important driver of obesity pathophysiology, and this aspect of adiposity is discussed below.

Visceral Adiposity

In addition to total body adiposity, increased visceral adipose tissue (VAT) mass also contributes to metabolic impairment (6). Deposition of VAT is a complex process that is not fully understood. Current evidence suggests that as subcutaneous adipose tissue (SAT) reaches its maximum capacity for expansion and storage of excess energy, fat is deposited intra-abdominally (visceral fat) and in other normally lean tissues such as the liver and pancreas (ectopic fat) (6-10). The chronic inflammatory state in obesity may further promote VAT accumulation through cytokine-induced interference of adipocyte maturation and promotion of lipolysis (11). Additional factors that play a role in fat accruing in the visceral cavity are age, race, sex, genetics, poor diet, and physical inactivity (6, 7). Accumulation of VAT results in poor health outcomes due to several key characteristics of this fat tissue.

Visceral adipose tissue (VAT) has metabolic properties that are distinct from subcutaneous adipose tissue (SAT) and promote cardiometabolic disease (3). VAT and ectopic fat depots have limited ability to store fatty acids and are highly lipolytic, leading to lipotoxicity, insulin resistance, inflammation, and ultimately, metabolic disease (6, 7, 10). Visceral fat is predominantly drained by the portal vein; so that with higher amounts of VAT, the liver must process greater quantities of free fatty acids and glycerol released from VAT. This results in reduced liver uptake of insulin, exacerbating hyperinsulinemia; increased production of very low density lipoprotein cholesterol, and increased hepatic glucose production (6). These metabolic changes connect VAT with glucose intolerance and type 2 diabetes. VAT also increases

inflammation through the release of TNF-α and IL-6 at higher rates than SAT and altered release of adipokines, namely decreased adiponectin secretion (6, 7, 11). Finally, excess VAT is a marker of additional ectopic fat. When SAT has limited expansion, lipid accumulation occurs in the visceral cavity as well as in the other tissues that are not designed to store fat, including the liver, heart, pancreas, and skeletal muscle (6). Lipid accumulation in these tissues impairs the function of these tissues, ultimately causing disease (3, 6). In total, VAT acts through several co-occurring processes that result in metabolic dysfunction and increase an individual's disease risk.

Both total adiposity and VAT are recognized as independent factors that promote metabolic diseases (3). Comprehensive investigation of obesity and fat distribution requires application of a broad range of assessment techniques. The knowledge gained through the use of new assessment methods may inform future clinical practices and improve efforts to identify individuals with obesity and design effective treatments to reduce disease occurrence.

Principles of Nutrition Assessment Applied to Research in Adiposity

A person's nutritional status, defined as an individual's health condition as it is influenced by the intake and utilization of nutrients (12), is the result of interrelated factors including the quality and quantity of food intake, physical health and functioning, and environmental factors (13, 14). Diet and nutrition are key influences on a person's health (15); therefore, nutritional assessments are important to consider when evaluating an individual's health and disease risk. Nutritional assessments are utilized in a broad range of research fields and are used to identify nutritional targets for disease treatment and prevention efforts, measure effectiveness of nutritional programs and interventions, and screen individuals at risk for malnutrition or overnutrition (13). These assessments are generally categorized as either indirect or direct

measurements (13). Indirect assessments measure factors that impact resources such as the social and physical environments, ecological elements, and economical factors. Direct assessments are conducted at the individual level and are grouped into anthropometric, biochemical, clinical, and dietary evaluations (13). Use of a wide range of these techniques can provide comprehensive assessment of the broad health and metabolic impacts of excess adiposity. In this dissertation, we applied direct nutritional assessments to explore the interrelationships between adiposity and fat distribution with detailed clinical and metabolic assessments.

Lifestyle Determinants Influencing Adiposity

Diet and physical activity play a key role in weight gain or weight maintenance and fat tissue partitioning, ultimately influencing risk of non-communicable diseases (15). While excess calorie intake leads to obesity (1), another important aspect of dietary intake that impacts adiposity and disease risk is the quality of food intake. A high quality diet is characterized by a greater intake of vegetables, fruits, whole grains, lean proteins, and healthy fats, and a lower intake of added sugars, sodium, trans-fats, and saturated fats (16). A poor diet is considered the leading risk factor for mortality in the United States (15). Dietary indices or dietary scores are composite measures of food groups chosen based on *a priori* criteria because they are associated with reduced disease risk (17), adhere to a particular dietary pattern, or represent concordance with a set of dietary guidelines (18-20). Several diet quality scores were utilized in this dissertation and discussed in detail in chapter 3, including the Alternate Healthy Eating Index (AHEI)(17), the Dietary Approaches to Stop Hypertension (DASH) score (21-23), the Mediterranean Diet Score (MDS)(18), and the Healthy Eating Index-2015 (HEI-2015)(19).

While the scores used have some overlap, each score reflects a unique dietary pattern linked to healthfulness and has distinct scoring components.

High diet quality assessed by AHEI, MDS, and DASH scores have been associated with reduced adiposity, as measured by BMI, waist-to-height ratio, and waist circumference (24, 25). Much of the research surrounding diet quality has focused on obesity-related diseases such as hypertension, type 2 diabetes, and cardiovascular disease. In a recent meta-analysis, high quality diets, as measured by the AHEI and the DASH score, were associated with a significant reduction in all-cause mortality, cardiovascular disease, cancer, type 2 diabetes, and neurodegenerative diseases (20). Highest quality diets were associated with up to a 22% reduction in disease risk (20). The Mediterranean diet has also been linked to reduced cardiovascular disease and mortality risk (18, 26, 27). Given the close link between excess adiposity and cardiovascular disease, type 2 diabetes, and some cancers, it is evident that high diet quality is an important lifestyle determinant of adiposity and disease risk. Higher diet quality has also been linked to improved metabolic health such as lower blood pressure, improved glycemic control, and lower oxidative stress (21, 25, 28, 29). Assessment of dietary quality considers the synergistic health effects of nutrients consumed together and plays an important role in metabolic health and disease risk; however, there is a need to study diet quality in the context of sensitive body composition measurements.

Another fundamental determinant of adiposity is physical activity. Participating in physical activity is protective against obesity and obesity-related diseases. To meet physical activity recommendations, adults must perform at least 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity aerobic physical activity each week (30). Despite the well-known health benefits of physical activity, it is estimated that 24% of adults are physically inactive

globally (31), and physical inactivity accounts for an estimated 6% of coronary heart disease and 7% of type 2 diabetes cases (32). Increasing physical activity levels is effective for reducing chronic disease prevalence in controlled research settings (33), but requires community support for large scale implementation (33).

Whereas physical activity is a behavior that increases energy expenditure, physical fitness is a functional attribute that is influenced by the amount of physical activity one performs. Assessing physical fitness provides an objective and accurate measure of physical activity participation (34). One measure of physical fitness is maximal aerobic capacity, referred to as VO_2 maximum (VO_2max) (35), which assesses maximal oxygen uptake by the entire cardiorespiratory system. It is well established that a higher VO_2max (i.e., more physically fit) is inversely related to cardiovascular disease, metabolic diseases, and all-cause mortality (32, 36-38). Increasing physical activity and improving physical fitness has also been shown to reduce total adiposity as well as visceral adiposity (6, 39). Despite being underutilized as a method for disease prevention and treatment, increased physical fitness is an important influence on adiposity.

Anthropometric Measures of Adiposity

Anthropometry is the study of human measurement and is a fundamental aspect required for obesity research (40). Simple anthropometric evaluations can be performed with low-cost tools such as scales, calipers, tape measures, and stadiometers. These tools are typically portable and feasible for use in field settings (40). These assessment methods also enable simple measurements of height, weight, and circumferences, which provide the basis for some of the most popularly used nutritional status indicators such as growth charts and mid-upper arm

circumference (41). The anthropometric index used for growth charts is body mass index (BMI), which is calculated as weight in kilograms divided by height in meters squared. Cut points of BMI are widely used to classify an individual as underweight, normal weight, overweight, or obese, with specific classes of obesity (41). BMI has served as an important tool for screening individuals clinically and for studying anthropometry and body fatness at population levels when cost and feasibility of other assessment methods limit their application (42). While these simple measures of anthropometry are highly cost-effective and feasible to employ on large scales, there are some reservations for use of these assessments.

Utilizing BMI as a key nutritional assessment for anthropometry neglects the distinct components of body weight, namely lean mass, fat mass, and bone mass. Obesity as defined by BMI cut points is remarkably heterogeneous, and individuals who have an obese BMI can have varying comorbidities and metabolic health (7, 43). Likewise, individuals with a normal weight BMI (<25 kg/m^2) can have varying degrees of adiposity, lean mass, and clinical risk factors (44). Additionally, important factors that influence the composition and distribution of a person's weight such as sex, age, race, and fitness level are neglected if solely using BMI (42). Finally, location of where fat is stored on the body is an important factor to consider when assessing an individual's disease risk (6). BMI assessment provides no indication of an individual's fat pattern deposition, and circumference measures provide limited information. While convenient and cost-effective, BMI is an insufficient marker to assess a person's metabolic disease risk (6). A move towards sensitive, accurate measures of anthropometry provides greater insight into body composition beyond measures of total weight.

Dual-Energy X-Ray Absorptiometry Assessment of Body Composition

Assessment of body composition using a multicomponent model allows investigation of the individual compartments of body weight beyond, which is not available using BMI. This enables accurate measurement of fat mass and other tissues for better understanding of their links to health and disease. Numerous methods exist to assess body composition, each with varying accuracy, cost, and feasibility (45). Dual energy x-ray absorptiometry (DXA) is a high-precision technique used to assess total and regional body composition (46). As subjects lie in a supine position on the DXA bed, the machine emits two low energy waves that pass through the subject. The attenuated energy rays are measured by a detector above the subject and converted to an electronic signal (45). Because different body tissues have varying densities, the attenuated energy rays can be used to measure fat mass, fat free mass, and bone mineral density with high precision and measurement stability (46). Limitations of DXA include the weight maximum of the scanning bed, lack of portability, and small amount of radiation exposure (45). Thus, DXA is an appropriate technique to use for quantification of fat mass and to study obesity with few limitations.

In addition to total body composition assessments, DXA also measures subregions including the arms, legs, trunk, and head based on specific well-defined cut points (46). Moreover, recent software advances now provide measurements of VAT (47). While excess total body fat is the primary characteristic of obesity, excess VAT, fat located within the abdominal cavity, is a key driver of metabolic disease risk, independent of total body fat (3, 48). Increased VAT is strongly linked to adverse clinical outcomes, including insulin resistance and cardiovascular disease (6). Thus, measurement of VAT is an important aspect of assessing adiposity. VAT as measured by DXA provides advantages over both computed tomography (CT) and magnetic resonance imaging (MRI) because it has lower radiation exposure than CT, has a

short (10 min) scan time, is calculated automatically, are of much lower cost than both CT and MRI, and DXA machines are readily available in clinical settings, with appropriate software to determine fat, lean, bone, and VAT mass (6, 47). DXA-derived VAT assessment has been validated against CT and shown to accurately measure VAT (47). By using body composition and fat distribution assessments derived from DXA, we are able to study the links between obesity and VAT with markers of metabolic health, clinical outcomes, and dietary intake. These additional assessments are introduced below.

Novel Biochemical Markers Linked to Adiposity

Obesity is often accompanied by comorbidities such as hypertension, dyslipidemia, and insulin resistance (2). Classic nutrition- and metabolism-related biochemical measures to monitor these obesity-related conditions include fasting glucose and insulin concentrations, lipid panels, and serum electrolyte panels. Dyslipidemia of obesity is characterized by elevated levels of fasting plasma triglycerides and low-density lipoprotein cholesterol along with low levels of high-density lipoprotein cholesterol (2). Insulin resistance often occurs in obesity and is closely associated with excess VAT (2, 6, 48). While these biomarkers provide valuable information regarding metabolic function and are readily available, they may not fully capture the complexity of metabolic diseases.

Aminothiol Redox

Oxidative stress is a key pathogenic component associated with obesity and additional metabolic diseases (49). One method to assess systemic oxidative stress is measurement of plasma aminothiols. The plasma aminothiol/disulfide couples, cysteine/cystine (Cys/CySS) and glutathione/glutathione disulfide (GSH/GSSH), are the most abundant low molecular weight

redox pools in extracellular and intracellular fluid, respectively. These redox couples are maintained independent of each other under stable, steady-state conditions in biological systems. The redox nodes function in discrete redox pathways to help regulate cellular signaling and function, and increased oxidative stress disrupts these pathways (49, 50). Measurement of traditional oxidative stress markers such as urinary isoprostanes, free radicals, and oxidized lipids reflects pro-oxidant macromolecular damage. In contrast, assessment of the plasma aminothiols provides insight into underlying mechanisms and pathway-specific oxidative damage (49-51). For example, GSH functions as an important antioxidant, and assessment of GSH reflects the availability of GSH for protection against oxidative reactions. Cys/CySS are regulated independently of GSH/GSSG and respond to inflammatory processes. Therefore, assessment of the plasma aminothiol redox couples provides an integrated picture of oxidative stress. Aminothiol redox imbalance is associated with adiposity, aging, atherogenesis, endothelial dysfunction, increased carotid intima media thickness, and type 2 diabetes (25, 49, 52-55). Thus, assessment of the plasma aminothiols can provide mechanistic insight linking oxidative stress to metabolic diseases (56-60).

High-Resolution Metabolomics

A novel biochemical assessment technique useful for phenotyping adiposity is high-resolution metabolomics (HRM). HRM is a state-of-the-art assessment method that measures low molecular weight (<2,000 Da) chemicals in human biosamples (61). Whereas traditional clinical biomarkers provide assessment of single, large molecules, HRM provides comprehensive detection of a broad range of metabolites, collectively referred to as the metabolic phenotype (62). In addition to the 40 essential nutrients, HRM also detects the estimated 2,000 intermediate metabolites (61) and thousands of endogenous lipid molecules and lipid intermediates (63).

Additionally, metabolites originating from the gut microbiome can be profiled in the plasma metabolome (64). HRM is beginning to be utilized as an objective assessment method for dietary intake. Targeted metabolomics has been used to identify biomarkers of intake of specific foods such as citrus fruits, salmon, red meat, cruciferous vegetables, coffee, and sugar sweetened beverages (65-67). Untargeted metabolomics has been utilized to study unique dietary patterns (68-70). Diet and nutrition are fundamentally complex as there are interactions between dietary intake with the gut microbiome, the genome, environmental pollutants, and disease states (61). Use of HRM may help to account for inter-individual variation in nutrient intake, digestion, and absorption when relating systemic metabolism to disease (71).

HRM provides the capability for investigators to comprehensively explore endogenous nutrition-related metabolism in various disease states such as obesity (72-76). Metabolomics has been used to identify specific metabolic signatures related to BMI and obesity (72, 73, 75, 77-79). A consistent finding among studies using metabolomics to study obesity has been elevated plasma branched chain amino acids (BCAAs), aromatic amino acids (AAAs), and C3 and C5 acylcarnitines (73, 80). The link between BCAA and C3 and C5 acylcarnitines is proposed to be a result of excess substrates in mitochondrial metabolism, which causes an overflow of these anaploretic molecules into the plasma (81). In line with altered mitochondrial metabolism, increased free fatty acids in the plasma is also a common finding for obesity research and linked to changes in mitochondrial beta-oxidation (72, 81). With regard to ectopic fat depots, increased plasma glutamate has been identified as the predominant signature of elevated VAT (82). These findings have helped advance the field of obesity research to define pathophysiologic mechanisms, which can be targeted in designing therapeutic strategies.

Advances in omics and bioinformatics tools also enable the integration of HRM data with

anthropometric, clinical, and other biochemical measures for comprehensive metabolic phenotyping (83, 84). The use of plasma HRM provides an objective, global assessment of a person's chemical profile and metabolism related to a disease state to inform targeted intervention and therapies (61, 85). As an objective assessment method, biochemical markers provide insight into metabolic regulation. Integration of classic biochemical measurements with novel assessments such as HRM enables mechanistic insight into metabolic health related to adiposity.

Normal Weight Obesity

There is increasing awareness of a group of people that appear to be lean according to BMI assessment but have high adiposity. These individuals have been termed as having normal weight obesity due to a normal weight BMI (BMI between 18.5-24.9 kg/m^2) but a percent high body fat. Although excess adiposity is the defining characteristic of obesity, there is no consensus on percent body fat cut points to classify obesity (86). There is established epidemiologic research showing BMI to be positively associated with mortality and obesity-related diseases such as cardiovascular disease and type 2 diabetes (86). However, research that utilized bioelectrical impedance analysis to assess fat mass and a BMI >30 kg/m^2 showed that BMI had a sensitivity of 42% to detect obesity (44, 87). Another study using DXA assessments reported that within the normal weight range of BMI, men had a body fat that ranged from 5.6-31.2% and women had body fat that ranged from 4.6-51.1% (88). This evidence shows that BMI may underestimate obesity and half of individuals with obesity may be misidentified by BMI guidelines.

As an under recognized high-risk population, individuals with NWO exhibit abnormal metabolic markers, including increased risk factors for chronic disease, and an increased mortality risk (89, 90). Evidence from diverse adult populations, comprising South Americans, Europeans, Asians, and North Americans, shows the presence of altered metabolic profiles in individuals with NWO. This includes hyperlipidemia, increased fasting glucose, increased insulin levels and insulin resistance, and high blood pressure (86, 91). Additionally, there is also evidence of altered adipokine levels such as leptin concentrations in individuals with NWO (92). One group found a decreased basal metabolic rate in women with NWO (93). There is also evidence of individuals with NWO exhibiting elevated markers of inflammation and oxidative stress, and lower antioxidant capacity than lean individuals (90, 94). These markers of increased inflammation and oxidative stress may indicate metabolic dysregulation that precedes metabolic changes that are clinically detected such as insulin resistance or hyperlipidemia (90, 91). Interestingly, people with NWO may or may not present with increased visceral or abdominal adiposity (86). Finally, several studies have found increased mortality risk in individuals with NWO. Romero-Corral *et al*. (87) found that women with NWO were 2.2 times higher cardiovascular disease mortality risk than lean women. Work from others suggests that individuals with normal weight central obesity have the highest mortality risk compared to other adiposity patterns (95). Despite no established definition for NWO, there is strong evidence of an altered metabolic state and increased disease risk in individuals with NWO.

There is limited research on lifestyle factors that may be driving the prevalence of NWO. Several studies have investigated diet and physical activity, which may play a role in NWO development (96-98). One group found intake of certain food groups, including sweets, cereals, fish, and vegetables, distinguished lean and NWO groups (96). Another found that individuals

with NWO had low intakes of antioxidants (97). While these findings are insightful for behaviors of individuals with NWO, a comprehensive assessment of diet quality in an NWO cohort has not been conducted. A study of young adults in China found lower levels of physical fitness in men and women with NWO based on several running and agility tests (98). However, in another study using self-reported physical activity data, individuals with NWO reported higher levels of physical activity participation than lean individuals (96). Thus, the role of diet quality and physical activity in NWO is largely unknown. There is a need to assess diet quality in individuals with NWO and provide objective measures of physical fitness in this population to understand if these factors impact NWO development.

Cystic Fibrosis

Special considerations must be made when conducting research in a specific clinical population. All evaluations must be interpreted within the context of the disease-specific physiology. For example, medical nutrition therapies are uniquely designed for different diseases and biomarkers may have disease-specific cut points (13). Cystic fibrosis (CF) is an autosomal recessive genetic disorder that affects 30,000 people in the United States. In CF, a genetic mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) protein causes dysfunction in the transport of chloride ions at many epithelia surfaces (99). This results in a muscus build up that affects multiple organ function including the lungs, pancreas, and liver (99, 100). Patients with CF often experience recurrent infection and inflammation, nutrient malabsorption, and skeletal muscle catabolism (100, 101). This cycle of catabolic milieu have prompted clinical care of persons with CF to focus on optimizing nutritional status.

Historically, improved nutritional status has been a crucial determinant of prognosis and survival for people with CF (102). To combat malnutrition in CF, nutrition therapies have promoted lifelong adherence to a high fat, high calorie diet (103) with little, if any, emphasis on diet quality. There is a lack of updated nutrition-related research in CF, but one recent study found that children with CF reported increased intake of foods with high calorie but low nutrient content (104). Monitoring of nutritional status in patients with CF relies heavily on achieving and maintaining a BMI at sex-specific goals. However, BMI does not assess fat, lean, or bone mass, which are individually related to clinical outcomes in CF populations. Therefore, sensitive body composition evaluations are needed to accurately measure nutritional status in people with CF.

Components of body composition have been linked to clinical outcomes in CF. Persons with CF have been reported to have low lean mass, which is not detected by BMI, and linked to worse disease severity, increased inflammation, and lower lung function (101, 105). Fat mass may be unrelated or negatively related to lung function (106-108). Additionally, a cross-sectional study of adults with CF found a 31% prevalence of NWO. Individuals with NWO had low fat-free mass (bone and lean mass) and worse lung function compared to adults with CF who were lean or overweight-obese (105). Several studies in CF have identified increased central fat accumulation (109, 110), which is an independent risk factor for metabolic disease in the general population (3). Although not evaluated in CF, in other clinical popualtions of patients with coronary disease and chronic kidney disease (CKD), NWO and normal weight central obesity are related to worse diseae prognosis and mortality risk, respectively (111, 112). The factors contributing to deposition of VAT in persons with CF are not well understood and there is a need to identify possible determinants.

Furthermore, improvements in the medical treatment of patients with CF has vastly increased life expectancy in this population, and there has been a parallel rise in the incidence of co-morbidities, such as obesity and CF-related diabetes (CFRD)(99). In the general population, increased abdominal adiposity is linked to higher risk of type 2 diabetes (3), however, knowledge is limited regarding the relationship between VAT and metabolic function in the CF community. Now, as CF transitions to a chronic disease with increasing occurence of comorbidities, updated body composition and nutrition-related research is needed to inform revised nutrition therapies that promote optimal health in people with CF.

Overall Goal and Significance

The goal of this dissertation was to gain greater insight into the relationships between adiposity and lifestyle factors, metabolic health, and clinical outcomes in adults with NWO and CF. We approached this goal in the following three chapters.

Overview of Chapters

In the fourth chapter, titled *Physical fitness but not diet quality distinguishes lean and normal weight obese adults*, we describe lifestyle determinants of adiposity, including diet quality scores and physical fitness levels, between adults categorized into three body composition subtypes: lean, normal weight obesity (NWO), or overweight-obesity. Individuals with an NWO phenotype have a normal body weight according to BMI guidelines but a high body fat percent (86). While there is strong evidence suggesting that individuals with NWO have abnormal biochemical markers (89), there is a lack of research characterizing lifestyle factors such as diet and physical fitness in people with NWO. In this project, we sought to address this

important knowledge gap by comparing AHEI (17), MDS (18), and DASH (21, 22) diet quality scores as well as VO2max levels between the three body composition subtypes.

In the fifth chapter, *Plasma high-resolution metabolomics differentiates adults with normal weight obesity from lean individuals*, we use HRM to examine differences in the plasma metabolome between adults classified as lean, as having NWO, or as having overweight-obesity. Past research shows evidence of metabolic dysregulation in adults with NWO, but there is a need to describe the nutrition- and metabolism-related dysfunction in NWO. To this end, the chemical profile generated from HRM, referred to as the metabolic phenotype, includes a broad range of chemical classes and metabolic pathways to provide a sensitive measure of nutrition-related metabolism and investigate NWO from a systems biology approach. This chapter compared the fasting plasma metabolome between adults in the three body composition groups.

In the sixth chapter, *Visceral adipose tissue is associated with poor diet quality and higher fasting glucose in adults with cystic fibrosis*, we studied a cohort of clinically stable adults with CF that were age-matched to adults without known illness. This chapter addresses the lack of updated nutrition-related research in the CF population by conducting detailed body composition assessment and collecting 3-day food records for analysis of nutrient intake and calculation of HEI-2015 to measure diet quality. Clinical assessments were also utilized including lung function of participants with CF and fasting glucose concentrations in all subjects. We related these nutritional assessments of dietary intake, clinical, and biochemical measures to body composition and fat distribution in the context of CF pathophysiology.

The novel information produced from this research can provide a basis for the development of strategies that modify nutritional components to improve overall health in adults with NWO and persons with CF.

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CHAPTER 3

EXTENDED METHODS

This chapter provides an overview of the parent studies and methods used in each chapter of this dissertation (Chapters 4-6). The current chapter includes the objectives and hypotheses for each chapter, purpose of the parent studies utilized, participant recruitment strategies, inclusion and exclusion criteria, as well as expanded descriptions of the assessment methods used in the respective chapters.

Specific Aims and Hypotheses

This dissertation focuses on describing relationships between adiposity and fat distribution with clinical, biochemical, and dietary assessments in general and clinical populations. The specific aims for each study were:

Specific Aim 1 (Chapter 4): Examine differences in lifestyle and classic biochemical markers among adults categorized into three body composition subtypes (lean, NWO, and overweight-obese).

We hypothesized that individuals with NWO would have similar diet quality scores and physical fitness levels, and clinical biochemical risk factors as individuals with overweight-obesity and lower diet quality scores and physical fitness levels than lean participants.

Specific Aim 2 (Chapter 5): Perform untargeted high-resolution metabolomics to investigate differences in the plasma metabolome of adults categorized as lean, as having normal weight obesity (NWO), or as having overweight-obesity.

We hypothesized that individuals with NWO would have metabolomic profiles that are similar to subjects with overweight-obesity and distinct from subjects who are lean.

Specific Aim 3 (Chapter 6): Examine differences in body composition, body fat distribution, and diet quality between adults with CF and healthy controls, as well as their relationships with CF clinical outcomes (lung function and fasting blood glucose concentrations).

We hypothesized that adults with CF would have increased VAT, which would be associated with poor diet quality and negative clinical outcomes in this clinical population.

Methods Specific to Chapters 4 and 5

Center for Health Discovery (CHD) Study Overview

In the United States, there is a huge expenditure for treating medical conditions, many related to lifestyle behaviors. In 2005, Emory and Georgia Tech established the Predictive Health Institute to challenge the current medical system by shifting the focus away from disease diagnosis and treatment and towards recognizing, maintaining, and sustaining optimal health. A key step to accomplish the goals of the Predictive Health Institute was establishing a research cohort, the Center for Health Discovery and Well-Being (CHD) cohort that would assist in defining and predicting health. Health is a dynamic and complex concept. The CHD study was developed to begin unraveling that complexity by assessing health indicators of major organ systems over time to examine their predictive ability for health and disease (1).

One important aim of the CHD study was to obtain new information on factors that predict health over time, including diet, exercise, body composition, and social and psychological factors in individuals followed for several years. The study also aimed to discover new biomarkers that may predict future disease and to define normal ranges of values for those

new markers. Data collected from the study also allows researchers to investigate multifaceted concepts of health to better define and predict health while avoiding disease. The information collected in the CHD study is extremely valuable for developing new and better ways to prevent disease and promote optimal health and functioning.

CHD Cohort Study Inclusion and Exclusion Criteria

The inclusion and exclusion criteria were designed to recruit an adult cohort with few known acute or uncontrollable chronic conditions.

Inclusion Criteria

1. Age 18 years and older.
2. No history of hospitalization due to an acute or chronic disease within the previous year (with exception of hospitalization for treatment of accidental trauma).
3. No history of severe Axis 1 psychosocial disorders within the previous year (e.g. delerium, dementia, schizophrenia, depression, bipolar disorder, hypochondriasis, dissociative disorder).
4. No history of addition of new prescription medications to treat a chronic disease condition (with exception of changes of anti-hypertensive or anti-diabetic agents) within the previous year.
5. No history of substance/drug abuse or alcoholism within the previous year.
6. Minimum of two years employment at Emory University or Emory Healthcare.
7. Currently working at least 20 hours/week.
8. Covered by Emory health insurance plans.

Exclusion Criteria

1. Current active malignant neoplasm; history of malignancy other than localized basal cell cancer of skin during previous 5 years.
2. Uncontrolled or poorly controlled autoimmune, cardiovascular, endocrine, gastrointestinal, hematologic, infectious, inflammatory, musculoskeletal, neurologic, psychiatric or respiratory disease.
3. Any acute illness (such as viral infection) in previous 12 weeks before baseline studies.
4. Likely inability to undergo the complete set of CHD assessments or participate in CHD educational and follow-up sessions over a several year period.
5. Inability to give informed consent.

CHD Participant Recruitment

The human resources department at Emory University identified employees who were eligible to participate in the CHD study. Employees were stratified to recruit a representative distribution of employees from faculty, Fair Labor Standards Act (FLSA)-exempt staff, and FLSA-nonexempt staff positions. To be eligible, employees had to be employed at Emory for at least two years and insured by university-sponsored health insurance plans. An alphabetic list of employees was generated, and every 10th employee was invited to participate in the study. An e-mail invitation to join the study was sent to eligible employees along with a description of the program. From the total list of eligible employees, 30% were reported to agree to be contacted for screening, and 10% were enrolled in the cohort (1).

Eligible participants were initially screened by telephone to ensure that they met all inclusion and exclusion criteria. Potential participants with positive screens were then e-mailed or mailed an informed consent and a brochure describing the CHD program. At the study visit,

participants signed an informed consent that explained the goals for the CHD, outlined the prospective data collection and analysis plans, described all of the specific measurements and assessments that could be performed, outlined the educational program, and gave permission for entry into a research subject registry from which they may be contacted by other investigators for additional ancillary studies. The informed consent also described that participants granted permission for investigators to perform some or all of the assessments and measurements at the discretion of the investigators or CHD staff. Finally, the consent also gave permission for the CHD staff and investigators to access additional participant health-related data for research purposes from other university-affiliated clinical care centers (1). These latter details were critical to help build a patient registry that could be utilized for current and future research. For the studies included in this dissertation, only baseline study visit assessments were included.

Assessments

Anthropometric measurements: Height and weight were measured without shoes. Height was measured with digital stadiometer and recorded to the nearest tenth of a centimeter. Weight was measured with a digital scale and recorded to the nearest tenth of a kilogram. Body mass index (BMI) was calculated from height and weight.

Body composition and fat distribution: A GE Lunar iDXA was used to measure body composition and fat distribution. Dual energy x-ray absorptiometry (DXA) is often considered a gold standard method for body composition assessment due to its high precision and accuracy (2). A three compartment model of body composition is assessed by DXA, which entails measurement of fat, lean, and bone tissues. DXA measures body composition by emitting two low dose x-ray beams with varying energy that pass through the body. The subject being assessed lies in a supine position on the bed. The x-ray beams are emitted from below the subject

and pass through the body. There is differential attenuation of the energy due to the varying densities of body tissue compartments. The attenuated x-rays are then measured by an energy-discriminating detector above the subject and converted to an electronic signal. Using well-established points of interest, regional body composition measurements are also calculated (3). The technology of DXA makes it possible to accurately measure body composition over a broad range of body sizes and types.

New software developments for DXA machines also enable measurement of visceral adipose tissue (VAT). Assessment of VAT provides a more in-depth view of body composition, with particular focus on VAT, which is closely tied to many metabolic diseases. Currently, the methods considered gold standard for VAT assessment are computed tomography (CT) or magnetic resonance imaging (MRI). Both of these assessments require access to highly specialized clinical equipment and time-intensive manual quantification of subcutaneous abdominal adipose tissue (SAAT) and visceral adipose tissue (4). CT also has a high dose of radiation associated with the assessment (4). DXA machines are available clinically, have high-precision and low radiation exposure. Now, CoreScan™ software also enable fully automated assessment of visceral adipose tissue, providing several advantages over VAT-assessment by CT and MRI.

Measurement of VAT was developed and described in detail by Kaul and colleagues (4). Briefly, to begin VAT measurement, an android region is automatically designated. The android region begins at the uppermost point of the iliac crest and extends 20% of the distance from the iliac crest to nearest point of the skull. VAT estimation by DXA is conducted through modeling algorithms. These algorithms were developed separately for males and females due to sex differences in abdominal fat distribution. A training dataset was used that included participants

with varying age, height, weight, and body mass index (BMI) to define the DXA modeling parameters. SAAT is quantified using measurements of abdominal thickness derived from x-ray attenuation, the width of the abdominal subcutaneous fat layer, and empirically derived geometric constants developed from comparing DXA and CT images. VAT is then computed by subtracting SAAT from total abdominal adipose tissue (4).

Food Frequency Questionnaires: The 2005 Block Food Frequency Questionnaire (FFQ) was used to assess habitual food intake from a wide range of nutrients and food groups. The full-length survey includes approximately 110 food items. The FFQ can be self-completed or administered by an interviewer and has an estimated time to completion of 30-40 minutes. The food list and nutrient data based used for the 2005 Block FFQ were updated from previous versions. The food list was developed from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) dietary recall data to comprise foods typically included in American diets. The nutrient database was developed from the USDA Food and Nutrient Database for Dietary Studies (FNDDS), version 1.0. Additionally, there are a series of questions included to provide adjusted values with greater accuracy for fat and carbohydrate intake. Pictures for each food are shown to enhance the accuracy of reporting individual portion size. The 2005 Block FFQ is a validated tool for dietary assessment and a popularly used tool (5-7).

Diet Quality Scores: The Alternate Healthy Eating Index (AHEI)(8), Mediterranean Diet Score (MDS)(9), and Dietary Approaches to Stop Hypertension (DASH) Score (10, 11) were all calculated from Block FFQ output. The detailed scoring criteria for each index are shown below as reported in Bettermann *et al*. 2018 (12).

Table 3.1. Dietary components used for the Alternative Healthy Eating Index (AHEI) Score calculation.

Dietary Component	Foods Included	Criteria for Points	
Vegetables, servings/d	All vegetables	0pts = 0 srv 10pts = 5 srv	
Fruit, servings/d	All fruits and juices	0pts = 0 srv 10pts = 4 srv	
Cereal and Grains, g/d	All cereals and grains	0pts = 0 g 10pts = 15 g	
Legumes, Nuts and Soy, servings/d	Peas, beans, nuts, nut butter, and soy products	0pts = 0 srv 10pts = 1 srv	
Ratio of white to red meat	Whole diet	0pts = ratio of 0 10 pts = ratio of 4	
Alcohol, servings/d	Wine, beer, and liquor	Females: 0 pts = 0, >2.5 srv 10 pts = 0.5-1.5 srv	Males: 0 pts = 0, >3.5 srv 10 pts = 1.5-2.5 srv
% kcal from trans fat	Whole diet	0 pts = $\geq$ 4% energy 10 pts = $\leq$ 0.5% energy	
Polyunsaturated to Saturated Fatty Acids Ratio	Whole diet	0 pts = ratio $\leq$ 0.1 10 pts = ratio $\geq$ 1	
Duration of Multivitamin Use	Prenatal vitamins and multivitamins	2.5 pts = < 5 years 7.5 pts = $\geq$ 5 years	

Table 3.2. Dietary components used for the Mediterranean Diet Score calculation.

Dietary Component	Foods Included	Criteria for 1 Point		Median[1]	
		Female	Male	Female	Male
Vegetables, servings/d	All vegetables except potatoes	≥ median intake	≥ median intake	2.17	1.98
Legumes, nuts, and soy, servings/d	Peas, beans, nuts, nut butter, and soy products	≥ median intake	≥ median intake	0.98	0.99
Fruits, servings/d	All fruits and juices	≥ median intake	≥ median intake	1.23	1.27
Total Grain, servings/d	Cereals and grains	≥ median intake	≥ median intake	3.95	5.31
Fish, servings/d	All fish	≥ median intake	≥ median intake	0.63	0.68
Ratio of monounsaturated to saturated fatty acids	Whole diet	≥ median intake	≥ median intake	1.39	1.33
Alcohol, servings/d	Wine, beer, and liquor	5-25 g/day	10-50 g/day		
Dairy products, servings/d	All dairy products	< median intake	< median intake	0.97	1.17
Meats, servings/d	Poultry, lunch meat, organ meat, red and white meat	< median intake	< median intake	1.97	2.27

[1]Median intake (servings/d) for dietary components were calculated stratified by gender.

Table 3.3. Dietary components used for the DASH Score calculation.

Dietary Component	Foods Included	Criteria for Points
Vegetables, servings/d	All vegetables	≥ 4 srv/day: 1 point 2-3 srv/day: 0.5 < 2 srv/day: 0
Fruit, servings/d	All fruit and juices	≥ 4 srv/day: 1 point 2-3 srv/day: 0.5 < 2 srv/day: 0
Total Grain, servings/d	All grains	≥ 7 srv/day: 1 point 5-6 srv/day: 0.5 < 5 srv/day: 0
Whole Grain, servings/d	Only whole grains	≥ 2 srv/day: 1 point 1 srv/day: 0.5 < 1 srv/day: 0
Legumes, Nuts, Soy, servings/wk	Peas, beans, nuts, nut butter, and soy products	≥ 4 srv/wk: 1 point 2-3 srv/wk: 0.5 < 2 srv/wk: 0
Dairy products, servings/d	All dairy products	≥ 2 srv/day: 1 point 1 srv/day: 0.5 < 1 srv/day: 0
Meat/Poultry/Fish, servings/d	Poultry, lunch meat, organ meat, red and white meat	≤ 2 srv/day: 1 point 3 srv/day: 0.5 ≥ 4 srv/day: 0
% kcal from fat	Whole diet	≤ 30%: 1 point 31-32%: 0.5 ≥33% : 0
% kcal from saturated fatty acids	Whole diet	≤ 10%: 1 point 11-12%: 0.5 ≥13% : 0
% kcal from sweets	Whole diet	≤ 3.2% = 1 point 3.3 – 5.0% = 0.5 point ≥.5.1% = 0 point
Sodium, mg/d	Whole diet	≤ 1500 mg/day: 1 point 1501 – 2400 mg/day: 0.5 ≥ 2401 mg/day: 0

VO2 Maximum: VO_2 maximum is a measure of maximal oxygen uptake for the entire cardiorespiratory system. It is strongly predictive of functional status and an individual's ability to perform work where a higher VO_2 maximum indicates better functional status. VO_2 maximum is also commonly used to assess physical fitness. The VO2 maximum test used in the CHD study

was a modified Balke protocol (13). The test is designed for participant to walk on the treadmill to exhaustion. At timed stages the test the walking speed (mph) and grade of slope (%) of the treadmill are increased.

The test is initiated with a 1-2 minute warm-up at 1.5 mph and 0% grade of slope. Then to begin the test, the treadmill is set up at the stage 1 speed (2.0 mph) and grade of slope (0%). At the respective times during the test, the speed and slope of treadmill are adjusted. After three minutes into the test the speed is adjusted to 2.0 mph and the slope to 3.5%, after six minutes into the test the speed is adjusted to 2.0 mph and the slope to 7.0%, as shown in the table below. At each stage of the test, the participant's blood pressure, heart rate, and rating of perceived exertion (RPE) are recorded. The technician starts the stopwatch at the start of the test and stops it when the participant is unable to continue. Following completion of the test, there is a short recovery period for the participant.

Table 3.4. Protocol used for VO2 maximum testing in CHD study.

Stage	Time (min)	mph	Slope %	Workload (ml/kg/min)
1	0-3	2.0	0	8.7
2	3-6	2.0	3.5	12.2
3	6-9	2.0	7.0	15.6
4	9-12	2.0	10.5	18.6
5	12-15	2.0	14.0	22.4
6	15-18	2.0	17.5	25.7
7	18-21	3.0	12.5	29.7
8	21-24	3.0	15.0	33.3
9	24-27	3.0	17.5	36.9
10	27-30	3.0	20.0	40.5
11	30-33	3.0	22.5	44.1

The calculation for VO2 maximum is shown below. The technician records heart rate 2 (HR2) and heart rate 1 (HR1) for the calculation below (ideally between 115 and 150 bpm). Workload 2 (WL2) corresponds to the stage of the protocol that HR2 was recorded and so forth for WL1 and HR1.

Calculation of estimated VO2 maximum using a multi-stage submaximal treadmill protocol:

1) Calculate slope (b) = $\frac{WL2 - WL1}{HR2 - HR1}$ 2) VO2 max = WL2 + b (HRmax – HR2)

Using a multi-stage model, heart rates and workloads are recorded from 2 or more stages. Where, HR2 is the steady state heart rate in the more intense stage completed. WL2 is the oxygen consumption (VO2) in ml/kg/min at the more intense stage completed. WL1 is the oxygen consumption (VO2) in ml/kg/min at the lower intensity stage completed HR1 is the steady state heart rate in lower intensity stage completed. HR max is age predicted heart rate maximum and calculated as 220 – age in years.

Laboratory Assessments

Fasting blood samples were collected to assess biomarkers relevant to glucose, insulin, lipids, inflammation, and aminothiol redox measures. Blood samples were obtained by a trained nurse and collected from the participant's upper arm at each study visit. A brief description of the laboratory techniques used to measure each marker included in the study is found below.

Fasting glucose: Plasma glucose concentrations were determined using spectrophotometry by Quest Diagnostics, Inc (Tucker, GA) with a reference range of 65-99 mg/dL.

Fasting insulin: Insulin levels were determined using immunoassay methods by Quest Diagnostics, Inc (Tucker, GA) with a reference range of 2.0-19.6 µIU/mL.

Fasting total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol: Total and HDL cholesterol concentrations were assessed using spectrophotometry by Quest Diagnostics, Inc (Tucker, GA) with reference ranges of ≤200 mg/dL for total cholesterol and >40 mg/dL for males and >50 mg/dL for females for HDL cholesterol for adults 20 years of age or older. LDL cholesterol was determined using enzymatic methods by Quest Diagnostics, Inc (Tucker, GA) with a reference range of <100 mg/dL for adults 20 years of age and older.

Fasting triglycerides: Plasma glucose levels were determined using spectrophotometry by Quest Diagnostics, Inc (Tucker, GA) with a reference range of <150 mg/dL for adults 20 years of age and older.

Cytokines: Serum tumor necrosis factor-α (TNF-α), interferon-gamma (IFNγ), interleukin-6 (IL-6), and interleukin-8 (IL-8) levels were determined using a Fluorokine® MultiAnalyte Profiling multiplex kit (R&D Systems, Minneapolis, MN) with a Bioplex analyzer (Bio-Rad, Hercules, CA).

Aminothiol Redox: Plasma glutathione, glutathione disulfide (GSSG), cysteine (Cys), cystine (CySS), and the CySS-GSH mixed disulfide (CySSG) were measured by high performance liquid chromatography following published methods (14) at Emory University. Following collection of a peripheral blood sample, a preservative solution containing iodoacetic acid was added to the sample and the blood was centrifuged. Supernatants were stored at – 80°C in 10% perchloric acid and 0.2M boric acid until derivatization with dansyl chloride and quantification of GSH, GSSG, Cys, CySS, and CySSG concentrations using high-performance liquid chromatography (HPLC) with fluorescence detection. The redox potential [E_h in millivolts

(mV)] of each redox couple (Eh GSSG and Eh CySS, respectively) was measured using the Nernst equation. Higher E_h values indicate more oxidative stress.

High-resolution metabolomics: Plasma high-resolution metabolomics (HRM) was performed in the Emory University Clinical Biomarkers Laboratory (15). Fasting blood samples were collected in EDTA tubes, centrifuged, and stored at –80°C. Thawed plasma samples (50 µL) are then treated with acetonitrile [high performance liquid chromatography (HPLC) grade, Sigma-Aldrich, St. Louis, MO] and an internal standard mixture containing 14 stable isotopic chemicals. These chemicals are selected to cover a wide range of chemical properties of small molecules and include: [$^{13}C_6$]-D-glucose, [^{15}N]-indole, [2-^{15}N]-L-lysine dihydrochloride, [$^{13}C_5$]-L-glutamic acid, [$^{13}C_7$]-benzoic acid, [3,4-$^{13}C_2$]-cholesterol, [^{15}N]-L-tyrosine, [trimethyl-$^{13}C_3$]-caffeine, [$^{15}N_2$]-uracil, [3,3-$^{13}C_2$]-cystine, [1,2-$^{13}C_2$]-palmitic acid, [^{15}N,$^{13}C_5$]-L-methionine, [^{15}N]-choline chloride, and 2'-deoxyguanosine-$^{15}N_2$,$^{13}C_{10}$-5'-monophosphate. To remove protein, samples were centrifuged at 14,000 x *g* for 5 minutes at 4°C and then loaded onto a Shimadzu® (Sil-20AC Prominence) autosampler until injection and kept at 4°C. Samples are analyzed in triplicate in batches of 20, and pooled reference samples (Qstd) and National Institutes of Standards and Technology (NIST) 1950 certified pooled plasma standard reference material are also prepared and included at the beginning and end of each batch.

Chromatography was performed on a Dionex Ultimate 3000 UHPLC using C18 liquid chromatography (LC) and positive electrospray ionization (ESI) to maximize the detection of low-molecular weight chemicals. Mass spectrometry was conducted on a Fourier transform mass spectrometer (MS, Q-Exactive HF, Thermo Fisher, Waltham, MA) with a mass-to-charge (*m/z*) scan range of 85-1,250.

The raw LCMS data files are extracted using apLCMS(16) and xMSanalyzer(17). The

apLCMS program uses the raw spectral data to identify peaks, filter noise, perform retention time alignment, and peak alignment (16). Additionally, xMSanalyzer works in conjunction with apLCMS. This package uses optimized feature detection methods to increase the number of detectable features with high accuracy. Specifically, xMSanalyzer is able to detect and extract features that may be present in low abundance and/or in a subset of the population being studied. This function is particularly important when studying dietary and environmental chemical exposures, which differ greatly between individuals. xMSanalyzer is also compatible with existing databases such as human metabolome database (HMDB) and can be used for feature annotation of known metabolites within biological pathways (17). To adjust for non-biological experimental variation in the batches analyzed, Combat software (18) was used. This framework is appropriate to use in smaller sample sizes and robust to outliers. By using this method, the data from multiple batches can be combined to increases statistical power (18). Finally, prior to statistical analyses, data pre-processing was performed to enhance reproducibility of results and reduce measurement variability. This included filtering of features with a coefficient of variation (CV) >50%, filtering of samples if there was a signal in at least 50%, and log10 transformation, quantile normalized, and mean centering.

Methods Specific to Chapter 6

Integration of Nutritional Metabolomics with Bioenergetics in Cystic Fibrosis Study (BEAM-CF) Overview

Cystic fibrosis (CF) is a catabolic, recessive genetic disease whose prognosis is highly dependent on maintenance of adequate nutritional status. As life expectancy increases, co-morbidities such as CF-related diabetes (CFRD) become more prevalent. It is thus increasingly important to identify pathophysiologic pathways that will inform nutritional therapies for adult

patients with CF. The overarching goal of the parent study was to test the hypothesis, using a variety of assessment methods, including high-resolution, liquid chromatography-mass spectrometry (LC-MS) metabolomics methods, that adults with CF have impaired β-oxidation compared to healthy controls. The parent study included extensive metabolic and nutritional assessments in the cohort, which were leveraged for this dissertation sub-study.

Participant Selection

Clinically-stable adult CF patients were recruited from outpatient Emory CF Clinics and among cohorts of patients with CF who were concurrently enrolled in studies performed in the Georgia Clinical and Translational Science Alliance (Georgia CTSA) Clinical Research unit. Healthy, age-matched volunteers were recruited through the use of approved flyers posted throughout Emory campus and surrounding areas and by word-of-mouth. Potential healthy volunteers were also identified (and invited via phone, email, or mail to participate in the study) through a database maintained by the Emory/Georgia Tech Predictive Health Institute, which includes individuals who have agreed to be contacted for future research studies. Participants were also recruited by advertising at health fairs, community events, and health and fitness stores and venues.

CF Inclusion Criteria

1. Must have a confirmed CF diagnosis with at least one Class I to III CFTR mutations.
2. Must be aged ≥ 18 years.
3. Must be on a clinically-stable medical regimen for 3 weeks.
4. Must have had no intravenous or oral antibiotics for at least 3 weeks.

CF Exclusion Criteria

1. Currently pregnant or breastfeeding.

2. Is unwilling or unable to discontinue enteral tube feeds for one night before the study visit, if applicable.

3. Has a most recent FEV1% <40%.

4. Has a history of drug (recreational or prescription) or alcohol abuse.

5. Has a pacemaker or any electronic implantable device.

6. Is unable to give informed consent.

The study was designed to enroll CF participants to maintain an even distribution of glucose tolerance groups: CF participants with confirmed diagnosis of CFRD for 1 year (and active treatment with insulin or other glucose lowering medication), with pre-diabetes or early untreated CFRD, and with normal glucose tolerance. Glucose tolerance status was determined based on an OGTT performed within the last 8 weeks (as standard of care or as part of a previous research study) or based on an OGTT performed during the study visit.

Healthy Controls Inclusion Criteria

1. Between the ages of 18-50 years.

2. Has ambulatory status.

3. No hospitalization in the previous year except for accidents.

Healthy Controls Exclusion Criteria

1. Is currently pregnant or breastfeeding.

2. Has a current active malignant neoplasm or history of malignancy (other than localized basal cell cancer of the skin) during the previous 5 years.

3. Has a current respiratory disease including asthma, chronic obstructive pulmonary disorder, or emphysema.

4. Has a current chronic autoimmune or pro-inflammatory disease.

5. Has a history of tuberculosis, HIV, or other chronic infection.

6. Has a previous diagnosis of type 1 or type 2 diabetes with active treatment with insulin or other glucose lowering medication.

7. Has advanced (≥ stage 3) renal disease.

8. Has a BMI ≥ 30 kg/m^2.

9. Has had an acute illness (such as a viral infection) within the past 2 weeks.

10. Is currently using prescription medications that would indicate presence of an acute or chronic medical condition that may influence study results.

11. Has a history of drug (recreational or prescription) or alcohol abuse.

12. Reports weight instability (± 10% body weight within the last 6 months) or current participation in weight loss or weight gain program.

13. Inability to provide informed consent.

Assessments

Anthropometric measurements: Height and weight were measured without shoes. Height was measured with a manual stadiometer and recorded to the nearest tenth of a centimeter. Weight was measured with a digital scale and recorded to the nearest tenth of a kilogram. Body mass index (BMI) was calculated from height and weight. Waist circumference was measured using a tape measure and recorded to the nearest tenth of a centimeter. Measurements were taken at the umbilicus, and the average of three assessments was recorded.

Clinical information: Descriptive clinical data including genotype, most recent FEV1%, HbA1c values, and pancreatic sufficiency were determined by self-report or through data extraction of medical records.

Oral glucose tolerance testing (OGTT): An OGTT was performed to verify glucose tolerance status if one has not been performed within eight weeks prior to the study visit. An OGTT was not performed if a participant with CF had a confirmed diagnosis of cystic fibrosis related diabetes (CFRD) and was being treated with insulin or other glucose lowering medications. If a participant with CF reported a past diagnosis of diabetes or it was noted in the medical record, but the subject was not actively being treated with insulin and/or oral glucose lowering medications, a finger stick blood glucose test was performed prior to the OGTT. If the finger stick glucose level was ≥ 126 mg/dL (7.0 mmol/L), diabetes status was confirmed and the OGTT was not be performed.

For the procedure, fasting peripheral blood samples were obtained by a trained nurse. Within five minutes following blood collection, participants consumed a drink containing 1.75 g of glucose per kilogram of body weight up to a maximum of 75 g of glucose. Another blood sample was taken two hours following consumption of the glucose-containing drink.

Fasting glucose and two-hour glucose: Plasma glucose concentrations were determined using enzymatic methods by Emory University Clinical Laboratory (Atlanta, GA) with a fasting glucose low and high reference range of 70-105 mg/dL, respectively. Glucose tolerance was categorized based on the following criteria from the American Diabetes Association.

Table 3.5. Glucose tolerance categories.

	Fasting plasma glucose mg/dl (mmol/l)	2-h OGTT glucose mg/dl (mmol/l)
NGT	<100 (5.6)	<140 (7.8)
IFG + NGT	100–125 (5.6–6.9)	<140 (7.8)
IGT	<100 (5.6)	140–199 (7.8–11.1)
IFG + IGT	100–125 (5.6–6.9)	140–199 (7.8–11.1)
CFRD FH−	<100 (5.6)	≥200 (11.1)
CFRD + IFG	100–125 (5.6–6.9)	≥200 (11.1)
CFRD FH+	≥126 (7.0)	≥200 (11.1)

Abbreviations: NGT, normal glucose tolerance; IFG, Impaired fasting glucose; CFRD, cystic fibrosis-related diabetes; FH- , without hyperglycemia; FH+, with hyperglycemia.

Body composition and fat distribution: Body composition and fat distribution were assessed using DEXA with CoreScan™ software, as described for the CHWB cohort above.

Three-day food records: Participants completed a food diary for three days (2 weekdays and 1 weekend day) where they recorded everything they ate and drank, including supplements. Participants were given food records prior to their study visit with pictures and instructions of how to accurately estimate food and drink consumption. Participant were asked to return completed food records on the day of their visit. On the day of the study visit, a registered dietitian reviewed the food record for accuracy and asked any questions to improve clarity in the dietary record.

Multiple days for records are considered the gold standard for dietary assessment. While not typically feasible in clinical settings, research subjects usually have the time and ability to complete these assessments. Some limitations of food records include that subjects may change how they typically eat or drink because they are recording their intake. The method is also time intensive for the subject and can become burdensome, especially if recording for longer amounts of time. Researchers should also be aware that the intake period recorded may not capture habitual dietary intake. Finally, analyzing food records can be time intensive and costly.

All dietary food records were analyzed using the Nutrient Data System for Research software (NDSR, Nutrition Coordinating Center, University of Minnesota, MN, USA; database version 2016) by a registered dietitian. NDSR software produces several different output files, including quantification of 174 nutrients that can then be used in subsequent statistical analyses.

Diet Quality Scoring: Diet quality was assessed by calculating the Healthy Eating Index-2015 score from the participants' three-day food record data and NDSR output. The HEI-2015 evaluates dietary adherence to the 2015 Dietary Guidelines for Americans (19). As shown in the table below, some components of the index are scored for adequacy of intake while others are scored for moderation in intake. The detailed scoring criteria for this index are shown below.

Table 3.6. Healthy Eating Index-2015 (HEI–2015[1]) components and scoring criteria.

Component	Maximum points	Standard for maximum score	Standard for minimum score of zero
Adequacy			
Total Fruits[2]	5	≥0.8 cup equiv. per 1,000 kcal	No Fruit
Whole Fruits[3]	5	≥0.4 cup equiv. per 1,000 kcal	No Whole Fruit
Total Vegetables[4]	5	≥1.1 cup equiv. per 1,000 kcal	No Vegetables
Greens and Beans[4]	5	≥0.2 cup equiv. per 1,000 kcal	No Dark Green Vegetables or Legumes
Whole Grains	10	≥1.5 oz. equiv. per 1,000 kcal	No Whole Grains
Dairy[5]	10	≥1.3 cup equiv. per 1,000 kcal	No Dairy
Total Protein Foods[6]	5	≥2.5 oz. equiv. per 1,000 kcal	No Protein Foods
Seafood and Plant Proteins[6,7]	5	≥0.8 oz. equiv. per 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids[8]	10	(PUFAs + MUFAs)/SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation			
Refined Grains	10	≤1.8 oz. equiv. per 1,000 kcal	≥4.3 oz. equiv. per 1,000 kcal
Sodium	10	≤1.1 gram per 1,000 kcal	≥2.0 grams per 1,000 kcal
Added Sugars	10	≤6.5% of energy	≥26% of energy
Saturated Fats	10	≤8% of energy	≥16% of energy

1. Intakes between the minimum and maximum standards are scored proportionately.
2. Includes 100% fruit juice.
3. Includes all forms except juice.
4. Includes legumes (beans and peas).

5. Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.
6. Includes legumes (beans and peas).
7. Includes seafood, nuts, seeds, soy products (other than beverages), and legumes (beans and peas).
8. Ratio of poly- and monounsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs)
Table available at: https://epi.grants.cancer.gov/hei/developing.html#f2

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CHAPTER 4

PHYSICAL FITNESS BUT NOT DIET QUALITY DISTINGUISHES LEAN AND NORMAL WEIGHT OBESE ADULTS

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Abstract

Background: Individuals with normal weight obesity (NWO) have increased cardiometabolic disease and mortality risk, but factors contributing to NWO development are unknown.

Objective: The objective of this study was to determine if diet quality scores and physical fitness levels differed between adults classified as lean, NWO, and overweight-obese.

Design: This cross-sectional study included metropolitan university and healthcare system employees. Body composition was measured by dual energy x-ray absorptiometry. Individuals with a body mass index (BMI) below 25 and body fat >23% for men and >30% for women were classified as having NWO. Alternate Healthy Eating Index (AHEI), Dietary Approach to Stop Hypertension (DASH) score, and Mediterranean Diet Score (MDS) were calculated from Block food frequency questionnaires. Cardiorespiratory fitness was assessed by measuring maximum oxygen consumption (VO2 maximum) during treadmill testing.

Participants/setting: This study included 693 adults (65% female, mean age 48.9 ± 11.5 years) enrolled between 2007 and 2013 in Atlanta, Georgia.

Statistical Analyses: Multiple linear regression analyses with post-hoc comparisons were used to examine relationships between the body composition groups and fitness, diet quality, and biomarkers. Multiple linear regression analyses were also used to examine relationships between diet quality scores and fitness with components of body composition.

Results: VO2 maximum was significantly lower in the NWO compared to the lean group (36.8 ± 0.8 vs. 40.8 ± 1.0 mL/min/kg, $p<0.05$). Individuals with NWO reported similar diet quality to lean individuals and more favorable AHEI and DASH scores than overweight-obese individuals ($p<0.05$). Diet quality and VO2 maximum were inversely associated with percent body fat and visceral adipose tissue ($p<0.05$) regardless of weight status. Individuals with NWO exhibited

higher fasting blood insulin concentrations, insulin resistance, LDL cholesterol, and triglyceride levels, and significantly lower HDL cholesterol levels than lean individuals ($p<0.05$).

Conclusions: Cardiorespiratory fitness was significantly decreased in individuals with NWO compared to lean individuals. Higher diet quality was associated with decreased total and visceral fat but did not distinguish individuals with NWO from lean individuals.

Research Snapshot

Research Question: Do diet quality scores and physical fitness levels differ between adults categorized as lean, as having normal weight obesity (NWO), or as having overweight-obesity?

Key Findings: In a large cohort of working adults, participants with normal weight obesity had lower physical fitness levels than lean individuals but reported similar diet quality to lean participants and higher diet quality scores than overweight-obese participants. For clinical biomarkers, individuals with NWO exhibited adverse metabolic panels that were similar to participants with overweight-obesity.

Introduction

Obesity is a principal, preventable risk factor for numerous well-characterized diseases such as cardiovascular disease, type 2 diabetes, and some cancers, which represent leading causes of death globally [1, 2]. When examining associations with disease, obesity is typically assessed by body mass index (BMI) to identify at-risk individuals. While BMI is an important measure used for epidemiology surveillance, clinical observations reveal that individuals classified as normal weight or obese using BMI may present with an unhealthy or healthy metabolic profile, respectively [3, 4]. Thus, BMI does not capture the large heterogeneity in cardiometabolic risk observed across individuals. This is partly due to BMI lacking the sensitivity needed to distinguish the proportions of fat and fat free mass that contribute to total body weight, and BMI provides no indication of fat mass distribution, which are important drivers of metabolic disease development [5, 6]. Further, BMI does not account for age, race, sex, or fitness level, which influence body composition [7]. Therefore, assessment of obesity and disease risk using BMI categories may misclassify individuals at risk for chronic disease.

Normal weight obesity (NWO) has emerged as a term to denote individuals who have a normal weight according to BMI guidelines but a disproportionately high body fat mass [8]. Studies have suggested the NWO body composition phenotype is associated with increased risk of chronic diseases and cardiometabolic abnormalities, including dyslipidemia, hypertension, glucose intolerance, and increased levels of inflammation and oxidative stress markers [8-15]. Further, individuals with NWO exhibit a higher mortality risk compared to their lean counterparts and metabolically healthy obese individuals [8, 13, 16, 17]. Despite this increase in disease risk, individuals with NWO may be overlooked by health professionals and/or misclassified as “healthy” when solely utilizing BMI as a screening tool [18].

Specific factors driving the prevalence of NWO are unknown. Lifestyle factors, such as diet quality and physical activity, are important factors influencing health and disease. A higher quality diet, characterized by a greater consumption of vegetables, fruits, whole grains, healthy fats, and lean proteins, is associated with lower chronic disease and mortality risk [19-23]. Diet quality indexes such as the Dietary Approach to Stop Hypertension (DASH) diet score, Alternate Healthy Eating Index (AHEI), and Alternate Mediterranean Diet Score (MDS) are associated with improvements in blood pressure, lower biomarkers of inflammation and oxidative stress, and reduced risk for type 2 diabetes and cardiovascular disease [19-28]. Physical inactivity is another major risk factor for chronic diseases and accounts for an estimated 9% of global premature mortality from leading cardiometabolic diseases[29, 30]. However, knowledge is limited on the role of diet quality and physical fitness in individuals with NWO. The objective of this study was to examine diet quality scores and physical fitness levels between adults categorized into three body composition subtypes (lean, NWO, and overweight-obese). It was hypothesized that individuals with NWO would have similar diet quality scores and physical fitness levels as individuals with overweight-obesity and lower diet quality scores and physical fitness levels than lean participants. Given that lifestyle behaviors are key drivers of metabolic health and disease[31], a secondary aim of this study was to provide extensive metabolic phenotyping of individuals with NWO.

Materials and Methods

Participants and Study Design

This study utilized the Emory-Georgia Tech Center for Health Discovery and Well Being Predictive Health Institute cohort (http://predictivehealth.emory.edu) based in Atlanta, Georgia, USA. This cohort is comprised of university and healthcare employees who were invited to

participate in the study[32]. Invited participants had to be employed for at least two years, and every tenth person from an alphabetized list was invited to join the study [32]. Data collection took place between December 2007 and January 2013. General exclusion criteria were having a poorly controlled chronic disease, acute illness, hospitalization within the previous year, and women who were pregnant or breastfeeding. The complete study protocol was previously described[32, 33]. The study was approved by the Emory Institutional Review Board, and all participants provided informed consent. Participants underwent extensive metabolic testing, including clinical laboratory analysis, dietary assessment, and exercise testing. Demographic information, educational attainment, and annual household income were self-reported. Participants were categorized as having a chronic disease (hypertension, hyperlipidemia, or diabetes mellitus) if they reported a previous diagnosis or were currently taking medications to treat hypertension, hyperlipidemia, or diabetes mellitus. Participants with available body composition and anthropometric data were included in this cross-sectional study (n=693).

Body Composition Analysis and Body Composition Subgroups

Body composition, including visceral adipose tissue (VAT), was assessed by dual energy x-ray absorptiometry (DXA) using a Lunar iDXA densitometer and CoreScan software (GE Healthcare, Madison, WI, USA). Height and weight were measured in light clothing without shoes using a digital scale and stadiometer (Tanita TBF-25, Tanita Health Management, Arlington Heights, IL). Height was recorded to the nearest eighth of an inch, weight was recorded to the nearest tenth of a pound, and both measures were converted to metric units. BMI was calculated as body weight in kilograms divided by height in meters squared (kg/m^2). Participants were categorized as either lean, NWO, or overweight-obese based on BMI and sex-specific body fat percent cut points. A body fat percent above 23 was considered elevated for

males, and a body fat percent above 30 was considered elevated for females based on previously published literature [34]. Lean participants had a BMI value between 18.5 and 24.9 kg/m^2 and a body fat percent below the sex-specific cut-off values. NWO was characterized as a BMI between 18.5 and 24.9 kg/m^2 and a body fat percent above the sex-specific cut-off values. Overweight-obese participants had a BMI ≥25 kg/m^2 and a body fat percent above the sex-specific cut-off values. A health professional trained in anthropometry assessed waist circumference (WC) to the closest millimeter using a tape measure. Three WC measurements were taken, and the average value is reported.

Diet Quality Scores, Dietary Food Groups, and Physical Fitness

Diet quality scores were calculated from dietary intake data assessed using 2005 Block food frequency questionnaires (FFQ, NutritionQuest, Berkeley, CA, USA), which reflected dietary intake over the past year[35-37]. Reported intakes that were less than 500 kcal per day or greater than 5,000 kcal per day were excluded for implausible values. All dietary data were energy adjusted per 1000 kcal. Three diet quality scores were calculated as previously described within this cohort[24]: AHEI[28], DASH[38] with adapted scoring of the sweets component[25, 39], and MDS [40]. The AHEI ranges from 0-87.5, DASH score ranges from 0-11, and MDS ranges from 0-9. For all diet quality scores, a higher score is indicative of a higher quality, more healthful diet. Independent of the diet quality scores, dietary food categories (i.e., grains, fiber, sugar, fruit, vegetables, and proteins) were also used to test for differences in reported intake of the food groups between lean, NWO, and overweight-obesity groups. Cardiorespiratory fitness was objectively measured by assessing maximal oxygen consumption (VO2 maximum, mL/min/kg) following a modified Balke protocol performed with a trained technician[41]. All VO2 maximum tests were conducted on a GE T2100 Treadmill (GE Healthcare, Waukesha, WI).

Clinical and Biochemical Markers

All blood samples were taken following an overnight fast, processed, and stored for analysis. Fasting lipid profile, metabolic panel, and inflammatory markers were analyzed commercially by Quest Diagnostics (Valencia, CA). Fasting insulin levels below the level of detection (<2 μIU) were replaced with a value of 1.9 for analyses. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (μIU/mL) x fasting glucose (mg/dL) divided by 405 [42]. An automated machine was used to measure systolic and diastolic blood pressure (Omron, Kyoto, Japan). Plasma aminothiol concentrations, including glutathione (GSH), glutathione disulfide (GSSG), cysteine (Cys), and cystine (CySS), were measured using high performance liquid chromatography following published protocols [43] at Emory University. The reduction-oxidation (redox) potentials (E_h) for the thiol/disulfide couples were calculated using the Nernst equation[43]. E_h provides a measure of the propensity of the redox couples to accept or donate electrons, and a higher value denotes increased oxidative stress. The inflammatory cytokines interleukin-6 (IL-6), IL-8, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were measured in fasting serum using Fluorokine® MultiAnalyte Profiling multiplex kits (R&D Systems, Minneapolis, MN) with a Bioplex analyzer (Bio-Rad, Hercules, CA).

Statistical Analyses

Continuous variables were summarized as mean ± standard deviation. Dichotomous variables were summarized as count (percentage). Continuous variables that did not appear to have a bell-shaped distribution were natural log transformed for analyses and back transformed for data presentation as geometric means. To account for zero values in cytokine analyses, a

constant of one was added to all values prior to log transformation. For categorical variables, χ^2 tests were used to test for differences between the body composition subtypes. Overall group differences in the diet quality scores, dietary food categories, and VO2 maximum between the body composition subtypes controlling for age (as a continuous variable), race (White=0, other=1), sex (male=0, female=1), and education (college degree, no=0, yes=1) were estimated using a multiple linear regression model. Post-hoc comparisons between the body composition subtypes were conducted using Tukey's honestly significant different tests. Within tables and figures, results of Tukey's post hoc analyses are denoted by superscript letters x, y, and z, where values that are not connected by the same letter are significantly different. Multiple linear regression analyses were also used to test for associations in dietary food categories and clinical and biochemical variables controlling for age, race, and sex with post-hoc comparisons conducted using Tukey's honestly significant different tests, as above. Multiple linear regression analyses were utilized to test for associations between components of body composition and VO2 maximum and the diet quality scores, controlling for age (as a continuous variable), race (White=0, other=1), sex (male=0, female=1), and education (college degree, no=0, yes=1). Presence of a chronic disease and reported annual household income were also considered as potential confounders but were not included in final models. The interaction between the body composition subtypes and race, age, sex, and education level on the outcomes was also examined. All analyses were conducted in JMP® Pro software version 13.0.0 (SAS Institute, Cary, NC)[44], using two-sided tests with an alpha significance value of 0.05.

Results

Demographic and clinical characteristics for all participants are shown in **Table 4.1**. BMI and DXA body composition data were available for 693 of the study participants of which 14% were classified as lean, 24% as having NWO, and 62% as having overweight-obesity. There were 14 participants (10 males) with BMI levels in the overweight category and percent body fat values below the sex-specific cut points; these individuals were categorized as lean. The lean group was younger than the NWO and overweight-obesity groups ($p<0.05$). There was a significant difference in sex distribution between the body composition subtypes ($p=0.02$). The proportion of individuals with a history of chronic disease was comparable between the three groups. The lean group had the highest proportion of participants report attaining a college degree or higher (90%) followed by participants with NWO (84%) and participants with overweight-obesity (80%, $p=0.047$). There were no differences in reported annual household income between the three groups ($p>0.05$).

Body Composition and Fat Distribution by Group

Body composition variables are shown in **Table 4.2.** In line with the applied definition of NWO, BMI was similar between the lean and NWO groups and was significantly lower than the overweight-obesity group ($p<0.05$). Total body fat percent, fat mass, VAT, and WC increased significantly across the three groups from individuals classified as lean to having NWO to having overweight-obesity ($p<0.05$). Individuals with NWO had the lowest lean body mass (LBM), which differed significantly between the three groups ($p<0.05$).

Diet Quality Scores by Group

On average, all diet quality scores reflected similar trends: the lean group reported the highest diet quality but was not significantly different from the NWO group (**Figure 4.1**). For AHEI and DASH scores, lean and NWO groups had similar diet quality scores ($p>0.05$), each

with significantly higher scores compared to the overweight-obese group ($p<0.05$). For the MDS, only the lean and overweight-obesity groups significantly differed ($p<0.05$). There was significant effect modification between education level and AHEI ($p=0.03$) and MDS ($p=0.04$). For individuals without college degrees, there were no differences in AHEI or MDS scores across the body composition subtypes. There was also significant effect modification between race and the DASH diet quality score ($p=0.008$). Among individuals who reported White race, DASH diet quality scores differed only between the lean and overweight-obesity groups with the NWO group reporting similar scores to both. These results should be interpreted with caution due to low numbers between groups for education level and race. Stratified analyses with sex as a biological variable are reported in Table 4.5 (supplementary).

In multiple linear regression analyses, all diet quality scores were significantly, inversely associated with measures of body fat and VAT ($p<0.001$ for all) but were not associated with measures of WC (**Table 4.3**). All diet quality scores were significantly, positively associated with percent lean mass ($p<0.001$ for all).

Dietary Food Categories by Group

In comparisons of reported daily food group consumption, participants with overweight-obesity reported significantly higher consumption of trans-saturated fats and potatoes and significantly lower consumption of fruits and legumes, nuts, and soy compared to lean and NWO participants (Table 4.6 [supplementary], $p<0.05$ for all). The NWO group reported lower consumption of total protein and fat, poultry, total grains, refined grains and significantly higher consumption of yellow/orange vegetables than the overweight-obese group ($p<0.05$ for all).

Physical Fitness by Group

Fitness levels were incrementally lower across the three groups ($p<0.05$), with the NWO and overweight-obesity groups having significantly lower fitness levels compared to the lean group (**Figure 4.2**). There was significant effect modification between VO2 maximum and age ($p=0.046$). Among individuals with NWO, there was a significant decline in VO2 maximum with aging. Analyses of VO2 maximum stratified by sex are shown in Table 6 (supplementary). In multiple linear regression analyses, VO2 maximum was inversely associated with all measures of total and abdominal adiposity ($p<0.001$ for all). Lean body mass percent was significantly, positively associated with VO2 maximum (**Table 4.3**, $p<0.001$).

Clinical and Biochemical Markers by Group

Fasting glucose concentrations were similar in the lean and NWO groups and significantly higher in the overweight-obesity group (**Table 4.4**, $p<0.05$). Fasting insulin concentrations and HOMA-IR were significantly different between each group, with the NWO group exhibiting values between the other two groups ($p<0.05$). Total cholesterol was higher in the NWO group compared to the lean group ($p<0.05$). LDL cholesterol levels were similar in the NWO and overweight-obesity groups and significantly elevated compared to the lean group ($p<0.05$). HDL cholesterol and triglycerides differed significantly between each group ($p<0.05$ for both). Systolic and diastolic blood pressure were significantly higher in the overweight-obese group compared to the lean and NWO groups ($p<0.05$). All plasma aminothiol concentrations were similar between lean participants and individuals with NWO ($p>0.05$) and reflected a less oxidized redox state compared to the overweight-obesity group who exhibited higher CySS, lower GSH, higher GSH redox potential, and higher CySS/GSH ratio. Inflammatory cytokines did not differ between the three groups ($p>0.05$ for all).

Discussion

NWO has been associated with cardiometabolic derangements that place seemingly lean individuals at risk for metabolic disease[8-15]. Notably, more adults in this cohort were classified as NWO than were classified as lean. Individuals with NWO had significantly lower physical fitness levels compared to their lean counterparts. Individuals with NWO reported higher diet quality than individuals classified as having overweight-obesity, although higher diet quality was inversely associated with measures of adiposity in all participants regardless of weight status. Furthermore, the metabolic panel of individuals with NWO indicated elevated risk factors for cardiometabolic disease, particularly in markers of insulin resistance and lipid concentrations.

Individuals with NWO have a higher chronic disease and mortality risk compared to normal-weight, lean individuals or metabolically healthy, obese individuals [8, 15-17], and the definition used to classify individuals with NWO plays an important role in establishing these risks. Oliveros and colleagues describe the history of investigating subtypes of obesity and summarize the metabolic dysfunction noted in individuals with NWO [9]. The prevalence of NWO has been reported as high as 30% and differs by race and sex [8, 10]. Importantly, there are no established percent body fat cut-points to define obesity [45], which contributes to the variability in reported prevalence [45, 46]. One study found the prevalence of NWO in women ranged from 1.4 to 27.8% when applying various thresholds. Using age- and sex-specific cut points may decrease the variability in prevalence [46]. In this cohort, there was a 24% prevalence of NWO among all participants, a 27% prevalence of NWO among women, and a 17% prevalence of NWO among men. Additional reports are generally consistent that women have a higher prevalence of NWO than men[46-49]. However, a recent nationwide study of Chinese adults noted a higher prevalence of NWO in males (9.5% vs 6.06%)[14], showing the importance of screening for NWO in both males

and females. Additionally, obesity misclassification by BMI may differ by sex [50]. One study showed males were more likely to be misclassified at a BMI between 25-27 kg/m^2, but women were more likely to be misclassified at a BMI between 24-26 kg/m^2 [50]. Altogether, while the prevalence and definition of NWO is variable, there is strong evidence from our data and others' that supports increased cardiometabolic and mortality risk factors in individuals with normal weight but high body fat and a need to effectively screen and identify these individuals[8-15, 17, 51].

The NWO group in our study had significantly lower levels of objectively measured cardiorespiratory fitness compared to the lean group. Similarly, a Chinese study using objective assessments reported impaired physical fitness and muscular strength in college-aged individuals with NWO [52]. Measures of self-reported physical activity data have shown mixed results [53, 54]. Circuit training has been successfully applied as a 10-week physical activity intervention in women with NWO [55], showing significant improvements in clinical measures and reductions in total and trunk body fat which resulted in participants no longer being classified as NWO [55]. Physical inactivity is a leading risk factor for chronic diseases [56], and increasing physical activity is an effective primary and secondary prevention strategy to reduce chronic disease risk [57]. The health benefits from exercise participation may exceed the effects of prescription medications [58]. Thus, there is a need to address the low levels of physical fitness in this population to reduce disease risk.

Much attention in NWO research is paid to increased adiposity, however, another important characteristic of NWO is decreased lean body mass. Physical activity, especially strength training, is integral for stimulating skeletal muscle protein synthesis and maintaining lean body mass, particularly as one ages [59]. Further, physical activity improves glucose metabolism and insulin sensitivity, and individuals with higher fitness levels have better insulin

sensitivity [60]. Skeletal muscle secretes a variety of myokines, especially during exercise, such as follistatin-like 1, fibroblast growth factor-21 (FGF-21), brain-derived neurotrophic factor (BDNF), myonectin, and interleukin-6 (IL-6), that have both local and systemic health-promoting effects [58]. These chemicals increase glucose uptake, promote uptake and lipolysis of free fatty acids in skeletal muscle and the liver, have neurocognitive benefits, promote angiogenesis, improve endothelial function, and protect against ectopic fat deposition [58]. Thus, strategies designed to increase lean body mass in individuals with NWO may be an important factor to target for health improvements through a variety of mechanisms.

Higher diet quality is associated with decreased chronic disease risk [19-23, 25-28]. In this study, despite differences in measures of insulin resistance, lipid concentrations, and fitness, individuals with NWO reported higher diet quality than individuals classified as overweight-obese. There were few similarities in reported food group intakes between NWO and overweight-obesity groups. Diet quality scores reflected suboptimal diet quality for all groups, although average AHEI score for all groups was above a previously reported U.S. average [61]. Few studies have investigated dietary intake or diet quality of individuals with NWO [53, 54, 62]. Mannisto *et al.* found components of dietary intake related to diet quality were associated with NWO, including lower intakes of cereals, fish, and root vegetables, and higher intakes of sugar [53]. Amani *et al*. found that individuals with NWO consumed lower amounts of antioxidant compounds compared to lean individuals and had similar total antioxidant capacity as individuals categorized as having overweight-obesity [62]. Further, the NWO group consumed higher total energy, less fiber, and fewer servings of fruit, legumes, and nuts and seeds compared to the lean group [62]. Notably, in our entire cohort, higher diet quality was associated with lower total body fat and VAT. In longitudinal studies, poor diet quality has been shown to predict higher visceral

adiposity, and interventions that increase physical activity and/or improve diet quality have been effective in reducing VAT and liver fat while improving cardiometabolic risk factors [63-65]. While diet quality may not differentiate individuals with NWO from lean individuals in this cohort, maintaining a higher diet quality may help prevent additional weight gain.

In the current study, individuals with NWO exhibited adverse metabolic biomarkers that were similar to the overweight-obesity group, including fasting insulin concentrations, HOMA-IR, total cholesterol, and LDL cholesterol. There is substantial evidence of cardiometabolic dysregulation in NWO cohorts, including dyslipidemia [9, 10, 12, 49, 66, 67], increased inflammation [12, 13], increased oxidative stress [11, 13], altered adipokine levels [8, 11], and the presence of metabolic syndrome components including hypertension, insulin resistance, and hyperglycemia [10, 11, 14, 16, 17, 47, 68-70]. Although individuals with NWO showed dysregulated insulin function and altered lipid levels in our cohort, there was no evidence of significant oxidative stress or inflammation in the NWO group compared to the lean group. We previously reported that higher diet quality is associated with lower levels of oxidative stress [24]. It is possible that individuals with NWO in the current cohort maintain a diet quality high enough to sustain aminothiol redox balance. While there is heterogeneity in reported metabolic profiles of individuals with NWO, there is consistent evidence of adverse metabolic health in these individuals, highlighting the need to screen for and prevent NWO[8-15, 17, 51].

Major strengths of this study were the use of sensitive body composition and fat distribution assessment methods in a large cohort of adults to classify body composition subtypes. This study also provides extensive clinical and metabolic phenotyping of individuals with NWO to add to the existing literature of the adverse clinical profiles presented in individuals with NWO. There are also some limitations to this study. FFQs are subject to recall

bias, have a high participant burden, and varying reliability [71, 72]. This is a cross-sectional analysis, and causality cannot be inferred in the reported relationships. Participants in this cohort reported high education and income levels, which may limit the generalizability of this population. Future research should examine the most appropriate cut points for defining obesity considering age, sex, and race. Of note, additional classifications exist for individuals with a normal weight but increased disease risk such as "metabolically obese, normal weight [3]", "lean, insulin resistant [73]," "lean, type 2 diabetics [74]," and "non-alcoholic steatohepatitis in lean individuals[75]." Many of these classifications are based on BMI, whereas NWO is classified by body fat percent and BMI. Indeed, many of these noted classifications are a subset of individuals with NWO with underlying obesity, abdominal adiposity, and inflammation as a driver of cardiometabolic disease [76, 77]. Finally, in addition to diet and physical fitness, numerous factors influence body weight and composition and metabolic health, including genetics, epigenetics, and environmental exposures, which are not addressed here [78, 79].

Conclusions

In conclusion, while diet quality was similar between individuals with NWO and lean individuals, physical fitness was significantly lower in the NWO group. Maintaining an adequately high diet quality may allow for individuals with NWO to maintain a normal weight, but lack of physical activity may prevent stimulation of muscle protein synthesis. Focus on increasing physical activity and physical fitness may be an important lifestyle factor to target for risk reduction in individuals with NWO.

Chapter 4 References

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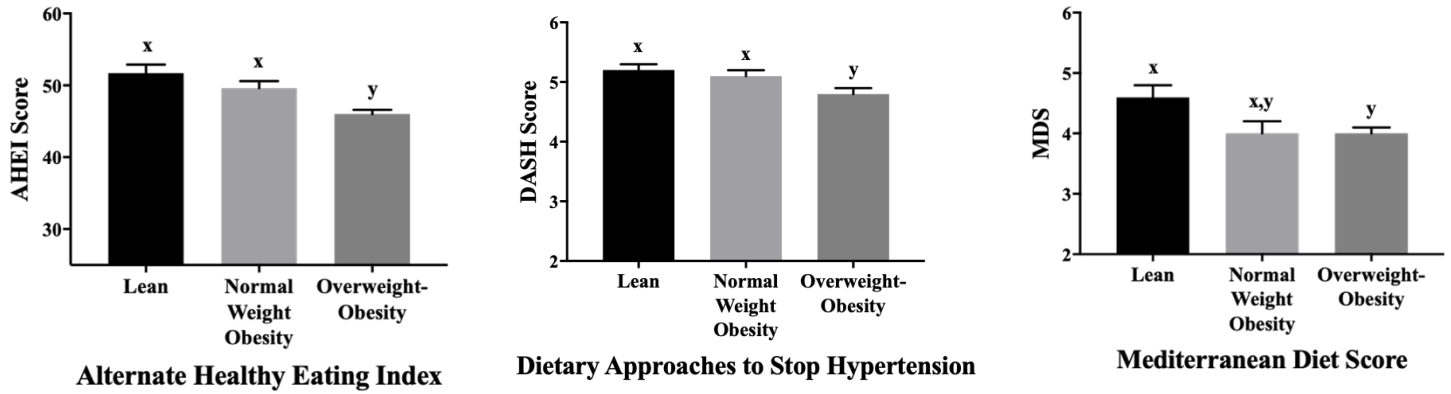

Figure 4.1. Average reported diet quality scores between three body composition subtypes [a]

Average (Mean ± SE, adjusting for age, race, sex, and education) diet quality scores for participants classified as lean, having normal weight obesity, or as having overweight-obesity enrolled in a large study of working adults in a metropolitan area of the Southeastern United States.

[a] n=98, 162, and 427 for the lean, normal weight obesity, and overweight-obesity groups, respectively

[b] There was significant effect modification by education status for alternate healthy eating index (AHEI) and Mediterranean diet score (MDS) variables. Among individuals without a college degree, there was no difference in AHEI scores or MDS between the lean, normal weight obesity, or overweight-obesity groups (p>0.05).

[c] There was significant effect modification by race for dietary approaches to stop hypertension (DASH) score. Among individuals who reported White race, the lean group reported significantly higher DASH diet quality scores compared to the overweight-obesity group ($p<0.05$). Values in the normal weight obesity group were similar to both the lean and overweight/obesity group ($p>0.05$).

[x,y] Results of Tukey's post hoc analyses are denoted by superscript letters x and y and indicate significant differences between groups for each row. Values that are not connected by the same letter are significantly different at $P < 0.05$.

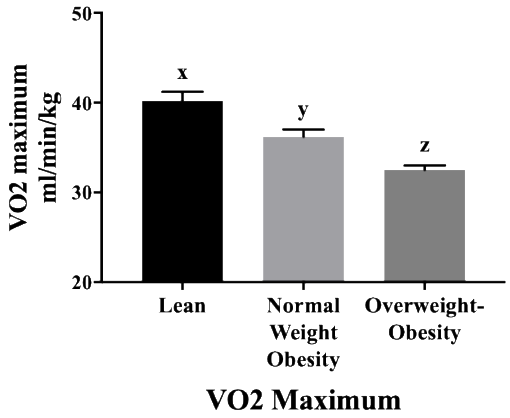

Figure 4.2. Average physical fitness levels between three body composition subtypes [a]
Average (Mean ± SE, adjusting for age, race, sex, and education) VO2 maximum values for participants classified as lean, having normal weight obesity, or as having overweight-obesity enrolled in a large study of working adults in a metropolitan area of the Southeastern United States.

[a] n= 91, 154, and 383 for the lean, normal weight obesity, and overweight-obesity groups, respectively

[b] There was significant effect modification between age and VO2 maximum. Among individuals with NWO, there was a significant decline in VO2 maximum with aging.

[x,y,z] Results of Tukey's post hoc analyses are denoted by superscript letters x, y, and z and indicate significant differences between groups for each row. Values that are not connected by the same letter are significantly different at $P < 0.05$

Table 4.1. Demographic characteristics of 693 adults participating in the Emory-Georgia Tech Predictive Health Initiative cohort according to body composition subtype [a]

Characteristic	Lean (n=100)	Normal Weight Obesity (n=164)	Overweight-Obesity (n=429)
Age (y)	43.3 ± 12.8[y]	49.1 ± 11.4[z]	50.0 ± 10.9[z]
Sex			
Female	61 (62)	122 (75)	268 (63)*
Male	38 (38)	42 (26)	162 (38)*
Race			
White	81 (83)	120 (74)	287 (68)
African-American, Asian, other	19 (17)	44 (26)	142 (32)
Presence of chronic disease (hypertension, diabetes, hyperlipidemia)	14 (14)	34 (21)	94 (22)
College degree or higher	90 (90)	137 (84)	342 (80)*
Annual household income [b]			
≤ $50,000/year	7 (8)	17 (11)	51 (12)
> $50,000-$100,000/year	23 (26)	37 (24)	126 (31)
> $100,000-$200,000/year	31 (34)	60 (39)	145 (35)
> $200,000/year	29 (32)	39 (25)	89 (22)

[a] Values are presented as mean ± standard deviation (SD) or n (%).
[b] n= 90, 153, and 411 for the lean, NWO, and overweight-obesity groups, respectively
[*] Results of χ^2 test showed a significant difference between the three groups, $p<0.05$.
[y,z] For continuous variables, results of Tukey post hoc analyses are denoted by superscript letters y and z and indicate significant differences between groups for each row. Values that are not connected by the same letter are significantly different at $P < 0.05$.

Table 4.2. Body composition and fat distribution measures among adult participants in the Emory University-Georgia Tech Predictive Health Initiative cohort classified by body composition subtype (n=693)[a]

Body composition measure	Lean (n=100)	Normal Weight Obesity (n=164)	Overweight-Obesity (n=429)
Body Mass Index (kg/m^2)	22.4 ± 0.5^x	23.0 ± 0.4^x	31.3 ± 0.2^y
Total body fat (%)	22.6 ± 0.5^x	31.1 ± 0.4^y	38.1 ± 0.2^z
Total body mass (kg)	65.6 ± 1.6^x	67.0 ± 1.2^x	90.5 ± 0.8^y
Lean body mass (kg)	48.5 ± 0.8^x	44.1 ± 0.6^y	52.3 ± 0.4^z
Fat mass (kg)	14.4 ± 1.0^x	20.3 ± 0.8^y	35.0 ± 0.5^z
Visceral adipose tissue (kg)[b]	0.17 ± 0.0^x	0.44 ± 0.03^y	1.21 ± 0.04^z
Waist circumference (cm)	79.7 ± 1.9^x	85.7 ± 1.6^y	101.0 ± 0.9^z

[a] Values are presented as mean ± SE adjusting for age, race, and sex or age and race when stratified by sex.

[b] Variable was natural log transformed for analyses and back transformed for data presentation.

[x,y,z] Results of Tukey post hoc analyses are denoted by superscript letters x, y, and z and indicate significant differences between groups for each variable. Within rows, values that are not connected by the same letter are significantly different at $P < 0.05$.

Table 4.3. Cross-sectional associations between body composition measures, diet quality scores, and physical fitness in 693 adult participants within the Emory Georgia-Tech Predictive Health Initiative cohort [β (p-value)][a]

Body Composition Measure	AHEI[c]	DASH[d]	MDS[e]	VO2 maximum (mL/min/kg)[f]
Body Fat %	**-0.16 (<0.001)**	**-1.54 (<0.001)**	**-0.73 (<0.001)**	**-0.33 (<0.001)**
Total Fat Mass (kg)	**-0.21 (<0.001)**	**-2.05 (<0.001)**	**-0.71 (0.008)**	**-0.49 (<0.001)**
Fat Mass Index (kg/m^2)	**-0.08 (<0.001)**	**-0.76 (<0.001)**	**-0.28 (<0.001)**	**-0.17 (<0.001)**
Visceral Adipose Tissue (kg)[b]	**-0.02 (<0.001)**	**-0.18 (<0.001)**	**-0.06 (0.003)**	**-0.03 (<0.001)**
Waist Circumference (cm)	-0.15 (0.05)	-1.00 (0.21)	-0.37 (0.43)	**-0.63 (<0.001)**
Lean Body Mass (kg)	-0.02 (0.45)	-0.42 (0.15)	0.17 (0.29)	**-0.11 (0.002)**
Lean Body Mass Index (kg/m^2)	-0.009 (0.26)	-0.15 (0.08)	0.03 (0.53)	**-0.04 (0.003)**
Lean Body Mass %	**0.17 (<0.001)**	**1.66 (<0.001)**	**0.77 (<0.001)**	**0.33 (<0.001)**

[a] All estimates are from linear regression analyses with body composition measures as a continuous outcome. Analyses were conducted individually for each measure of body composition. All estimates are adjusted for age, race, sex, and education.
[b] Variable was log transformed for analyses
[c] AHEI, Alternate Healthy Eating Index
[d] DASH, Dietary Approaches to Stop Hypertension Score
[e] MDS, Mediterranean Diet Score
[f] VO2, volume of oxygen consumption

Table 4.4. Clinical outcomes and markers of oxidative stress and inflammation in 693 adults within the Emory-Georgia Tech Predictive Health Initiative cohort classified according to body composition subtype [a]

	Lean (n=100)	Normal Weight Obesity (n=164)	Overweight-Obesity (n=429)
Blood glucose (mg/dL) [b,c]	86.0 ± 1.3^{x}	86.0 ± 1.0^{x}	90.2 ± 0.7^{x}
Insulin (μIU/mL) [b,d,e]	2.4 ± 0.2^{x}	3.1 ± 0.2^{y}	5.4 ± 0.2^{z}
HOMA-IR [b,d,f]	0.5 ± 0.04^{x}	0.7 ± 0.04^{y}	1.2 ± 0.05^{z}
Total cholesterol (mg/dL) [c,g]	185.0 ± 3.8^{x}	196.9 ± 3.0^{y}	191.7 ± 1.9^{x,y}
LDL-C (mg/dL) [c,g]	98.4 ± 3.4^{x}	113.0 ± 2.7^{y}	113.0 ± 1.6^{y}
HDL-C (mg/dL) [g]	72.1 ± 1.7^{x}	65.2 ± 1.3^{y}	56.5 ± 0.8^{z}
Triglycerides (mg/dL) [c,h]	72.2 ± 6.0^{x}	93.8 ± 4.8^{y}	112.4 ± 3.0^{z}
Systolic blood pressure (mmHg) [i]	114.9 ± 1.5^{x}	116.9 ± 1.2^{x}	125.9 ± 0.7^{y}
Diastolic blood pressure (mmHg) [i]	72.3 ± 1.1^{x}	75.6 ± 0.9^{x}	79.2 ± 0.5^{y}
Cysteine, μM [k]	8.9 ± 0.2	9.2 ± 0.2	9.4 ± 0.1
Cystine, μM [k]	78.7 ± 1.8^{x}	79.7 ± 1.4^{x}	87.9 ± 0.9^{y}
Glutathione, μM [b,k]	1.77 ± 0.07^{x}	1.76 ± 0.05^{x}	1.50 ± 0.03^{y}
Glutathione disulfide, μM [b,k]	0.05 ± 0.003	0.05 ± 0.003	0.05 ± 0.001
E_h Cysteine, mV [k,l]	-69.7 ± 0.6	-70.3 ± 0.5	-69.5 ± 0.3
E_h Glutathione, mV [k,l]	-137.3 ± 1.1^{x}	-136.9 ± 0.8^{x}	-134.0 ± 0.5^{y}
Cystine/Glutathione ratio [b,k]	43.5 ± 2.06^{x}	44.6 ± 1.63^{x}	57.3 ± 1.29^{y}
Interleukin-6 (pg/mL) [b,mn]	1.13 ± 0.16	1.14 ± 0.12	1.31 ± 0.09
Tumor necrosis factor-α (pg/mL) [b,m,o]	3.25 ± 0.16	3.47 ± 0.17	3.64 ± 0.11
Interleukin-8 (pg/mL) [b,m,q]	7.87 ± 0.55	7.96 ± 0.43	7.91 ± 0.26
Interferon gamma (pg/mL) [b,m,r]	0.35 ± 0.07	0.32 ± 0.048	0.41 ± 0.04

[a] Values are presented as mean ± SE adjusting for age, race, and sex.
[b] Variable was natural log-transformed for analyses and back-transformed for data presentation.
[c] n=162 and 427 for the normal weight obesity (NWO) and overweight-obesity groups, respectively

[d] n=162 and 426 for the NWO overweight-obesity groups, respectively
[e] To convert μU/mL insulin to pmol/L, multiply μU/mL by 6.945. To convert pmol/L insulin to μU/mL, multiply pmol/L by 0.144.
[f] HOMA-IR, homeostasis model assessment of insulin resistance.
[g] To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.0259. To convert mmol/L cholesterol to mg/dL, multiply by 38.7. n=161 and 426 for the NWO overweight-obesity groups, respectively
[h] To convert mg/dL triglycerides to mmol/L, multiply mg/dL by 0.0113. To convert mmol/L triglycerides to mg/dL, multiply mmol/L by 88.6.
[i] n=163 in the NWO group, n=429 in the overweight-obese group
[k] aminothiol redox measures: n=93, 155, and 412 for the lean, NWO, and overweight-obesity groups, respectively
[l] E_h millivolts, redox potential
[m] cytokine measure
[n] n=93, 155, and 415 for the lean, NWO, and overweight-obesity groups, respectively
[o] n=95, 159, and 418 for the lean, NWO, and overweight-obesity groups, respectively
[q] n=94, 157, and 415 for the lean, NWO, and overweight-obesity groups, respectively
[r] n=93, 156, and 415 for the lean, NWO, and overweight-obesity groups, respectively
[x,y,z] Results of Tukey post hoc analyses are denoted by superscript letters x, y, and z and indicate significant differences between groups for each value. Within each row, values that are not connected by the same letter are significantly different at $P < 0.05$.

Table S4.5 (supplementary): Comparisons of diet quality scores and physical fitness among 693 adults within the Emory University-Georgia Tech Predictive Health Initiative classified into three body composition subtypes and stratified by sex[a]

Diet Quality Score or Fitness Measure	Lean	Normal Weight Obesity	Overweight-Obesity
Female participants			
Alternative Healthy Eating Index[b]	55.0 ± 1.5^{x}	51.4 ± 1.1^{x}	47.3 ± 0.7^{y}
DASH[b,c]	5.2 ± 0.1^{x}	5.0 ± 0.1^{x}	4.7 ± 0.1^{y}
Mediterranean Diet Score[b]	5.0 ± 0.2^{x}	$4.4 \pm 0.2^{x,y}$	4.0 ± 0.1^{y}
VO2 Maximum (mL/min/kg)[d]	37.4 ± 1.2^{x}	32.6 ± 0.8^{y}	28.9 ± 0.5^{z}
Male participants			
Alternative Healthy Eating Index[b]	50.1 ± 1.9	48.1 ± 1.7	47.0 ± 1.1
DASH[b,c]	5.3 ± 0.2	5.3 ± 0.2	5.0 ± 0.1
Mediterranean Diet Score[b]	4.6 ± 0.3	4.6 ± 0.3	4.4 ± 0.2
VO2 Maximum (mL/min/kg)[d]	44.4 ± 1.7^{x}	$41.4 \pm 1.5^{a,y}$	38.1 ± 1.0^{y}

[a] Values are presented as mean ± SE adjusting for age, race, and sex.
[b] n=62, 121, and 267 in the lean, NWO, and overweight-obesity groups, respectively, in females and n=36, 41, and 160 in the lean, NWO, and overweight-obesity groups, respectively, in males
[c] DASH, Dietary Approaches to Stop Hypertension
[d] VO_2, volume of oxygen. n=57, 116, and 232 in the lean, NWO, and overweight-obesity groups, respectively in females and n=34, 38, and 151 in the lean, NWO, and overweight-obesity groups, respectively in males
[x,y,z] Results of Tukey post hoc analyses are denoted by superscript letters x, y, and z and indicate significant differences between groups for each row. Within rows, values that are not connected by the same letter are significantly different at $P < 0.05$.

Table S4.6 (supplementary). Comparison of reported daily intake of food groups between 689 adults within the Emory-Georgia Tech Predictive Health Initiative cohort categorized into body composition subtypes [a]

Food Group Variable	Lean (n=98)	Normal Weight Obesity (n=161)	Overweight-Obesity (n=430)
Total Fat (grams)	62.9 ± 2.8 ^{y,z}	58.5 ± 2.0 ^{y}	64.8 ± 1.3^{z}
Saturated Fat (grams)	17.5 ± 0.8 ^{y,z}	16.2 ± 0.6 ^{y}	18.6 ± 0.4^{z}
Monounsaturated Fat (grams)	24.9 ± 1.1 ^{y,z}	23.3 ± 0.8 ^{y}	25.7 ± 0.5^{z}
Polyunsaturated Fat (grams)	15.3 ± 0.7	14.1 ± 0.5	15.2 ± 0.3
Trans-saturated Fat (gram)	1.5 ± 0.1 ^{y}	1.4 ± 0.1 ^{y}	1.8 ± 0.1^{z}
Total Grains (ounce equivalents)	4.5 ± 0.2^{y,z}	3.9 ± 0.2^{y}	4.3 ± 0.1^{z}
Whole Grains (ounce equivalents)	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.04
Refined Grains (ounce equivalents)	3.0 ± 0.2^{y,z}	2.7 ± 0.1^{y}	3.2 ± 0.1^{z}
Total Dietary Fiber (grams)	20.6 ± 1.0^{y}	18.6 ± 0.7 ^{y,z}	17.9 ± 0.4^{z}
Added Sugars (teaspoon equivalents)	9.0 ± 0.6	8.3 ± 0.4	8.6 ± 0.2
Total Fruits (cups)	1.3 ± 0.1^{y}	1.3 ± 0.1^{y}	1.0 ± 0.03^{z}
Whole Fruit (cups)	1.0 ± 0.1^{y}	0.9 ± 0.1^{y}	0.8 ± 0.03^{z}
Total Vegetables (cups)	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
Yellow/Orange (cups)	0.1 ± 0.01^{y,z}	0.2 ± 0.01^{y}	0.1 ± 0.01^{z}
Dark Leafy Greens (cups)	0.5 ± 0.05	0.5 ± 0.03	0.5 ± 0.02
Potatoes (cups)	0.1 ± 0.01^{y}	0.1 ± 0.01^{y}	0.2 ± 0.01^{z}
Total Proteins (grams)	64.7 ± 2.7^{y,z}	58.6 ± 1.9^{y}	65.5 ± 1.3^{z}
Milk and Dairy Products (milk equivalents)	1.0 ± 0.1	0.9 ± 0.05	1.0 ± 0.03
Eggs (count)	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.01
Beef, Pork, and Lamb (ounces)	0.6 ± 0.1	0.6 ± 0.05	0.9 ± 0.04
Poultry (ounces)	0.5 ± 0.1^{y,z}	0.4 ± 0.04^{y}	0.6 ± 0.03^{z}
Fish/Seafood (ounces)	0.6 ± 0.1	0.5 ± 0.05	0.6 ± 0.04
Beans/Legumes/Nuts/Soy (servings)	3.0 ± 0.3^{y}	2.6 ± 0.2^{y}	2.1 ± 0.1^{z}

[a] Values are presented as adjusted geometric mean ± SE. All variables were log transformed for analyses and back transformed for data presentation.

^{x,y,z} Results of Tukey post hoc analyses are denoted by superscript letters x, y, and z and indicate significant differences between groups for each row. Within each row, values that are not connected by the same letter are significantly different at $P < 0.05$.

CHAPTER 5

PLASMA HIGH-RESOLUTION METABOLOMICS DIFFERENTIATES ADULTS WITH NORMAL WEIGHT OBESITY FROM LEAN INDIVIDUALS

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Format adapted for this dissertation

What is already known about this subject?

- Obesity increases a person's disease and mortality risk.
- Body mass index (BMI) is typically used to assess obesity and categorize disease risk.
- Individuals with a normal weight but high body fat (normal weight obesity, NWO) have increased disease and mortality risk but may be overlooked when screening for obesity by BMI.

What does this study add?

- This study characterizes the plasma metabolome of individuals with NWO compared to individuals classified as lean or having overweight-obesity using high-resolution metabolomics.
- Individuals with NWO exhibited altered plasma metabolomic profiles similar to individuals with overweight-obesity, including oxidized linoleic acid and related metabolites and dysregulated amino acid metabolism.
- High-resolution metabolomics may be a helpful tool to identify individuals who have increased disease risk despite having a normal BMI.

Abstract

Objective: We explored underlying metabolism-related dysfunction by examining metabolomic profiles in adults categorized as lean, having normal weight obesity (NWO), or overweight-obesity.

Methods: Subjects (n=179) had fasting plasma analyzed using liquid chromatography and high-resolution mass spectrometry for high-resolution metabolomics (HRM). Body composition was assessed by dual energy x-ray absorptiometry. NWO was defined as a body mass index (BMI) <25 and body fat >30% for females and >23% for males. Differentiating metabolomic features were determined using linear regression models and likelihood ratio tests, with false discovery rate (FDR) correction. *Mummichog* was utilized for pathway and network analyses.

Results: A total of 222 metabolites significantly differed between the groups at FDR q=0.2. Linoleic acid, beta-alanine, histidine, and aspartate/asparagine metabolism pathways were significantly enriched (all, $p<0.01$) by metabolites that were similarly upregulated in the NWO and overweight-obesity groups compared to the lean group. Network analysis linked branched chain amino acids and amino acid metabolites as elevated in the NWO and overweight-obesity groups compared to the lean group (all, $p<0.05$).

Conclusions: Metabolomic profiles of individuals with NWO reflected similar metabolic disruption as individuals with overweight-obesity. HRM may help identify people at risk for developing obesity-related disease, despite normal BMI.

Keywords: Amino acids, body composition, body mass index, diet, fat distribution, fatty acids, nutrition, overweight, linoleic acid, lipid metabolism

Introduction

Obesity is a leading risk factor for major diseases including cardiovascular disease, type 2 diabetes, and cancer and health conditions such as depression, obstructive sleep apnea, and decreased physical functioning(1). Individuals with obesity have excess fat mass and metabolic dysregulation resulting in increased all-cause mortality risk(1). Body mass index (BMI), calculated using simple anthropometric measures of height and weight, is used clinically to define obesity as a BMI above 30 kg/m^2. While BMI is useful for identifying individuals at extreme levels with very high or low adiposity, BMI values in more moderate ranges are not well correlated with body fatness(2, 3). This is because BMI utilizes total body weight and does not account for body composition components such as lean mass and fat mass, which independently influence disease risk.

Within the range of intermediate BMI values is a group of individuals with a body composition phenotype termed normal weight obesity (NWO)(4). These individuals have a BMI within the normal weight range (18.5-24.9 kg/m^2) but exhibit excess fat mass. The current reported estimates for NWO are as high as 30%(4, 5). Individuals with NWO have an increased risk of cardiometabolic disease and mortality compared to individuals who are normal weight and lean and individuals who are metabolically healthy with obesity(6). Previous studies show that individuals with NWO have elevated cardiometabolic disease risk factors demonstrated by hyperlipidemia, hypertension, glucose intolerance, insulin resistance, increased inflammation, increased oxidative stress, and decreased physical functioning(7). Although there is a growing literature of metabolic dysregulation in NWO, there is a need to define the nutrition and metabolism-related pathophysiology of NWO.

High-resolution metabolomics (HRM) is an innovative platform that is useful for

exploring obesity-related disease from a systems-biology approach(8). HRM is a powerful tool for nutrition research because it enables the profiling of thousands of small-molecular weight metabolites in human biosamples and allows the investigation of important questions regarding the complex metabolite interactions which derive from diet, endogenous nutrient metabolism, the microbiome, and exogenous chemicals(8). Metabolomics has been used to identify specific metabolic signatures related to BMI and obesity(9, 10). However, there is little known regarding the metabolomic profiles of individuals with NWO in comparison to other body composition subtypes utilizing HRM. In this study, we used HRM to investigate differences in the plasma metabolome between three body composition subtypes: lean, NWO, and overweight-obesity. We hypothesized that individuals with NWO would have metabolomic profiles that are similar to subjects who are overweight-obese and distinct from subjects who are lean.

Methods

Subjects and Study Design

Emory University and Emory Healthcare employees were randomly invited to join the Emory-Georgia Tech Center for Health Discovery and Well Being Predictive Health Institute (http://predictivehealth.emory.edu) cohort study between December 2007 and December 2010. Participants underwent extensive dietary, metabolic, and other phenotypic assessments, as described in detail elsewhere (11). All subjects provided written informed consent and the study was approved by the Emory University Institutional Review Board. Exclusion criteria were the addition of a new prescription medication for chronic disease treatment within the previous year (other than anti-hypertensive or anti-diabetic agents), acute illness within 12 weeks of the study visit, hospitalization for an acute or chronic disease within the previous year, history of substance/drug or alcohol abuse, current active malignant neoplasm, women who were pregnant

or breastfeeding, or having an uncontrolled (non-medicated) or poorly controlled autoimmune, cardiovascular, endocrine, gastrointestinal, hematologic, infectious, inflammatory, musculoskeletal, neurologic, psychiatric, or respiratory disease (11). All data included in this analysis were collected at baseline visits. The current study included a subset of individuals with available baseline plasma HRM data. Demographic, education, and income information were self-reported. Subjects were classified as having a history of chronic disease (yes/no) if they reported a current diagnosis of diabetes, hypertension, or hyperlipidemia, or if subjects were currently taking anti-hypertensive, anti-diabetic, or lipid-lowering medications.

Clinical Markers, Physical Fitness, and Diet Quality Scores

Fasting concentrations of glucose, insulin, and lipids were measured by Quest Diagnostics (Valencia, CA). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to Matthews *et al*.(12). Systolic and diastolic blood pressure were measured using an automated machine (Omron, Kyoto, Japan). Physical fitness (VO2 maximum) was assessed using a GE T2100 Treadmill (GE Healthcare, Waukesha, WI) following a modified Balke protocol. Subjects completed the Cross-Cultural Activity Participation Study (CAPS) (13) to determine if individuals met the 2007 American College of Sports Medicine/American Heart Association physical activity and strength guidelines. Dietary intake was assessed with the 2005 Block food frequency questionnaire (NutritionQuest, Berkeley, CA). Any reported intakes below 500 calories or above 5,000 calories were considered implausible and excluded. Three validated diet quality scores, Alternate Healthy Eating Index (aHEI)(14), Dietary Approach to Stop Hypertension (DASH)(15), and Alternate Mediterranean Diet Score (MDS)(16), were calculated from FFQ output, as previously described(17).

Body Composition Analysis and Body Composition Subgroups

Whole and regional body composition were assessed by dual energy x-ray absorptiometry (DXA) using a Lunar iDXA densitometer and enCORE (v.12.2) with CoreScan® software (GE Healthcare, Madison, WI, USA). BMI was calculated from height and weight measured using an electronic scale and stadiometer (Tanita TBF-25, Tanita Health Management, Arlington Heights, IL). Participants were then classified into one of three body composition subtypes (lean, NWO, or overweight-obesity) based on sex-specific body fat percent values and BMI. For males, a body fat percent above 23 was considered elevated, and for females, a body fat percent above 30 was considered elevated based on published literature(18). Participants were categorized as having a lean body composition subtype if BMI was between 18.5 and 24.9 kg/m^2 and body fat percent was below the sex-specific cut-off values. NWO was defined as a BMI between 18.5 and 24.9 kg/m^2 and a body fat percent above the sex-specific cut-off values. Lastly, overweight-obesity was categorized as a BMI $\geq$25 kg/m^2 and a body fat percent above the sex-specific cut-off values. Waist circumference was measured three times by a health professional trained in anthropometry using a tape measure, and the average value is reported.

Plasma High-Resolution Metabolomics (HRM)

Plasma HRM was performed on 179 fasted individuals using published methods(19) in the Emory University Clinical Biomarkers Laboratory. In brief, fasting plasma previously stored at –80°C was treated with acetonitrile and an internal standard mixture using an established protocol(19). Following protein precipitation, fasting plasma samples were analyzed in triplicate with a Fourier transform mass spectrometer (MS, Dionex Ultimate 3000, Q-Exactive HF, Thermo Fisher, Waltham, MA) using C18 liquid chromatography (LC) and positive electrospray

ionization (ESI) to maximize the detection of low-molecular weight chemicals. After analysis of all participant samples and quality control samples, LC/MS data was extracted using the R-based packages apLCMS(20) and xMSanalyzer(21) to provide a mass to charge (*m/z*) feature table of detected ions denoted by relative retention time and accurate mass. Batch correction was completed by ComBat(22). Data pre-processing included: 1) filtering of features based on coefficient of variation (CV); 2) filtering of samples based on Pearson correlation between averaged technical replicates and percent missing values (features were retained only if there was a signal in at least 50% of samples); and 3) log10 transformation, quantile normalized, and mean centering. A total of 9,967 metabolomic features were included in this analysis following data filtering.

Metabolite Identification

The R package xMSannotator was used for metabolite annotation, which uses multiple criteria to provide a score-based annotation(23). Identities of multiple endogenous metabolites, including the amino acids, have been confirmed by comparing coelution with an authentic standard(24) in the Emory Clinical Biomarkers Laboratory and are equivalent to a Level 1 identification according to the Schymanski *et al*. criteria(25). Additional annotations were made with a high or medium confidence (≥ Level 2) with M+H adducts. When identity confirmation was not available, metabolites were annotated by searching metabolite databases such as Human Metabolome Database (http://www.hmdb.ca) and Metlin (https://metlin.scripps.edu) for metabolite *m/z* matches. For selected features which could not be annotated based on MS1 data only, ion dissociation spectra (MS/MS) were collected on a Thermo Scientific Fusion Mass Spectrometer for MS/MS spectral library matching using the mzCloud database

(https://www.mzcloud.org).

Statistical Analyses and Bioinformatics

Descriptive statistics (mean ± SD) were performed for clinical variables. Distributions were assessed for normality, and any non-normally distributed clinical variables were natural log-transformed for use in parametric statistics and back-transformed for data presentation. Analysis of covariance (ANCOVA) tests, adjusting for age, race, sex, and history of chronic disease (yes or no), were used to test for overall group differences in clinical, body composition, and lifestyle factors. Post-hoc comparisons between specific groups were assessed with Tukey's honestly significant different tests. Fisher's exact tests were used for comparison of categorical variables due to small numbers in the variable levels. HRM bioinformatics analyses were performed using R. HRM analyses used multiple linear regression analyses with likelihood ratio tests, adjusting for age, sex, race, and history of chronic disease to determine differences between the three body composition groups (lean, NWO, and overweight/obesity). False discovery rate (FDR) was controlled for with the Benjamini-Hochberg procedure (q= 0.2). Metabolites that significantly differed between the groups were analyzed by the *Mummichog* pathway enrichment and modular analysis program(26). Significantly enriched metabolic pathways that included less than four metabolites were excluded from findings. Modular analyses are also produced from *Mummichog*, which are unbiased from established biological pathways and construct independent networks of highly correlated metabolites(26). To test for differences in significantly enriched pathways and network metabolites between body composition subtypes, intensity values for individual metabolites within each pathway and network were compared, adjusting for age, race, sex, and history of disease. Post-hoc analyses of

differing metabolites also controlled for group differences in VO2 maximum. In a subset of the cohort (n=86), sensitivity analyses were performed on metabolites of interest between individuals classified as having NWO and overweight-obesity using student's T tests. Individuals were matched by age (within two years), race, and sex. Out of 43 individuals classified as having NWO, 32 were matched to individuals classified as having overweight-obesity on all three criteria and 11 were matched on two of the three criteria. Statistics comparing clinical variables and individual metabolite intensity values were performed in JMP Pro (version 13, SAS Institute Inc., Cary, NC).

Results

Demographic and clinical characteristics for all subjects are shown in **Table 5.1**. Distributions of age and race did not significantly differ between the three groups ($p=0.07$ and $p=0.3$, respectively). There were significantly more females in the NWO group ($p<0.05$) compared to the lean and overweight-obesity groups. The overweight-obesity group had a significantly higher proportion of individuals with a history a chronic disease compared to the lean or NWO groups ($p=0.01$). In general, the population was highly educated and reported a high annual household income, which were similar between all groups ($p>0.05$ for both). Fasting plasma glucose, total cholesterol, LDL cholesterol, and diastolic blood pressure did not significantly differ between the groups ($p>0.05$). Fasting insulin, HOMA-IR, and triglycerides, were similar between the lean and NWO groups ($p>0.05$) but were significantly higher in the overweight-obesity group ($p<0.05$). Systolic blood pressure differed only between the NWO and overweight-obesity groups ($p<0.05$). HDL cholesterol levels did not differ between lean and NWO groups but were significantly lower in the overweight-obesity group ($p<0.05$). The proportion of subjects in each group with adverse clinical biomarkers is shown in Supplemental

Table 5.1 (Table S5.1).

Body Composition, Diet Quality, and Physical Fitness

Body composition and lifestyle variables are presented in **Table 5.2**. Per body composition subtype classification, BMI was similar between the lean and NWO groups ($p>0.05$) but was significantly higher in the overweight-obesity group ($p<0.05$). Body fat percent increased significantly from subjects classified as lean to NWO to overweight-obesity ($p<0.05$). Although lean body mass did not differ between the lean and overweight-obesity groups, it was significantly lower in the NWO group ($p<0.05$). Visceral adipose tissue (VAT) increased significantly with each group, while waist circumference was only significantly higher in the overweight-obesity group ($p<0.05$). VO_2 maximum was highest in the lean group and significantly lower in the NWO and overweight-obesity groups ($p<0.05$). Based on self-reported data, a greater proportion of the lean group completed moderate-vigorous aerobic activity ($p<0.05$), but there were no differences between groups for strength training ($p>0.05$). MDS and aHEI diet quality scores were similar across all groups ($p>0.05$). DASH diet quality score was significantly higher in the lean group compared to the overweight-obesity group ($p<0.05$).

High-Resolution Metabolomics

Of the 9,967 filtered metabolomic features, 1,533 features were significantly associated with the body composition subtypes at $p<0.05$ (**Figure 5.1A**). Following FDR correction, there were 222 significantly associated metabolites ($q=0.20$), which were used as input for *Mummichog* (26) pathway enrichment and modular analyses. Significantly enriched pathways are shown in **Figure 5.1B**. There were ten significantly enriched pathways predominantly related

to lipid and amino acid metabolism. Representative metabolites within the significantly enriched pathways are shown in **Figure 5.2**. All metabolites included in Figure 2 were matched by an M+H adduct and have a Level 1 or Level 2 annotation with high or medium confidence (23, 27). Metabolites within the linoleic acid metabolism pathway, such as linoleic acid and oxidized linoleic related metabolites, were higher in the NWO and overweight-obesity groups compared to the lean group ($p<0.05$ for all). Metabolites within beta-alanine, histidine, and aspartate/asparagine metabolism were significantly elevated in the NWO and overweight-obesity groups compared to the lean group. Glutathione and glutamate metabolism contained metabolic features that were similarly elevated in the NWO and overweight-obesity groups compared to the lean group, and metabolites that were elevated in only the overweight-obesity group compared to the lean group. The significantly enriched pathways lysine metabolism, glycine and serine metabolism, and urea cycle contained metabolites that were higher in the overweight-obesity group compared to the lean group. Following further adjustment with VO2 maximum, lysine levels were similar between all three groups. No other findings in pathway analyses changed after adjusting for VO2 maximum, as shown in Figure 2. Significantly enriched pathways with all tentatively annotated metabolic features are shown in Supplemental Table 5.2 (Table S5.2).

Figure 5.3 depicts a module analysis of metabolites significantly differing between the body composition subtypes ($p<0.05$ for all metabolites). The network was predominantly comprised of amino acids and amino acid-related metabolites (17 out of 21 metabolites) including the branched chain amino acids (BCAA) leucine/isoleucine, cystine, pyruvate, histidine, 5-oxoproline, ornithine, and putrescine, which were significantly elevated in the NWO and overweight-obesity groups compared to the lean group. Additional amino acid metabolites such as the BCAA valine, 3-methyl-2-oxobutanoic acid (a valine-related metabolite), the

aromatic amino acids (AAA) tyrosine and threonine, glutamate, and phenylpyruvate (a phenylalanine-related metabolite), were higher in the overweight-obesity subtype compared to the lean subtype, but these metabolite intensities in the NWO group did not differ from either of the other groups. Following additional adjustment for VO2 maximum, 5-oxoproline levels were significantly elevated in the overweight-obese group compared to the lean group but did not differ significantly in the NWO group. All of the metabolic features tested followed the same pattern after adjusting for VO2 maximum, as noted in Figure 3.

In sensitivity analyses of matched subjects classified as NWO and overweight-obesity, there were no changes in statistical findings from the results reported above and shown in Figure 5.2 and Figure 5.3; all metabolite intensities remained similar between the NWO and overweight-obese groups.

Discussion

In this Atlanta-based cohort, we found that adults with an NWO phenotype had metabolomic profiles that were similar to individuals who have overweight-obesity and distinct from individuals who are lean. In particular, linoleic acid, beta-alanine, histidine, and aspartate/asparagine metabolism, and some BCAA, were upregulated in the NWO and overweight-obesity subtypes compared to the lean subtype. We also found dysregulation of amino acid metabolism related to valine, tyrosine, and phenylalanine in the overweight-obesity group compared to the lean group.

Analysis of classic clinical measures showed similar profiles between individuals with NWO and individuals who are lean for lipid levels, insulin resistance (via HOMA-IR), and blood pressure. While other studies have shown elevated clinical measures in individuals with NWO compared to lean individuals(7, 28), we did not find those distinctions in this cohort. Only

triglyceride concentrations were similar between individuals with NWO and overweight-obesity. All other clinical variables were comparable between NWO and lean groups. While the lean group exhibited higher physical fitness, further adjustment of VO2 maximum did not alter our main findings, therefore, we conclude the differences in metabolite intensities is more likely due to differences in the body composition subtypes rather than differences in fitness levels. The combined results of the clinical measures and HRM in individuals with NWO shows that HRM may be a more sensitive measure to detect metabolism-related dysfunction prior to altered clinical measures in middle-aged adults.

Linoleic acid is an essential omega-6 poly-unsaturated fatty acid (n-6, PUFA) whose effects on cardiometabolic health have been debated(29, 30). In our study, linoleic acid metabolism was significantly upregulated in the NWO and overweight-obesity groups compared to the lean group, indicating disruption of this metabolic pathway with elevated adiposity. Additional studies have shown increased total linoleic acid in subjects with a BMI >30 kg/m^2 (31), while others reported decreased levels of linoleic acid but increased levels of linoleic acid-related metabolites(32, 33). As it can be converted to arachidonic acid, linoleic acid has been suggested to promote pro-inflammatory pathways(34, 35). Evidence suggests that obese individuals may have a greater pro-inflammatory response to linoleic acid consumption compared to lean individuals(34, 36). Previous studies have shown that individuals with NWO have increased circulating pro-inflammatory biomarkers(7). Through their actions on PPARϒ (peroxisome proliferator-activated receptor gamma) activation(37), the oxidized linoleic acid metabolites, 9-HODE and 13-HODE, may promote both inflammation and adipocyte differentiation(34). Further, through competition with the shared Δ6 desaturase enzyme, a high intake of linoleic acid may blunt the anti-inflammatory effects of alpha-linolenic acid (ALA, a

precursor to docosahexanoic acid and eicosapentanoic acid)(34, 38). In aggregate, upregulated linoleic acid metabolism may be indicative of increased inflammation in settings of excess adiposity.

Previous studies have reported elevated amino acid concentrations in individuals with obesity. Our findings show similarly increased levels of amino acids and related metabolites, including histidine, in individuals with NWO compared to individuals classified as lean. Studies utilizing principal components analyses to investigate relationships between cardiometabolic health and the plasma metabolome have identified histidine as a significantly associated metabolite (39, 40), although others have found a negative association or no relationship between histidine with BMI and obesity(9, 31, 41). Metabolites enriched within histidine and beta-alanine overlapped with glutamate metabolism and may represent anaplerotic substrates(31). Lysine metabolism was upregulated in the overweight-obesity group. Lysine is an essential amino acid that is needed to synthesize carnitine for fatty acid transport into the mitochondria for oxidation. Both carnitine and lysine have been shown to be elevated in obesity(41), and here we report higher levels of carnitine in subjects who have NWO and overweight-obesity compared to subjects who are lean. Acylcarnitines, especially C3 and C5 acylcarnitines(42), have been found to be elevated in obesity, perhaps as a result of incompletely oxidized BCAAs. Finally, pathways related to nitrogenous waste excretion, aspartate and asparagine metabolism, were dysregulated in the NWO and overweight-obesity groups compared to the lean group, in line with other obesity and cardiometabolic disease research(43). Our findings of altered amino acid metabolism are in line with published reports regarding obesity pathophysiology and represent new findings for individuals with NWO.

In line with previous obesity-related research(42, 44), we found dysregulation of BCAAs,

AAAs, and related metabolites associated with greater adiposity in the modular analysis. There is now a well-established metabolic signature of obesity including elevated concentrations of BCAAs and AAAs (particularly tyrosine and phenylalanine) related to insulin resistance, mitochondrial oxidative capacity overload(42) and, ultimately, increased risk of developing type 2 diabetes(44). The altered flux of BCAA catabolism exceeds mitochondrial oxidative capacity and ultimately leads to release of BCAAs into the blood(42). The increase in AAA may be due to competition for the same cellular transport protein used by large neutral amino acids. Elevated levels of glutamate, alanine, and pyruvate in obese individuals, which we also show in subjects with NWO, may also be linked to altered BCAA metabolism and overload of the Krebs cycle(42). Glutamate is produced in the first step of BCAA catabolism and increased concentrations of glutamate may shift pyruvate towards conversion to alanine(42). In summary, we found altered BCAA and AAA metabolism in subjects with NWO and overweight-obesity, which may reflect the underlying pathophysiology of insulin resistance and mitochondrial energy metabolism overload.

In this study, individuals with NWO had significantly lower lean body mass compared to the lean and overweight-obesity groups, and individuals with NWO had significantly higher VAT compared to lean individuals. Furthermore, individuals with NWO and overweight-obesity had significantly lower fitness levels compared to lean individuals. Relevant to our metabolomics findings, resistance and aerobic training in overweight, insulin resistant adults showed reductions in whole plasma molar sum of the BCAAs and improved clearance of acyl groups(45). Thus, the plasma metabolomic differences observed between individuals with NWO and those who are lean may reflect a combination of differences in body composition and fitness, or other variables that were not assessed.

To our knowledge, this is the first study to examine plasma metabolomic profiles of individuals with NWO and fills an important gap in knowledge about this population. This novel approach allowed for the comparison of detailed health profiles between groups beyond classic clinical laboratory assessments. Furthermore, the use of pathway enrichment analysis provides context to associations of disease with metabolic pathways instead of single metabolites. Pathway analysis also provides the advantage of being downstream from genetic changes and allows insight into products of genetic or epigenetic alterations. A limitation of the study was its cross-sectional nature, which impedes our ability to infer causality in the results. Health status, education, and income were collected by self-report, and therefore may be subject to recall bias. This cohort is predominantly composed of individuals who reported a high education and income, which may not be reflective of the general United States population. Our power to determine differences in outcomes between groups may have been limited by small numbers. For example, several metabolites in the NWO group had intermediate values that were between lean and overweight-obese subjects but were not statistically significantly different. This may be due to small numbers between groups or heterogeneity in the metabolic health of individuals with NWO. Finally, there are no established cut points to define obesity based on body fat percent and applying another threshold to define obesity in this population may have yielded different results.

This study reports novel findings in this adult population that individuals with NWO have altered metabolomic profiles, denoting underlying metabolic dysfunction similar to individuals with overweight-obesity, despite having a normal BMI and generally normal clinical biomarkers. Specifically, linoleic acid and amino acid pathways were dysregulated in the NWO and overweight-obesity subtypes compared to the lean subtype. Thus, the plasma metabolome may be a useful measure of health status to detect perturbations that predict early metabolic changes.

Larger, prospective studies are needed to determine if HRM can identify normal weight individuals at risk for obesity-related diseases and if targeted interventions in individuals with NWO can reduce such risk.

Chapter 5 References

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Table 5.1. Demographic and clinical characteristics

	Lean (n=26)	NWO (n=43)	Overweight/ Obesity (n=110)
Age (y)	47.3 ± 2.0	47.8 ± 1.6	50.6 ± 1.0
Female [n (%)]	15 (58)	35 (81)[a]	66 (60)
White [n (%)]	23 (88)	35 (81)	79 (72)
Education			
Less than high school	1 (4)	-	-
Completed high school	1 (4)	-	4 (4)
Some college	1 (4)	5 (12)	21 (19)
Four years of college	6 (23)	12 (28)	25 (23)
Any graduate school	17 (65)	26 (60)	60 (55)
Annual household income			
≤ $50,000/year	1 (4)	1 (3)	14 (14)
> $50,000-$100,000/year	6 (24)	6 (15)	33 (32)
> $100,000-$200,000/year	10 (40)	21 (53)	31 (30)
> $200,000/year	8 (32)	12 (30)	25 (24)
Chronic disease [n (%)]	5 (19)	6 (14)	38 (35)[a]
Plasma glucose (mg/dL)	95.7 ± 4.8	93.1 ± 4.0	94.9 ± 3.1
Plasma insulin (μIU/mL)*	2.7 ± 0.5[a]	3.6 ± 0.5[a]	5.5 ± 0.6[b]
HOMA-IR*	0.6 ± 0.1[a]	0.8 ± 0.1[a]	1.3 ± 0.1[b]
Total cholesterol (mg/dL)	193.9 ± 9.4	201.4 ± 8.0	197.8 ± 6.1
LDL-C (mg/dL)	105.3 ± 8.2	117.8 ± 7.0	118.4 ± 5.3
HDL-C (mg/dL)	72.7 ± 3.8[a]	63.5 ± 3.3[a]	55.5 ± 2.5[b]
Triglycerides (mg/dL)	81.1 ± 11.4[a]	101.2 ± 9.7[a,b]	117.2 ± 7.4[b]
Systolic Blood Pressure (mmHg)	119.4 ± 3.6[a,b]	118.1 ± 3.1[a]	126.1 ± 2.3[b]

Diastolic Blood Pressure (mmHg)	74.7 ± 2.5	75.5 ± 2.1	79.9 ± 1.6

Values are mean ± SE or n (%). Values not connected by the same letter are significantly different at $p < 0.05$. Plasma variables adjusted for age, sex, race, and history of chronic disease. Abbreviations: NWO, group with normal weight obesity; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.
*Variables were natural log-transformed for analyses and back transformed for data presentation and reported as geometric mean ± SE.

Table 5.2. Body Composition Variables, Physical Fitness, and Diet Quality Scores

	Lean (n=26)	NWO (n=43)	Overweight-Obesity (n=110)
BMI (kg/m^2)	23.9 ± 0.9[a]	24.3 ± 0.7[a]	30.8 ± 0.6[b]
Total body mass (kg)	69.7 ± 2.9[a]	68.5 ± 2.5[a]	87.6 ± 1.9[b]
Lean body mass (kg)	50.3 ± 1.5[a]	44.4 ± 1.2[b]	51.1 ± 1.0[a]
Fat mass (kg)	16.5 ± 1.8[a]	21.5 ± 1.5[b]	33.6 ± 1.1[c]
Total body fat (%)	23.1 ± 1.0[a]	31.3 ± 0.8[b]	37.7 ± 0.6[c]
Visceral adipose tissue (kg)*	0.22 ± 0.04[a]	0.5 ± 0.1[b]	1.3 ± 0.2[c]
Waist circumference (cm)			
Males	82.3 ± 3.2[a]	85.8 ± 3.2[a]	97.6 ± 2.6[b]
Females	77.0 ± 2.9[a]	77.5 ± 2.1[a]	91.7 ± 1.7[b]
VO2 Maximum (mL/min/kg)	42.9 ± 2.3[a]	34.6 ± 2.0[b]	34.2 ± 1.5[b]
Met MVPA Guidelines‡	16 (64)[a]	14 (33)[b]	45 (41)[b]
Met Strength Guidelines‡	10 (38)	8 (19)	23 (21)
Mediterranean Diet Score	4.7 ± 0.4	4.1 ± 0.4	3.9 ± 0.3
DASH Diet Score	5.5 ± 0.3[a]	5.0 ± 0.2[a,b]	4.9 ± 0.2[b]
Alternative Healthy Eating Index	48.6 ± 2.6	46.8 ± 2.2	45.0 ± 1.7

Values are mean ± SE or n (%). Values not connected by the same letter are significantly different at $p< 0.05$. Variables adjusted for age, sex, race, and history of chronic disease. Abbreviations: NWO, group with normal weight obesity; BMI, body mass index; MVPA, moderate to vigorous physical activity; DASH, dietary approaches to stop hypertension.
*Variable was natural log-transformed for analyses and back transformed for data presentation and reported as geometric mean ± SE.
‡ Met the 2007 guidelines of 30 minutes of moderate activity exercises at least 5 times per week, 20 minutes of vigorous activity three times per week, or a combination of both. Subjects reported meeting the strength guidelines by performing muscular strengthening exercises at least twice per week.

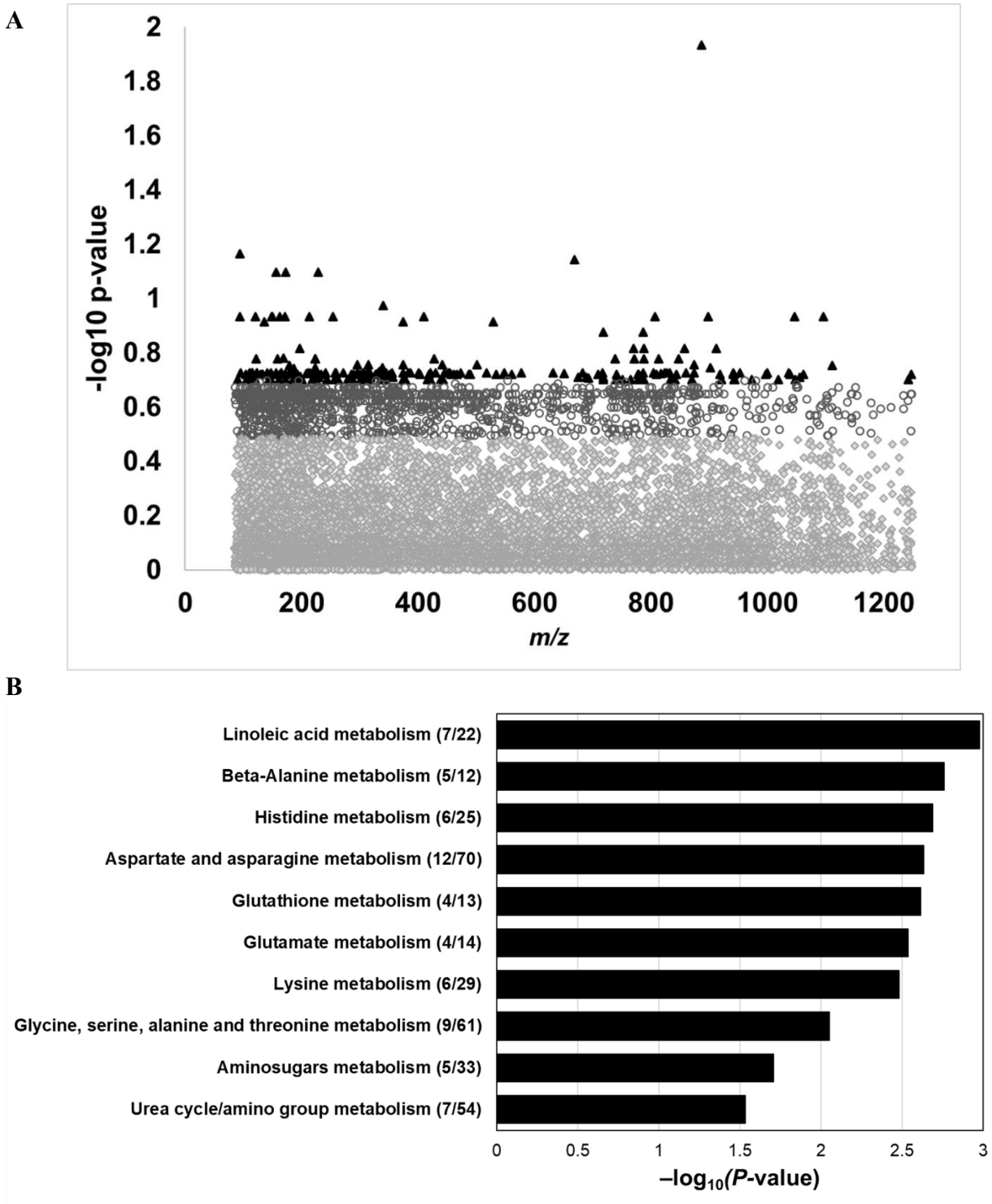

Figure 5.1. Panel A. Manhattan plot of metabolites significantly different between body composition subtypes. There were 1,533 metabolic features that were significant at a p<0.05 (grey open circles) and 222 metabolic features that were significant at an FDR q<0.2 (black triangles). Panel B. Pathway enrichment analysis of the 222 metabolites significantly associated with the body composition subtypes at a q<0.2.

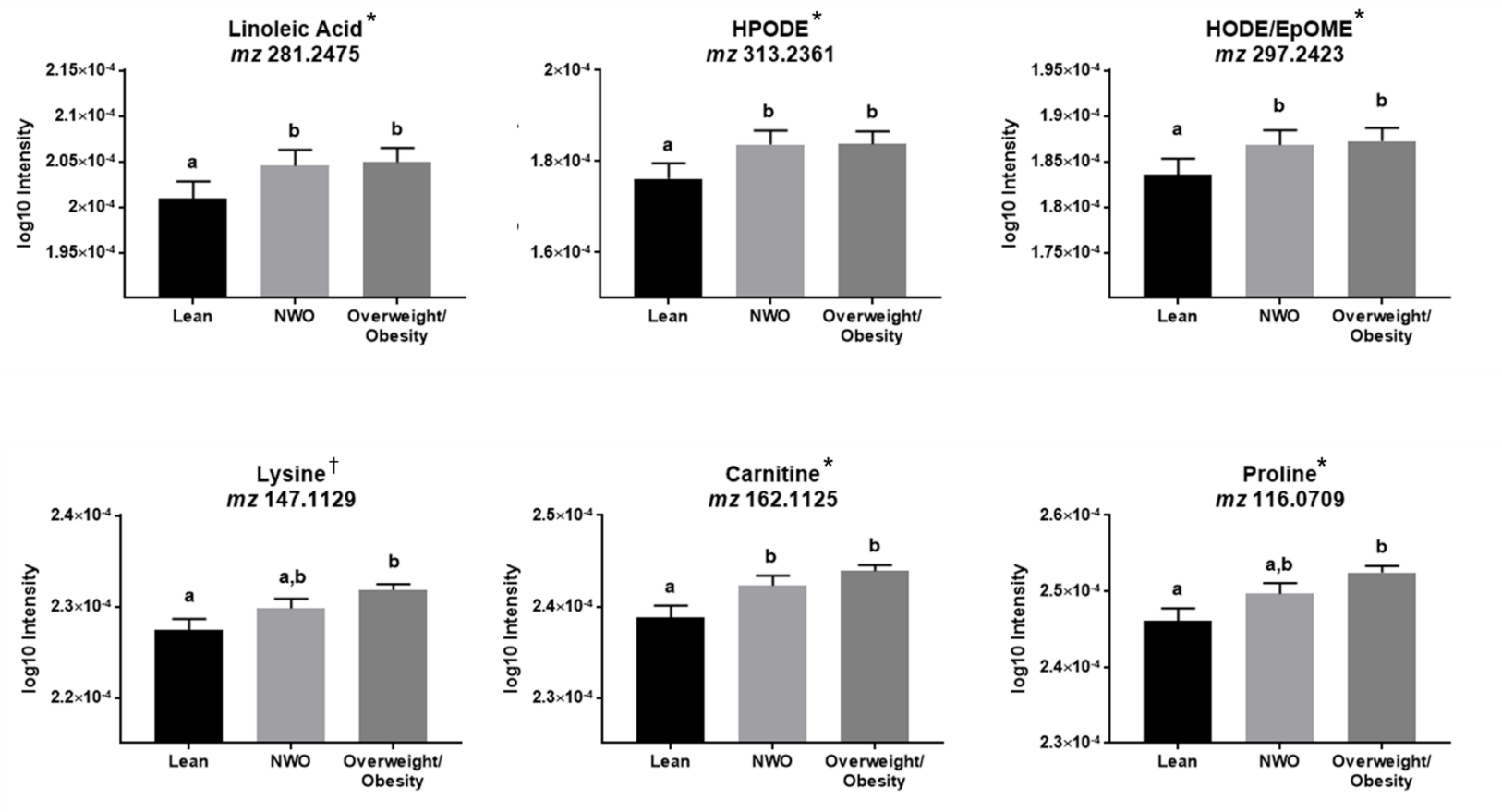

Figure 5.2. Representative metabolites within significantly enriched metabolic pathways. All metabolites have been matched by an M+H adduct in positive electrospray ionization mode. Abbreviations: NWO, normal weight obesity; HPODE, hydroperoxy-octadecadienoic acid, an intermediate of linoleic acid metabolism and precursor for the oxidized metabolite octadecadienoic acid;

HODE, hydroxyoctadecadienoic acid, a derivative of linoleic acid; EpOME, Epoxyoctadecenoic acid, a peroxidation product of linoleic acid.

*Indicates that findings were confirmed in post-hoc analyses with further adjustment for VO2 maximum, and in a subset of the cohort (n=86) with subjects categorized as having normal weight obesity or overweight-obesity matched by age, race/ethnicity, and sex.

† Following further adjustment with VO2 maximum, metabolite was similar between all three groups.

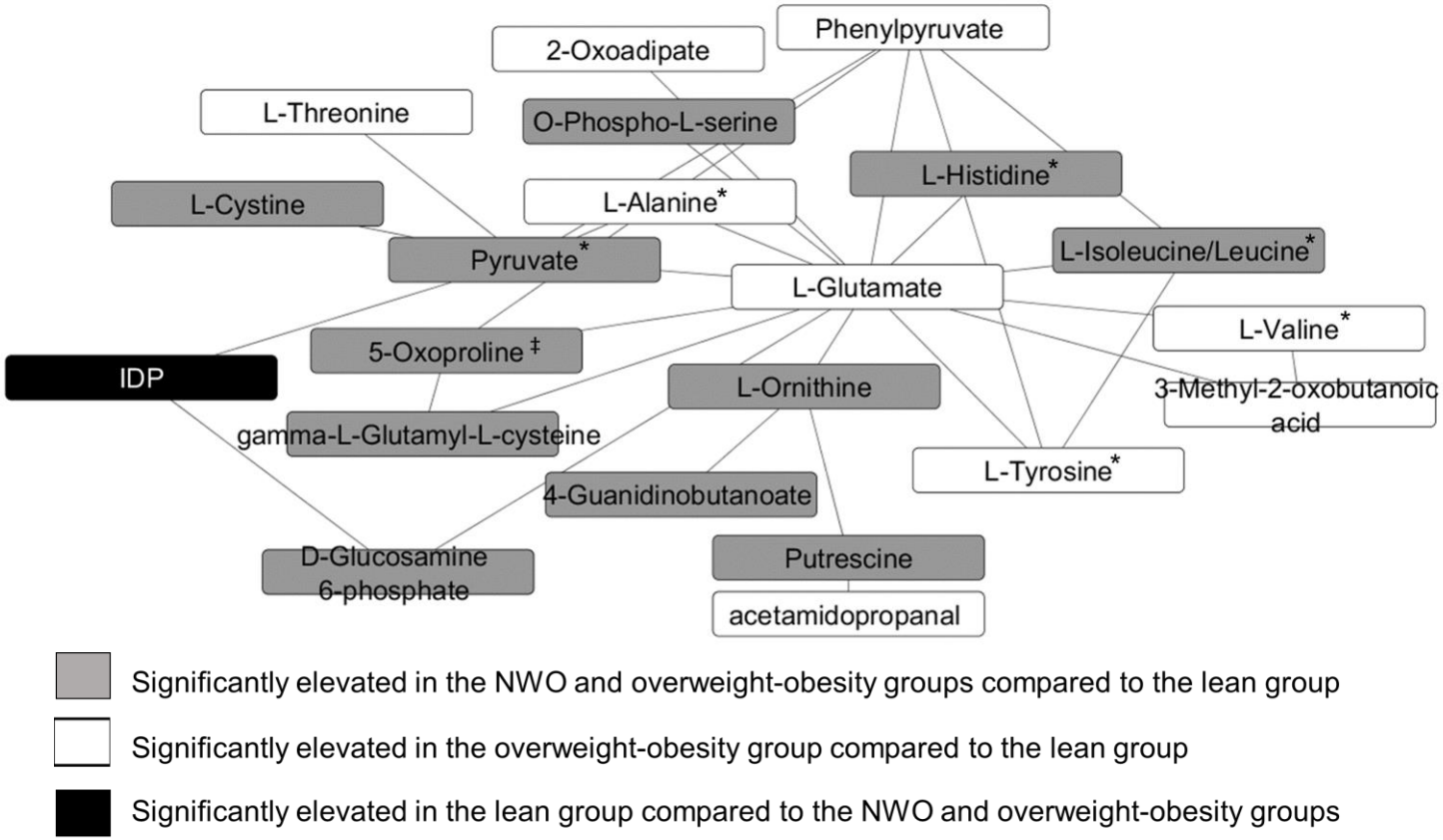

Figure 5.3. Modular analysis of correlated metabolic features that were significantly associated with the three body composition subtypes. Abbreviations: IDP, Inosine diphosphate

*Indicates that findings were confirmed in post-hoc analyses with further adjustment for VO2 maximum, and in a subset of the cohort (n=86) with subjects categorized as having normal weight obesity or overweight-obesity matched by age, race/ethnicity, and sex.

‡ Following further adjustment with VO2 maximum, metabolite was significantly elevated in the overweight-obesity group compared to the lean group.

Table S5.1 (supplementary). Adverse clinical biomarkers

	Lean (n=26)	NWO (n=43)	Overweight/ Obese (n=110)	p-value
Plasma glucose >125 mg/dL	1 (4)	-	3 (3)	0.47
HDL cholesterol <50 mg/dL (females) or <40 mg/dL (males)	1 (4)	5 (12)	18 (17)*	0.16
Triglycerides >150 mg/dL	-	5 (12)	25 (23)	0.006
Systolic Blood Pressure >140 mmHg	3 (12)	3 (7)	17 (15)	0.33
Diastolic Blood Pressure >90 mmHg	2 (8)	-	18 (16)	0.004

All data is presented as n (%).
- indicates no subjects had adverse biomarkers
* 109 subjects

Table S5.2 (supplementary). Significantly enriched pathways and putative annotation of metabolic features within each pathway produced from *Mummichog* analyses

Significantly Enriched Pathway	Individual Metabolites	P-value
Linoleic metabolism	Linoleic acid, 13(S)-HODE, 13-OxoODE, 9,10-DHOME, 12(13)-EpOME, 9(S)-HPODE, 13(S)-HPODE	0.001
Beta-alanine metabolism	Glutamate, Beta-alanine, Histidine, Ornithine, 5-oxoproline	0.002
Histidine metabolism	Glutamate, Histidine, Beta-alanine, 5-oxoproline, Uric acid, Ornithine	0.002
Aspartate and asparagine metabolism	Glutamate, 5-oxoproline, Proline, Putrescine, Carnitine, Ornithine, Lysine, Gamma-L-glutamyl cysteine, Acetamidopropanal, Acetyalaminobutanal, 4-Guanidinobutanoate, Pyrroline-hydroxy-carboxylate	0.002
Glutathione metabolism	5-oxoproline, Glutamate, Alanine, Gamma-L-glutamyl cysteine	0.002
Glutamate metabolism	Glutamate, Alanine, Gamma-L-glutamyl cysteine, Pyruvate	0.003
Lysine metabolism	Glutamate, Carnitine, Lysine, Pipecolinic acid, 2-oxoadipate, Unknown	0.003
Glycine, serine, alanine, threonine metabolism	Glutamate, Choline, Betaine, Pyruvate, Glycerate, Allotheronine, Alanine, Ornithine, Threonine	0.009
Aminosugars metabolism	Glutamate, Inosine diphosphate, Glucosamine-6-phosphate, Pyruvate, CMP-N-glycolyneuraminate	0.02
Urea cycle/amino group metabolism	Glutamate, Proline, Ornithine, Thiopurine, N-acetyl-glutamate, Sarcosine, N4-Acetylamino-butanal	0.03

Metabolic feature annotation is based on output from *Mummichog* analyses. *Mummichog* utilizes the collective power of mapping statistically significant metabolic features to biological pathways to detect the most likely match for metabolites. Importantly, one mass to charge ratio (*mz*) may match to several metabolites. *Mummichog* maps all possible identifications for an *m/z* and then bases

the final output on what pathway is enriched in a significant manner. The possible matches outside of the enriched pathway are distributed at random, eliminating the possibility of additional pathways.

CHAPTER 6

VISCERAL ADIPOSE TISSUE IS ASSOCIATED WITH POOR DIET QUALITY AND HIGHER FASTING GLUCOSE IN ADULTS WITH CYSTIC FIBROSIS

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Abstract

Background

Body fat distribution and diet quality influence clinical outcomes in general populations but are understudied in patients with cystic fibrosis (CF). The aim of this pilot study was to assess body fat distribution and diet quality in relation to fasting glucose and lung function in adults with CF.

Methods

Subjects were 24 adults (ages 18-50) with CF and 25 age-matched controls. The Healthy Eating Index 2015 (HEI-2015) was calculated from 3-day food records and data were adjusted/1000 kcal intake. Whole and regional body composition, including visceral adipose tissue (VAT), was assessed by dual energy X-ray absorptiometry.

Results

Subjects with CF reported more added sugar intake [26.1 (IQR 18.1) vs. 12.9 (12.5) g/1000kcal, $p<0.001$] and had lower HEI-2015 scores [48.3 (IQR 9.9) vs. 63.9 (27.3), $p<0.001$] compared to controls. There were no differences in BMI, total body fat, or lean body mass (LBM) between subjects with CF and controls ($p>0.05$ for all), although subjects with CF had higher VAT than control subjects [0.3 (IQR 0.3) vs 0.1 (0.3) kg, $p=0.02$]. Among subjects with CF, VAT was positively associated with added sugar intake ($p<0.001$) and fasting blood glucose ($p=0.04$). Lung function was positively associated with BMI ($p=0.005$) and LBM ($p=0.03$) but not with adiposity indicators.

Conclusions

These novel data link body fat distribution with diet quality and fasting glucose levels in adults with CF, whereas LBM was associated with lung function. This study highlights the importance

of increasing diet quality and assessing body composition and fat distribution in the CF population.

Keywords

Cystic Fibrosis, Diet Quality, Body Composition, Fat Distribution, Nutrition, Healthy Eating Index

Abbreviations

BMI – body mass index

HEI – Health Eating Index

LBM – lean body mass

VAT – visceral adipose tissue

LBMI – lean body mass index

FMI – fat mass index

Funding

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Highlights

- Adults with CF reported lower diet quality compared to healthy controls
- Visceral adipose tissue (VAT) was higher in adults with CF compared to controls
- Added sugar intake was positively associated with VAT in adults with CF
- VAT was positively associated with fasting glucose levels in adults with CF
- Lung function was positively associated with lean body mass but not body fat mass

Introduction

Poor nutritional status plays a key role in the progression of cystic fibrosis (CF)(1). The high risk of malnutrition in patients with CF has prompted the long-standing clinical practice of prescribing unrestricted high-calorie, high-fat diets to meet sex-specific body mass index (BMI) goals(2). In the general population, an emphasis on dietary quality above individual macronutrients is the basis for dietary recommendations to reduce the risk of chronic disease(3). However, evidence-based research is lacking to guide CF-specific nutritional recommendations regarding diet quality. Recent advances in the management of CF have led to prolonged life expectancy, yet the long-term clinical and metabolic effects of an unrestricted diet in adults with CF are unknown. A recent study in children with CF revealed a disproportionately high intake of energy-dense, yet nutrient-poor, foods(4). With a growing prevalence of glucose intolerance and CF-related diabetes (CFRD) in an aging CF population, studies are needed to determine if diet quality plays a role in the metabolic health of these patients.

Body mass index (BMI) positively correlates with lung function and survival in the CF population (5, 6); however, clinical studies have identified a discordance between BMI and body composition measures(7, 8). BMI lacks the sensitivity needed to assess optimal body composition for health, particularly in clinical populations such as patients with CF(9). The sole use of BMI as a nutritional index in patients with CF can result in the misidentification of at-risk patients, including those with a “healthy” BMI but concomitant depletion of lean body mass (LBM)(8) or patients with normal weight obesity (NWO, defined as a normal weight BMI but elevated percent body fat)(7). Further, BMI measurements alone provide no indication of body fat distribution. Excess accumulation of adipose tissue in the abdominal area is linked to adverse cardiometabolic health outcomes in the general population(10) and is correlated with adverse

pulmonary outcomes in patients with chronic obstructive pulmonary disease and other non-CF lung diseases(11, 12). Two previous studies found elevated visceral adipose tissue (VAT) in patients with CF compared to healthy controls(13, 14). However, the clinical implications of elevated VAT in CF are unknown. It is also unknown if dietary factors, such as poor diet quality, contribute to VAT deposition in individuals with CF.

The aims of this study were to: 1) describe and compare diet quality and body fat distribution between adults with CF and healthy controls, and 2) investigate the inter-relationships between dietary intake, fat distribution, and clinical outcomes (lung function and fasting blood glucose concentrations). We hypothesized that increased VAT would be associated with poor diet quality and negative clinical outcomes.

Materials and Methods

Subjects and Study Design

This pilot, cross-sectional study included twenty-four adults with clinically-stable CF and twenty-five age-matched healthy controls within the Atlanta, Georgia, United States region. The Emory Institutional Review Board approved the study, and all participants provided written informed consent prior to participation. Testing was conducted in the Emory University Hospital Clinical Research Network unit of the Georgia Clinical and Translational Science Alliance. Inclusion criteria for participants with CF were having a confirmed diagnosis of CF by a chloride sweat test and/or CFTR genetic test, confirmation that at least one of the CFTR mutations was Class I, II, or III, and a stable medical regimen for at least three weeks, including no recent pulmonary exacerbation involving administration of oral or intravenous antibiotics or glucocorticoids. Exclusion criteria were current pregnancy, inability or unwillingness to discontinue enteral tube feeds for one night prior to the study visit, the most recent forced

expiratory volume in 1 second expressed as a percentage of the predicted value (FEV1%) <40%, or recreational or prescription drug or alcohol abuse. Healthy control volunteers were recruited by flyers and word-of-mouth and were aged-matched within 18 months to a subject with CF. Inclusion criteria for healthy controls were 18-50 years of age and absence of any hospitalization in the previous year aside from accidents. Exclusion criteria were presence of a chronic infection, respiratory disease, cardiometabolic disease, acute illness within the previous two weeks, history of malignancy in the past 5 years, weight instability (+/- 10% of body weight within six months), drug or alcohol abuse, or BMI >30 kg/m^2.

Following a 10-hour overnight fast, participants without previously diagnosed CFRD (n=13) and all control subjects completed a 2-hour oral glucose tolerance test (OGTT), which included a fasting baseline blood glucose draw and a two-hour blood draw following ingestion of 75 g glucose. Patients with a CFRD diagnosis (n=11) only had a fasting blood glucose draw. Glucose concentrations were determined in the Emory University Hospital Clinical Laboratory. Glucose tolerance was determined based on the results of the OGTT(15) or from a previous clinical diagnosis of CFRD. CF glucose tolerance subtypes based on previously published literature were also determined (16). For subjects with CF, available HbA1c values assessed within the last three months were obtained from the electronic medical record (EMR). Due to the limited number of CF subjects with 2-hour glucose (n=13) or HbA1c% (n=20) values, analyses examining clinical outcomes with diet and body composition utilized fasting glucose concentrations. For subjects with CF, spirometry was performed at the Emory University Hospital Adult CF Clinic following the American Thoracic Society/European Respiratory Society guidelines for pulmonary function testing (17). Absolute values from spirometric testing was compared to population reference values for determination of FEV1 percent predicted

(FEV1% predicted). Clinical spirometry data was extracted from the EMR for baseline FEV1% predicted and to calculate average rate of FEV1% decline in the last three years. Baseline FEV1% was calculated by averaging the best FEV1% value within each quarter of the calendar year. Mean rate of lung function decline was calculated by finding the difference in FEV1% between each year and averaging those values. Best FEV1% in the previous year was also assessed. Lung function was not assessed in healthy controls.

Body Composition and Fat Distribution

Whole and regional body composition was assessed by dual energy x-ray absorptiometry (DXA) using a Lunar iDXA densitometer. Fat mass index and LBM index were calculated to correct for height as kg/m^2. VAT was determined via DXA using an automated software program (CoreScan® GE Healthcare, Madison, WI, USA) that segments images into VAT and subcutaneous adipose tissue compartments in the abdominal region and has been validated against computed tomography and magnetic resonance imaging in non-CF populations(18). Subjects with a normal weight BMI ($<25\ kg/m^2$) and body fat percent above sex-specific cut points (>23% and >30% body fat for males and females, respectively) were classified as NWO, while those with BMI values $\geq 25\ kg/m^2$ (7). Waist circumference was measured using a measuring tape by a registered dietitian trained in anthropometry.

Dietary Intake

Prior to their study visit, all participants completed a three-day food record of two consecutive weekdays and one weekend day. A registered dietitian reviewed each record and asked probing questions for details that may have been missed. All records were analyzed using the Nutrition Data System for Research software (NDSR, Nutrition Coordinating Center, University of Minnesota, MN, USA; database version 2016). Food record data were not available

for one control subject. The Healthy Eating Index-2015 (HEI) score was calculated for each participant to assess diet quality, based upon the current Dietary Guidelines for Americans 2015-2020 (3, 19). It considers adequate amounts of high-quality foods such as vegetables, fruits, and whole grains as well as limited amounts of poor quality diet components such as added sugars, refined grains, and saturated fats (Supplementary Table 6.1). HEI-2015 scores range from 0-100, with a score of 100 indicating the highest diet quality. Components of the HEI-2015 are important for the health of all people and in line with recommendations from the World Health Organization (20). To account for differences of total caloric intake, individual dietary and macronutrient data were adjusted per 1000 kilocalorie (kcal) intake. Dietary intake in subjects with CF was also compared to current CF nutrition guidelines(2). Total energy requirements were calculated according to the Institute of Medicine's Dietary Reference Intakes (DRI) equations, accounting for sex, age, height, weight, and a physical activity coefficient of 1.0(21).

Statistical Analyses

Descriptive statistics were performed on all variables. Wilcoxon sum rank tests were used to compare dietary intake and body composition between subjects with CF and controls. Multiple linear regression analyses were used to test for associations between body composition, dietary intake, and clinical outcomes, adjusting for age and sex. Variables that were not normally distributed were log-transformed for use in regression analyses. Analyses were performed among all subjects [also adjusting for health status (CF or control)] and within subjects with CF only. Additional exploratory analyses were performed to assess for interactions between health status and outcomes, and to assess potential confounding effects of added sugars on group differences. All analyses were conducted in JMP® Pro software version 13.0.0 (SAS Institute, Cary, NC), using two-sided tests with an alpha significance value of 0.05.

Results

Demographic and clinical characteristics for all participants are presented in **Table 6.1**. The distribution of sex and race and the mean age were similar between groups. The majority of subjects with CF were homozygous (50%) or heterozygous (46%) for ΔF508. All subjects with CF had pancreatic insufficiency. The median FEV1% of subjects with CF indicated mild to moderate lung disease, and average rate of decline was 1.2% each year. Median fasting glucose concentrations were higher in subjects with CF compared to controls ($p=0.04$). Among CF participants, 29% had normal glucose tolerance, 25% had impaired glucose tolerance, and 46% had CFRD (median CFRD HbA1c= 7.1%). One healthy control had impaired fasting glucose (IFG). Among subjects with CF, four had IFG and two had fasting hyperglycemia (Supplementary Table 6.2). Five subjects with CF were taking CFTR modulators.

Dietary Intake in Subjects with CF versus Control Subjects

As shown in **Table 6.2,** participants with CF reported a higher median total energy intake compared to controls (2,673 vs 1,914 kcal/day, $p<0.001$), although intake of total dietary fat, carbohydrate, and protein did not differ between groups ($p>0.05$). On average, subjects with CF reported consuming 136% of the estimated DRI for daily calorie requirements, 35.2% of total daily caloric intake from fat, and protein intake at 1.9 gm/kg body weight/day. All but one subject with CF (96%) met total energy requirement recommendations for CF. Despite similarities in total macronutrient intake between subjects with CF versus controls, there were significant differences specific macronutrient substrates. Subjects with CF consumed more trans-fatty acids ($p=0.002$) and added sugars ($p<0.001$), and consumed less dietary fiber ($p<0.001$) compared to healthy controls. Subjects with CF had significantly lower HEI-2015 scores compared to healthy controls ($p<0.001$), indicating worse overall diet quality.

Body composition and fat distribution in subjects with CF versus Control Subjects

Table 6.3 compares body composition and fat distribution between participants with CF and control subjects. Measures of total body composition, including BMI, lean body mass (LBM), body fat percent, and total fat mass, were similar between groups. Participants with CF had significantly more VAT than controls (p=0.02). Among subjects with CF, VAT was greater in males compared to females [median (IQR): 0.35 (0.34) kg vs 0.12 (0.025) kg, p=0.02]. Waist circumference was similar between subjects with CF and control subjects. Among subjects with CF, 29% exhibited NWO, and 8% were considered overweight-obese, a similar distribution as the controls (32% NWO and 12% overweight-obese).

Relationships between diet, body composition, and clinical variables

Among subjects with CF, there was a significant, positive association between fasting blood glucose concentrations and VAT, independent of age and sex (p=0.04; **Figure 6.1A, Table 6.4**). FEV1% predicted was positively, independently associated with BMI (p=0.005) and LBM (p=0.03) but was not associated with any indicators of adiposity (all p>0.05, Table 6.4). Results were similar when using best FEV1% in the previous year. There were no significant associations between average rate of FEV1% decline and body composition variables (all p>0.05, Table 6.3). Added dietary sugar intake was significantly, positively associated with VAT among CF subjects ($\beta=0.61 \pm 0.30$, p=0.047, **Figure 6.1B**). The interaction term between health status and added sugar was not significant (p=0.88). The difference in VAT between participants with CF and controls became non-significant after controlling for added sugar intake (p=0.37). Additional dietary variables were not significantly associated with body composition or clinical outcomes (Supplemental Table 6.3).

Supplemental Tables 6.4 and 6.5 provide the relationships between diet, body composition, and fasting glucose among all subjects. Among all subjects, added sugar and saturated fat were positively associated with VAT ($p=0.005$ and 0.03, respectively), while dietary fiber and protein were inversely associated with VAT ($p=0.006$ for each). Whole grain intake and HEI-2015 scores were inversely associated with total body fat ($p=0.04$ and 0.03, respectively). Neither dietary intake nor body composition variables were significantly associated with fasting glucose among all subjects (all $p>0.05$).

Discussion

To our knowledge, this is the first study to examine associations of dietary intake and diet quality with body composition and clinical outcomes in adults with CF. Our data showed that patients with CF reported poorer diet quality compared to healthy controls. Further, subjects with CF had significantly greater amounts of VAT, which was positively associated with added sugar intake and fasting glucose levels. Together, these data indicate a need for increased surveillance of diet quality and body fat distribution in patients with CF.

Although most patients with CF achieved the recommended energy intake, mean reported dietary fat intake was below the recommended intake, consistent with a recent large European study of pediatric patients with CF (22). Additionally, our novel findings demonstrate poor diet quality in adults with CF compared to healthy controls, attributed to higher intakes of added sugar, trans-fatty acids, and refined grains, and lower intakes of total fiber and whole grains. Similarly reflective of low diet quality, Sutherland et al. recently reported that children with CF in Australia consumed a disproportionately high intake of energy-dense, yet nutrient-poor, foods(4). In addition to meeting recommended macronutrient guidelines, registered dietitians and other healthcare providers should emphasize the importance of choosing foods that enhance diet

quality in their patients with CF. Future studies should identify causes for lower quality food choices by patients with CF to better design effective interventions.

We found a significantly greater amount of VAT in adult subjects with CF compared to healthy controls, despite comparable measures of BMI, total fat mass, and LBM. To date, only two published studies, using DXA(13) or a computed tomography scan(14), have investigated body fat distribution in patients with CF. Both studies reported greater VAT in adolescents and adults with CF compared to healthy controls, even in CF subjects considered to be malnourished (14). In our study, waist circumference did not differ between CF and controls, indicating the need for more sensitive measures of visceral adiposity that distinguish between subcutaneous and visceral adipose tissue. In agreement with known effects of VAT on glucose intolerance in non-CF populations(23), our novel data suggest that VAT in CF is positively associated with fasting glucose concentrations. Elevated fasting glucose is reflective of hepatic insulin resistance and/or dysregulation of hepatic glucose production, both of which are exhibited in subjects with CF (16). However, fasted glucose values are typically in the normal range, presumably due to compensatory mechanisms in CF(16). Our data suggest that VAT may alter this adaptive response. Although findings require confirmation in larger, longitudinal studies, the clinical implications of altered endocrine function are important.

Dietary factors such as added sugars have been hypothesized to promote VAT accumulation (24). Our findings demonstrated that the high intake of added sugars in adults with CF was associated with increased VAT. In addition, adjustment for differences in added sugar intake mitigated significant differences in VAT between groups. Thus, current clinical dogmas favoring unrestricted diets in CF may promote VAT accumulation in this population. Future studies to

decrease added sugars while maintaining a high-calorie intake may be important to consider based on our data in adult patients with CF.

Experimental data also provide a mechanistic basis for a CF-specific dysregulation of body fat deposition. The transcription factor PPARγ is critical for functional lipid storage capabilities of adipocytes(25), yet is decreased with CFTR deficiency(26). Elevated pro-inflammatory cytokines, characteristic of CF, may also impair adipocyte lipid storage capabilities(27). In addition, Bederman *et al.*(28) reported impaired *de novo* lipogenesis in CF mouse models resulting in low subcutaneous adipose tissue stores, and increased hepatic triglycerides following a high-fat diet. These studies suggest an inability of CF adipose tissue to store excess lipids, which, in turn, may be deposited as ectopic fat. Although our study did not include a sufficient number of participants on CFTR modulators to make comparisons, an investigation of the role of CFTR modulators on lipid handling and fat distribution is warranted.

Currently, the primary clinical determinant of adequate nutritional status in patients with CF is the maintenance of a goal BMI or associated growth chart percentile(2). While BMI is a widely utilized and validated tool to assess nutritional status in patients with CF, it has severe limitations because it only reflects body size. BMI does not differentiate between metabolically active components of body weight (LBM vs. fat mass) or provide an indication of body fat topography such as VAT. In this study, LBM but not body fat, was positively associated with lung function, as shown previously(7). Body composition and fat distribution assessment are more sensitive determinants of nutritional status and may facilitate more targeted interventions compared to BMI.

In this study, we used DXA to assess total body composition and fat distribution. Although magnetic resonance imaging (MRI) and computed tomography (CT) are considered gold-

standard for assessing fat distribution, their costs and radiation exposure (with CT) limit their clinical utility. Other available clinical tools to assess body composition such as bioelectrical impedance analysis do not provide information about VAT. As it is recommended that patients with CF begin regular assessments of bone mineral density assessment using DXA scanning at age eight(2), the addition of total and regional body composition with VAT assessment should not be difficult to implement. However, DXA-derived VAT assessment will require further validation in populations with CF before its recommendation for routine clinical application.

A major strength of this study was the careful matching of subjects with CF versus healthy controls, which allowed for group comparisons of body fat distribution without the added bias of differences in total body composition. Furthermore, we describe novel associations of body fat distribution against dietary intake and clinical outcomes in adults with CF. It is possible that there are limitations in the generalizability to non-United States populations; however, high intakes of added sugar have been reported in nationally representative European populations (29), and implications of poor diet quality are a world-wide concern (20). As this was a pilot study, any null findings may have resulted from a lack of power; although these data will be useful for informing larger, adequately-powered studies. Further, as this was a cross-sectional study, we cannot infer causality in the reported outcomes. Finally, there are inherent limitations in dietary intake assessment, such as recall bias and social desirability bias, which may influence diet data.

Conclusions

In this pilot study, adults with CF consumed poor-quality diets and had increased amounts of VAT compared to age-matched healthy controls. The low diet quality scores in participants with

CF were primarily driven by increased intakes of added sugars, refined grains, trans-fatty acids, and low intakes of whole grains and dietary fiber. These preliminary data further suggest that VAT was associated with poor diet quality and elevated fasting glucose concentrations. Thus, adults with CF may have an increased propensity to store metabolically-detrimental ectopic fat, which may be exacerbated by an unrestricted high-calorie diet. These findings highlight the importance of assessing nutritional status using body composition and fat distribution. Larger, prospective studies are needed to determine if body fat distribution and dietary intake are causally related to clinical outcomes, and if they better identify at-risk patients with CF and provide targets for future interventions.

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1

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Table 6.1. Demographic and Clinical Characteristics

Variable	CF group	Control group	p-value
n	24	25	
Age (y)	26.4 (13.7)	25.5 (8.4)	0.52
Males n (%)	12 (50.0%)	9 (36.0%)	0.39
Caucasian n (%)	21 (87.5%)	20 (80.0%)	0.55
Genotype			
ΔF508 homozygous n(%)	12 (50%)	-	
ΔF508 heterozygous n(%)	11 (46%)	-	
Other	1 (5%)	-	
Pancreatic Insufficiency n (%)	24 (100%)	-	
FEV1 (% predicted)	74.9 (28)	-	
FEV1 Average Rate of Decline (%)	-1.0 (2.3)	-	
Fasting Glucose (mg/dL)	91.5 (26.5)	79.5 (14.8)	**0.04**
Glucose Tolerance n (%)			
Normal	7 (29%)	25 (100%)	
Impaired	6 (25%)	-	
Diabetes (CFRD)	11 (46%)	-	
HbA1c %[1]	6.0 (1.7)	-	

Values are presented as median (IQR) or n (%).

[1] n=20 in CF subjects; no data for healthy controls

Abbreviations: FEV1, forced expiratory volume; CFRD, cystic fibrosis related diabetes; HbA1c, hemoglobin A1c

Table 6.2. Dietary Intake in Participants with CF versus Control Subjects

	CF group (n=24)	Control group (n=24)	p-value
Total Calories (kcal)	2,673 (1,131)	1,914 (881)	**<0.001**
% Daily Recommended Intake	133.7 (36.5)	91.1 (37.4)	**<0.001**
Total Fat (g)	39.7 (9.2)	39.7 (10.1)	0.95
% Calories from Fat	35.2 (7.9)	34.5 (7.7)	0.98
Total Saturated Fat (g)	13.5 (5.8)	10.8 (5.8)	0.07
Trans Fatty Acids (g)	1.0 (0.9)	0.6 (0.4)	**0.002**
Total Carbohydrate (g)	114.8 (19.9)	113.4 (28.8)	0.49
% Calories from Carbohydrates	46.2 (7.8)	45.0 (10.4)	0.34
Added Sugar (g)	26.1 (18.1)	12.9 (12.5)	**<0.001**
Total Dietary Fiber (g)	7.2 (3.2)	13.9 (9.1)	**<0.001**
Whole Grains (%)	18 (16)	23 (49)	**0.16**
Refined Grains (%)	82 (16)	77 (49)	**0.16**
Total Protein (g)	43.6 (9.1)	40.0 (19.2)	0.72
% Calories from protein	17.5 (4.8)	15.5 (7.7)	0.43
Healthy Eating Index-2015 Score	48.3 (9.9)	63.9 (27.3)	**<0.001**

Values are presented as median (IQR). All dietary data reported in grams were adjusted per 1000 kcal/day.

Table 6.3. Body Composition of Participants with CF versus Control Subjects

	CF group (n=24)	Control group (n=24)	p-value
Height (cm)	167.6 (12.7)	170.1 (18.2)	0.39
Weight (cm)	62.7 (18.6)	63.6 (14.2)	0.67
Body Mass Index (kg/m^2)	21.6 (3.1)	21.8 (2.9)	0.66
Lean Body Mass (kg)	43.8 (13.0)	43.2 (18.8)	0.64
Lean Body Mass Index (kg/m^2)	15.7 (2.7)	15.2 (2.3)	0.99
Total Fat Mass (kg)	12.1 (9.2)	16.1 (7.1)	0.13
Fat Mass Index (kg/m^2)	4.5 (4.0)	5.9 (2.1)	0.28
Body Fat (%)	23.7 (14.4)	26.9 (8.6)	0.30
Waist Circumference (cm)[1]	81.5 (10.8)	78.8 (15)	0.26
Visceral Adipose Tissue (kg)	0.3 (0.3)	0.1 (0.3)	**0.02**

Values are presented as median (IQR).
[1]n=14 for both groups

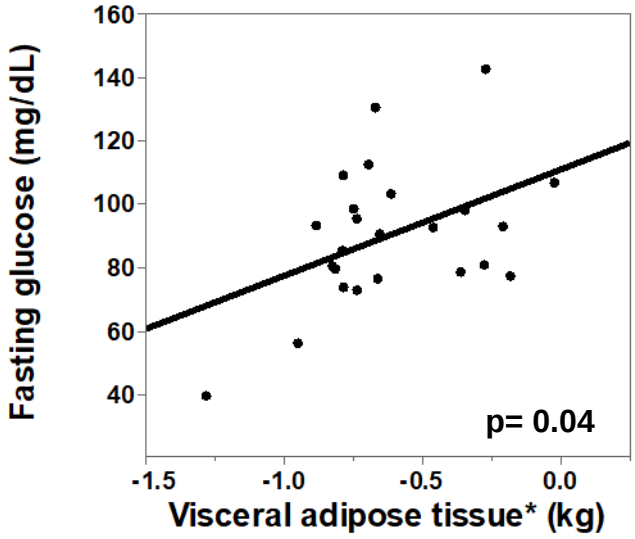
Table 6.4. Multiple linear regression analyses of body composition (independent variables) and clinical outcomes (dependent variables) in participants with CF

	BMI (kg/m^2)	LBM (kg)	LBM Index (kg/m^2)	Total Fat Mass (kg)	Fat Mass Index (kg/m^2)	Body Fat (%)	Waist Circumference (cm)	VAT (kg)[1]
FEV1% Average Rate of Decline	0.2 ± 0.2 (0.3)	0.002 ± 0.08 (0.98)	0.37 ± 0.32 (0.26)	0.11 ± 0.09 (0.3)	0.38 ± 0.27 (0.17)	0.11 ± 0.09 (0.2)	0.09 ± 0.09 (0.4)	2.4 ± 1.7 (0.2)
FEV1% predicted	**4.0 ± 1.3 (0.005)**	**1.3 ± 0.6 (0.03)**	**8.0 ± 2.0 (<0.001)**	1.04 ± 0.8 (0.2)	3.3 ± 2.2 (0.14)	0.64 ± 0.7 (0.4)	1.0 ± 1.4 (0.5)	12.6 ± 14.5 (0.4)
Fasting glucose (mg/dL)	-0.81 ± 1.7 (0.6)	-0.47 ± 0.7 (0.5)	-1.38 ± 1.89 (0.47)	-0.15 ± 0.9 (0.9)	0.31 ± 1.5 (0.84)	0.09 ± 0.8 (0.9)	0.31 ± 0.5 (0.6)	**33.4 ± 14.9 (0.04)**

Analyses were conducted in CF subjects only, adjusting for age and sex, and reported as β ± SE (p-value). Abbreviations: FEV, forced expiratory volume; BMI, body mass index; LBM, lean body mass; VAT, visceral adipose tissue.

[1]Data were log transformed for use in regression analyses

A



B

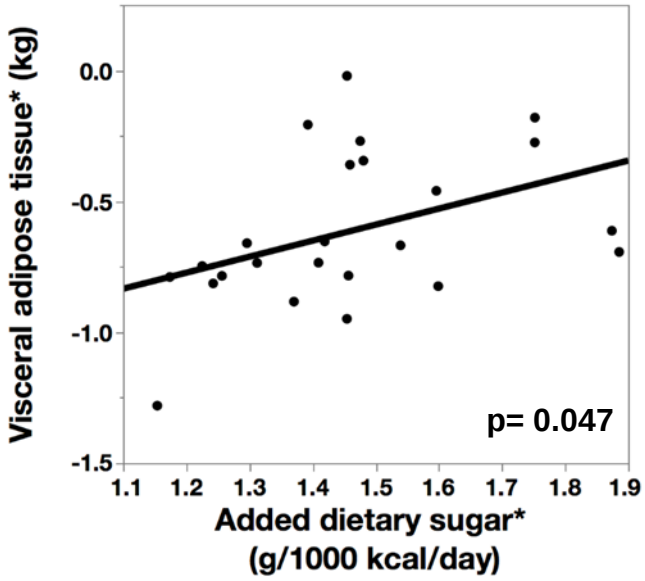


Figure 6.1. Panel A. Positive association between VAT and fasting blood glucose (mg/dL) in CF participants (β=33.4 ± 14.9, p=0.04), controlling for age and sex. Panel B. Positive association between added sugar intake and visceral adipose tissue (VAT) among CF subjects (β=0.61 ± 0.30, p=0.047), independent of age and sex. *VAT and added sugars are log10-transformed.

Table S6.1 (supplementary). HEI–2015[1] Components & Scoring Standards

Component	Maximum points	Standard for maximum score	Standard for minimum score of zero
Adequacy:			
Total Fruits[2]	5	≥0.8 cup equiv. per 1,000 kcal	No Fruit
Whole Fruits[3]	5	≥0.4 cup equiv. per 1,000 kcal	No Whole Fruit
Total Vegetables[4]	5	≥1.1 cup equiv. per 1,000 kcal	No Vegetables
Greens and Beans[4]	5	≥0.2 cup equiv. per 1,000 kcal	No Dark Green Vegetables or Legumes
Whole Grains	10	≥1.5 oz. equiv. per 1,000 kcal	No Whole Grains
Dairy[5]	10	≥1.3 cup equiv. per 1,000 kcal	No Dairy
Total Protein Foods[6]	5	≥2.5 oz. equiv. per 1,000 kcal	No Protein Foods
Seafood and Plant Proteins[6,7]	5	≥0.8 oz. equiv. per 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids[8]	10	(PUFAs + MUFAs)/SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation:			
Refined Grains	10	≤1.8 oz. equiv. per 1,000 kcal	≥4.3 oz. equiv. per 1,000 kcal
Sodium	10	≤1.1 gram per 1,000 kcal	≥2.0 grams per 1,000 kcal
Added Sugars	10	≤6.5% of energy	≥26% of energy
Saturated Fats	10	≤8% of energy	≥16% of energy

1: Intakes between the minimum and maximum standards are scored proportionately.
2: Includes 100% fruit juice.
3: Includes all forms except juice.
4: Includes legumes (beans and peas).
5: Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.

6: Includes legumes (beans and peas).
7: Includes seafood, nuts, seeds, soy products (other than beverages), and legumes (beans and peas).
8: Ratio of poly- and monounsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs)
Table available at: https://epi.grants.cancer.gov/hei/developing.html#f2

Table S6.2 (supplementary). Classification of glucose tolerance among all subjects

	Fasting plasma glucose mg/dl (mmol/l)	2-h OGTT glucose mg/dl (mmol/l)	Number in each category (%)	
			CF group (n=24)	Control group (n=24)
NGT	<100 (5.6)	<140 (7.8)	7 (29)	23 (96)
IFG + NGT	100–125 (5.6–6.9)	<140 (7.8)	NA	1 (4)
IGT	<100 (5.6)	140–199 (7.8–11.1)	4 (17)	NA
IFG + IGT	100–125 (5.6–6.9)	140–199 (7.8–11.1)	2 (8)	NA
CFRD FH−	<100 (5.6)	≥200 (11.1)	7 (29)	NA
CFRD + IFG	100–125 (5.6–6.9)	≥200 (11.1)	2 (8)	NA
CFRD FH+	≥126 (7.0)	≥200 (11.1)	2 (8)	NA

Abbreviations: NGT, normal glucose tolerance; IFG, Impaired fasting glucose; CFRD, cystic fibrosis-related diabetes; FH- , without hyperglycemia; FH+, with hyperglycemia. Classification of glucose tolerance subtypes based on Frohnert et al. (16)

Table S6.3 (supplementary). Multiple linear regression of diet (independent variable) versus body composition and fasting glucose (dependent variables) in participants with CF [β ± SE (p-value)]

	Energy (kcal)[2]	Dietary Fat (g)[2]	Saturated Fat (g)[2]	Trans-saturated fat (g)[2]	Total CHO (g)[2]	Added sugars (g)[1,2]	Fiber (g)[2]	Whole grains (%)	Protein (g)[2]	HEI-2015
LBM (kg)	3.0 ± 2.5 (0.24)	0.38 ± 0.20 (0.07)	0.81 ± 0.39 (0.05)	1.4 ± 2.3 (0.55)	-0.11 ± 0.07 (0.14)	5.3 ± 7.1 (0.46)	-0.87 ± 0.62 (0.18)	-0.76 ± 10.9 (0.95)	0.25 ± 0.22 (0.28)	-0.24 ± 0.18 (0.19)
Total Fat Mass (kg)	1.7 ± 2.1 (0.43)	0.13 ± 0.18 (0.48)	0.3 ± 0.4 (0.41)	-1.75 ± 1.93 (0.38)	-0.03 ± 0.06 (0.62)	8.5 ± 5.7 (0.15)	-0.68 ± 0.53 (0.21)	-4.4 ± 9.1 (0.63)	0.06 ± 0.19 (0.76)	-0.14 ± 0.15 (0.37)
Body Fat (%)	0.7 ± 2.3 (0.78)	0.04 ± 0.20 (0.85)	0.17 ± 0.39 (0.66)	0.17 ± 0.39 (0.66)	-0.01 ± 0.07 (0.94)	5.6 ± 6.4 (0.39)	-0.39 ± 0.58 (0.51)	-3.0 ± 9.8 (0.76)	-0.04 ± 0.21 (0.84)	-0.08 ± 0.17 (0.66)
VAT(kg)[1]	0.11 ± 0.11 (0.33)	0.004 ± 0.01 (0.71)	0.01 ± 0.02 (0.61)	-0.1 ± 0.1 (0.34)	-0.0004 ± 0.003 (0.90)	**0.61 ± 0.29 (0.047)**	-0.03 ± 0.03 (0.32)	-0.07 ± 0.48 (0.88)	-0.005 ± 0.01 (0.64)	-0.002 ± 0.008 (0.80)
Fasting glucose (mg/dL)	-4.8 ± 8.4 (0.57)	-0.48 ± 0.73 (0.51)	-0.6 ± 0.73 (0.68)	3.1 ± 7.7 (0.69)	0.29 ± 0.25 (0.25)	28.2 ± 23.0 (0.24)	1.5 ± 2.1 (0.49)	29.5 ± 35.6 (0.42)	-0.79 ± 0.73 (0.29)	0.39 ± 0.61 (0.52)

All analyses were conducted in subjects with CF only and included age and sex in the models. [1]Log10-transformed for analysis. [2]Dietary data are
adjusted per 1000 kcal/day. Abbreviations: CHO, carbohydrates; HEI, Healthy Eating Index; LBM, lean body mass; VAT, visceral adipose tissue

Table S6.4 (supplementary). Multiple linear regression analyses of body composition (independent variables) and fasting glucose (dependent variable) in all subjects [β ± SE (p-value)]

	BMI (kg/m^2)	Lean Body Mass (kg)	Total Fat Mass (kg)	Body Fat (%)	WC (cm)	VAT (kg)*
Fasting glucose (mg/dL)	-0.49 ± 1.06 (0.65)	-0.41 ± 0.45 (0.37)	0.14 ± 0.5 (0.81)	0.25 ± 0.48 (0.60)	0.05 ± 0.5 (0.91)	13.5 ± 8.2 (0.10)

Analyses are adjusted for age, sex, and health status (CF or healthy control).
Abbreviations: BMI, body mass index; WC, waist circumference, VAT, visceral adipose tissue

Table S6.5 (supplementary). Multiple linear regression of diet (independent variable) versus body composition and fasting glucose (dependent variables) in all subjects [β ± SE (p-value)]

	Calories (kcal)[2]	Total Dietary Fat (g)[2]	Saturated Fat (g)[2]	Trans-saturated fat (g)[2]	Total Carbohydrates (g)[2]	Added sugars (g)[1,2]	Fiber (g)[2]	Whole grains (%)	Total Protein (g)[2]	HEI-2015
Lean Body Mass (kg)	2.1 ± 1.5 (0.18)	**0.25 ± 0.12 (0.048)**	**0.60 ± 0.23 (0.01)**	0.76 ± 1.4 (0.6)	-0.08 ± 0.04 (0.07)	-0.78 ± 3.9 (0.84)	-0.004 ± 0.19 (0.98)	3.5 ± 3.8 (0.36)	0.03 ± 0.06 (0.59)	-0.03 ± 0.08 (0.73)
Total Fat Mass (kg)	1.2 ± 1.3 (0.38)	0.16 ± 0.10 (0.13)	**0.46 ± 0.20 (0.03)**	0.71 ± 1.19 (0.55)	-0.01 ± 0.04 (0.79)	6.2 ± 3.1 (0.05)	-0.31 ± 0.15 (0.05)	**-6.4 ± 3.1 (0.04)**	-0.06 ± 0.05 (0.27)	**-0.13 ± 0.06 (0.03)**
Body Fat (%)	0.32 ± 1.5 (0.83)	0.09 ± 0.12 (0.48)	0.34 ± 1.36 (0.69)	0.56 ± 0.39 (0.66)	0.02 ± 0.04 (0.65)	**7.1 ± 3.5 (0.049)**	**-0.37 ± 0.17 (0.04)**	**-8.9 ± 3.4 (0.01)**	-0.09 ± 0.06 (0.14)	**-0.15 ± 0.07 (0.03)**
Visceral Adipose Tissue (kg)[1]	0.09 ± 0.08 (0.27)	0.008 ± 0.007 (0.26)	**0.03 ± 0.01 (0.03)**	0.03 ± 0.08 (0.69)	0.001 ± 0.002 (0.76)	**0.66 ± 0.18 (0.005)**	**-0.03 ± 0.009 (0.006)**	-0.22 ± 0.20 (0.28)	**-0.009 ± 0.003 (0.006)**	-0.007 ± 0.004 (0.08)
Fasting glucose (mg/dL)	-3.5 ± 4.7 (0.45)	-0.10 ± 0.38 (0.80)	-0.23 ± 0.76 (0.76)	2.1 ± 4.3 (0.63)	0.17 ± 0.12 (0.17)	11.3 ± 11.8 (0.34)	0.49 ± 0.58 (0.41)	8.3 ± 11.8 (0.48)	-0.19 ± 0.19 (0.31)	0.06 ± 0.23 (0.8)

Analyses were conducted in all subjects and included age, sex, and health status (CF or Healthy Control) in the models. [1]Log10-transformed for analysis. [2]Dietary data is adjusted per 1000 kcal/day. Abbreviations: HEI, Healthy Eating Index

CHAPTER 7

DISCUSSION

Excess adiposity leads to broad health impacts, adversely affecting psychological, physiological, and metabolic functions (1). Advanced methodologies allow improved insight into our understanding of the pathophysiology of obesity and altered body fat distribution. While significant knowledge has been gained over the past decades, there is still much to be discovered about the health-related impacts of adiposity, especially in populations without overt overweight or obesity. In this dissertation, we add to the existing knowledge on obesity pathophysiology by investigating an obesity phenotype, normal weight obesity (NWO), in a large, cross-sectional cohort of adults. We also examined NWO and body composition and fat distribution in a clinical population of adults with cystic fibrosis (CF).

Key Findings

We integrated metabolic, clinical, and dietary assessments to characterize relationships with total adiposity and adipose tissue distribution. To examine these connections, we investigated a large cohort of working adults [Center for Health Discovery (CHD) cohort] categorized as lean, NWO, and overweight-obese, as well as a clinical population of adults with CF. The NWO subtype is characterized by a normal weight according to body mass index (BMI) but high body fat percent and high risk of metabolic disease (2). Findings in the CHD cohort (Chapter 4) indicated that adults with NWO exhibited significantly lower VO2 maximum levels but reported similar diet quality to lean individuals as measured by the Alternate Healthy Eating Index (AHEI), Mediterranean Diet Score (MDS), and Dietary Approaches to Stop Hypertension

(DASH) diet quality score. Additionally, females were more likely to have NWO than males, and associations with physical fitness and diet quality between the body composition subtypes were driven by female participants. Regardless of body composition subtype, higher dietary quality was associated with lower total body fat and visceral adipose tissue (VAT, metabolically detrimental fat stored within the abdominal cavity (3). These findings are in line with current research that identifies low levels of physical activity and poor diet quality as risk factors for obesity. However, most of the past and current research focuses on obesity assessed by BMI. Here, we report novel findings that low physical fitness is also related to NWO. Our results also support the need to maintain and improve the quality of dietary intake to reduce VAT accumulation and weight gain in adults.

Subsequently, in a subset of the CHD cohort, characterization of the plasma metabolome using high-resolution metabolomics (HRM), detected perturbations in metabolic pathways of adults with NWO, which reflected metabolic dysfunction similar to adults with overweight-obesity (Chapter 5). Linoleic acid metabolism was upregulated, including elevated levels of pro-inflammatory linoleic acid-derived metabolites, in adults with NWO and overweight-obesity compared to lean individuals. Additionally, plasma amino acid levels, including branched chain amino acids (BCAA) and aromatic amino acids (AAA), were increased with greater adiposity. Increased plasma BCAAs and AAAs have been implicated as a cause and consequence of insulin resistance (4). Our novel findings in adults with NWO are in line with other obesity-related research (5) and provide additional insight into possible mechanisms of inflammation related to diet and adiposity that may serve as targets for further study.

To better understand the role of excess adiposity in a clinical population who exhibits high levels of inflammation and is at high risk for metabolic dysfunction, we also investigated

body composition subtypes and body fat distribution in a cohort of adults with CF (Chapter 6). Among participants with CF, almost 30% of participants had NWO. Adults with CF also had significantly higher amounts of VAT compared to age-matched healthy participants, which was positively related to fasting blood glucose concentrations. Most of the CF scientific literature and recommendations focus on the use of BMI to assess nutritional status and the need to maximize calorie and fat intake through an unrestricted diet (6). Our findings highlight the importance of assessing body composition and add to the limited research investigating body fat distribution in this clinical population (7, 8). Participants with CF also had lower diet quality scores compared to their age-matched counterparts, which had not been previously reported in the CF community. Poor diet quality among participants with CF was driven by increased reported intake of added sugar, refined grains, and saturated fat compared to controls. Further, added sugar intake was positively associated with greater VAT, and significant group (CF vs. healthy) differences in VAT were negated when models were adjusted for differences in added sugar. This original finding is consistent with other non-CF obesity and fat distribution literature (9, 10), and implies the need for updated diet education and improved diet quality in people living with CF.

Role of Diet Quality and Physical Fitness on Body Composition

In this dissertation work, lower diet quality was associated with increased total adiposity and visceral adiposity in both middle aged adults in the CHD cohort and young adults with CF. Poor diet quality has been estimated as the leading cause of death in the United States and is recognized as a major contributor to the onset and progression of cardiovascular diseases, neoplasms, diabetes, urogenital, blood, and endocrine diseases (11). There is ongoing debate of how dietary macronutrient distribution impacts body composition and fat distribution (12);

however, strong evidence suggests that dietary quality influences the partitioning of adipose tissue to anatomic regions. For example, refined carbohydrates can lead to increased accumulation of visceral and liver fat in adult males (13). Independent of BMI, increased VAT is tightly linked to increased metabolic disease and mortality in adults (3, 14). While a multitude of factors are related to body composition, the results of this dissertation indicate that in middle aged adults and adults with CF, diet quality has an important influence on body composition and fat distribution, which impact disease risk.

Adults with NWO in the CHD cohort (chapter 4) had lower levels of physical fitness compared to lean adults. Low physical fitness is an indicator of decreased physical functioning and is an independent risk factor for mortality. Finding ways to improve physical fitness can improve health substantially (15, 16). The considerable metabolic benefits of exercise exceed some of the improvements achieved through medications (15). Research also suggests that energy balance and weight maintenance are best achieved with a high level of physical activity (17) both through increasing energy expenditure and possibly through changes in appetite regulation that lead to reduced energy intake (12, 18). While physical fitness was not assessed in the CF population in the current dissertation work, this is an important area to address in the future. Research shows that high intensity exercise may improve lung function in people with CF (19) whereas aerobic and resistance exercise may promote preservation of lean mass rather than improvements in clinical outcomes, but updated research is needed (20). The benefits of exercise have been clearly demonstrated and finding ways to increase physical activity in people with NWO and CF could result in improved body composition, metabolic health, and clinical outcomes in these populations.

Role of Sex on Body Composition

A greater proportion of women were classified as having NWO than men in the CHD cohort, indicating that women may be more likely to be misclassified for disease risk when solely using BMI guidelines. The development of obesity and VAT accumulation in women is different from men, and the management of obesity in women should be distinct from men (21). Important female-specific factors that influence total body fat and body fat distribution in women include reproductive stage and pregnancy (21), which should be considered when designing intervention studies.

Research in female cohorts illustrates the unique relationships between diet and physical activity with body composition as women age. In post-menopausal women, women with normal weight and central obesity according to waist circumference had similar mortality risk as women with obesity and central obesity (22). Importantly, in the CHD cohort, waist circumference was comparable between women categorized as lean and NWO, indicating that some women may have increased abdominal obesity that is undetected by waist circumference. Greater perivascular adipose tissue (PVAT), an ectopic fat depot, has also been associated with decreased physical functioning in women as they age (23). In line with this finding, physical fitness was inversely associated with VAT in the CHD cohort, and women with NWO had lower fitness than lean women. Finally, research in postmenopausal women determined that a diet with greater consumption of whole grains and legumes and lower intake of refined grains was negatively related to BMI, waist circumference, and waist-to-height ratio (24). Altogether, evidence indicates that dietary choices and physical activity are critical factors for middle aged women to attenuate or prevent weight gain, redistribution of abdominal fat, and loss of physical functioning, which are associated with multiple adverse health and disease consequences (25).

Future intervention research should target middle-aged and older women and help improve physical fitness and dietary intake and evaluate changes in physical functioning and body composition and fat distribution.

Novel Metabolic Pathways Linked to Body Composition

HRM provides comprehensive metabolic assessment through broad detection of low molecular weight chemicals (26). In this dissertation, untargeted metabolomics was applied to characterize nutrition-related metabolism in an obesity phenotype, and the primary pathway differentiating the body composition subtypes was linoleic acid (an n-6 fatty acid) metabolism. Experimental models have shown that linoleic acid-derived metabolites are pro-inflammatory (27-29), can induce osteoclast formation and bone resorption (30, 31), and promote mesenchymal stem cell differentiation to adipocytes at the expense of osteoblast differentiation (32, 33). Preliminary findings (manuscript under review) in the CHD cohort also implicated linoleic acid and related metabolites, as predominant correlates of bone mineral density (BMD). Together, these findings support the concept that pro-inflammatory lipid molecules related to linoleic acid may promote adiposity while having adverse effects on bone health.

Additional HRM findings in our cohort of middle-aged adults provided indications of mitochondrial dysfunction in participants with NWO and overweight-obesity. Previous work has demonstrated a link between surplus mitochondrial metabolism substrates with increased plasma BCAAs, AAAs, and short chain acylcarnitines, and insulin resistance (34). We found increased levels of plasma BCAAs and AAAs associated with adiposity in the NWO and overweight-obesity groups, in agreement with other obesity research (4). Mitochondria play an integral role in both cellular energy metabolism and disease processes. Indeed, obesity, cardiovascular

disease, type 2 diabetes, neurodegenerative diseases, and diseases of aging are associated with mitochondrial dysfunction (35-38). Impaired mitochondrial function promotes disease processes through increased production of oxidant species that exacerbates the inflammatory state of obesity (39). Whether body fat distribution affects mitochondrial function to a greater degree compared to whole body adiposity is unknown but should be studied given the detrimental metabolic effects of VAT. Additional research should also focus on sex-specific relationships between adiposity and mitochondrial function. Our preliminary findings in a separate small cohort of adults suggested that individuals with NWO have impaired oxidative phosphorylation (40) and total adiposity was associated with *in vivo* and *ex vivo* indexes of mitochondrial energy metabolism in males, but not in females (unpublished data). Whether confirmation of such findings would translate into sex differences in effects of excess adiposity on disease risk require further study.

Clinical Implications and Future Directions for NWO

In the CHD cohort subset (n=179), while some classic clinical laboratory values were similar between lean and NWO participants, HRM provided a more sensitive detection of metabolic disruption. There is heterogeneity in the metabolic health of individuals with NWO based on classic laboratory values. Using HRM to predict individuals with NWO who are likely to develop metabolic diseases would help focus intervention and treatment efforts using a more precision medicine-based approach. In other disease states, such as type 2 diabetes mellitus, the plasma metabolome was a useful indicator to identify increased risk of developing diabetes (41-44). The plasma metabolome has also been shown to dynamically change with therapeutic interventions. For example, both medical interventions such as bariatric surgery and lifestyle

interventions, such as resistance and aerobic training have shown improvements in both the plasma metabolome, reflected by decreases in plasma BCAA levels, and linked clinical outcomes such as insulin sensitivity (45-47). More research is needed to understand the mechanisms that lead to improved metabolic profiles and if HRM can be used to predict disease risk based on total adiposity and adipose tissue distribution.

An ongoing area of research is attempting to elucidate the mechanisms driving deposition of VAT and establish temporality in the relationships between whole body adiposity, deposition of VAT, and development of insulin resistance. There are two primary hypotheses that have been proposed: the adipose tissue expandability hypothesis and the portal hypothesis. The expandability hypothesis suggests that individuals have a limited capacity to expand their subcutaneous adipose tissue (48). Once that limit is reached, metabolic disturbances ensue, including lipid spill over and accumulation of ectopic fat, altered adipokine secretion, and increased cytokine production, which can modulate insulin action (49). On the other hand, the portal hypothesis postulates that fat stored in the omental and mesentery depots, which is highly pro-inflammatory and lipolytic, causes hepatic insulin resistance and hepatic steatosis because these fat tissues are drained by the portal vein. The exaggerated release of free fatty acids and pro-inflammatory cytokines from VAT may cause direct insult to the liver and impact live function (50). While these theories seem to present as mutually exclusive, both whole body adiposity and VAT are associated with insulin resistance, and there is evidence to support both theories. While we did not directly explore these relationships in this dissertation, our findings implicated increased body fat with higher insulin resistance, although we cannot establish directionality in these occurrences. Future research should continue to explore the role of whole

body adiposity and VAT deposition linked to the onset of insulin resistance and type 2 diabetes mellitus.

Although this dissertation focused on adiposity, a second differentiating factor of persons with NWO is diminished lean mass. Our findings in the CHD cohort (Chapter 4) showed that lean mass, measured by DXA, was significantly lower in individuals with NWO compared to the both the lean and overweight-obesity groups. Individuals with overweight-obesity tend to have higher lean mass due to the load bearing effect of increased body weight (1); however, body weight in individuals with NWO is insufficient to confer this load bearing benefit. The detrimental effects of sarcopenia, defined as the loss of muscle mass and concomitantly decreased muscle strength, on clinical outcomes and mortality in older adult populations have been extensively studied (51, 52). Although long term studies are required for confirmation, it is possible NWO is a risk factor for sarcopenia. Important clinical targets for individuals with NWO may be to increase lean mass and improve physical fitness. Future research should determine effective strategies for increasing lean mass and improving physical fitness among those with NWO, both among generally healthy adults, and in clinical populations such as the CF cohort we studied in this dissertation work. Next steps in NWO research should also investigate the specific role of lean mass on metabolic health in males and females by using tools such as HRM paired with clinical outcomes.

While diet quality, assessed from reported food intake, was not different between lean and NWO participants in the CHD cohort (Chapter 4), it should not be neglected as a factor in intervention efforts due to its known relationship with disease. Our HRM findings of significantly elevated plasma linoleic acid and related metabolites among individuals in the NWO and overweight-obesity groups compared to the lean group (Chapter 5) may provide a

more objective indication of diet quality. Highly processed foods, which are not differentiated from whole foods in most diet intake analysis programs or in diet quality scores, constitute a major source of dietary linoleic acid in Western diets (53). Thus, it is possible that the elevated plasma linoleic acid observed with increased adiposity in our study reflect greater consumption of processed foods. In a recent randomized controlled trial, ad libitum intake of ultra-processed, poor quality foods resulted in greater fat mass and weight gain than unprocessed food consumption, emphasizing the benefits of higher quality diets to prevent or reduce weight gain (54).

The relationships of total and individual dietary polyunsaturated fatty acids (PUFA) intake with health is complex, and its metabolic effects continue to be debated (55-58). Metabolism of linoleic acid may be influenced by nutrient-nutrient interactions, as well as the ratio of omega-6 (n-6) to omega-3 fatty acid (n-3) intake. As an essential fatty acid, adequate amounts of dietary linoleic acid are necessary for normal health and functioning, but excessive intake, which is typical in Western-style diets, may be deleterious for health and promote adiposity (29, 55, 59). These negative effects largely stem from n-6 fatty acid promotion of inflammation and oxidative stress and suppression of the predominantly anti-inflammatory effects of n-3 PUFAs and related metabolites (60). A high dietary intake of n-6 linoleic acid is reflected by higher amounts of pro-inflammatory n-6 derived oxylipins (27) and increases the availability of ARA as a signaling molecule. Further, as detailed above, pro-inflammatory linoleic acid-derived oxylipins such as HODE and HPODE have been shown to increase adipocyte differentiation and decrease osteoblast differentiation *in vitro* (33, 61, 62). Recently, consumption of an omega-3 fatty acid supplement with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was shown to increase n-3 derived oxylipins and suppress the

effects of n-6 oxylipins (63). Additional experimental and clinical trials are required to determine if higher intakes of n-3 fatty acids and/or lower intakes of n-6 fatty acids have beneficial effects on lowering inflammation and/or regulating adiposity.

Our plasma HRM findings in adults with NWO implicated impaired mitochondrial function. Mitochondrial dysfunction is linked to cardiovascular disease, neurodegenerative disorders, age-related disease, and metabolic disorders (38). Mitochondria are sensitive to changes in cellular environments such as increases in oxidative stress, which commonly occurs in obesity. Future research projects may investigate if NWO is associated with mitochondrial functions such as oxidative phosphorylation, in males and females and how oxidative stress and redox signaling impact mitochondrial energy metabolism. Additionally, because metabolism varies in specific disease states such as CF, examining these relationships with mitochondrial function in clinical populations is also needed. Better understanding of these interactions will help elucidate disease mechanisms and aid in constructing improved strategies for disease prevention and treatment.

Clinical Implications and Future Directions for Cystic Fibrosis and Other Catabolic Illnesses

There is a lack of nutrition and body composition research in patients with CF, and this dissertation addresses both topics. Almost 30% of adults with CF were categorized as NWO, which was consistent with findings form a previous cohort of adults with CF (64). Additionally, participants with CF reported low diet quality and increased amounts of VAT, which was positively associated with added sugars and fasting glucose levels. These results support the inclusion of body composition assessment as routine clinical care of patients with CF to monitor fat, lean, and visceral fat mass. While CF nutrition recommendations do not currently focus on

diet quality (65), these findings may warrant a nutritional intervention that includes diet education to improve diet quality and characterizes changes in body composition and VAT. For example, other studies have demonstrated reductions in abdominal obesity by improving diet quality in adults (66, 67). Notably, about 50% of adults with CF develop CF related diabetes (CFRD), which is related to increased morbidity and mortality (68). Determining factors that influence the onset and management of CFRD is critical (68). Such factors may include excess visceral adiposity, as indicated by the current dissertation work in CF (69), and/or dietary influences, as indicated by a recent study linking dietary glycemic index to derangements in blood glucose levels in individuals with CF (70). Longitudinal studies are needed that assess relationships between diet, physical activity, and body composition and fat distribution with clinical outcomes in patients with CF. Dietary intervention studies that promote high diet quality, including greater intake of whole grains, high-quality protein, and unsaturated fats, and lower intake of added sugar are warranted and should be tested for its effects on body composition, fat distribution, and diabetes risk in this population.

As utilized in characterizing metabolism related to the body composition subtypes, HRM represents a unique frontier for CF research. HRM can be applied in CF to broadly profile underlying metabolism related to disease exacerbation and treatment. For example, HRM was recently used to assess the effects of a high-dose vitamin D intervention in adults with CF who were hospitalized for an acute pulmonary exacerbation, with findings indicating a potential anti-catabolic effect of vitamin D in this population (71). HRM may also be used to study differences in metabolism related to glucose tolerance status in patients with CF compared to healthy adults or adults with type 1 or type 2 diabetes. Future studies should investigate nutrition-related

metabolism linked to clinical outcomes that can help inform nutritional interventions and updated dietary recommendations for CF.

In addition to CF, there are other catabolic disease states that would benefit from inclusion of sensitive body composition assessment in clinical settings, including tuberculosis (TB), human immunodeficiency virus (HIV), critically ill and other hospitalized patients, patients with chronic infections, and cancer patients undergoing extensive chemotherapy. Similar to persons with CF, these patients often experience depleted lean mass, which is associated with increased morbidity and mortality (51, 52, 72). Some patients with these diseases may develop sarcopenia, defined as severe loss of muscle mass and function, which greatly impacts their prognosis and survival (73). Evaluation of body composition in these patients, potentially linked to changes in plasma HRM, would provide earlier detection of lean mass loss, sarcopenia, and malnutrition compared to BMI for prompt nutritional intervention efforts.

Strengths and Limitations

Strengths of the studies outlined in this dissertation include the range of sophisticated assessment methods to provide novel metabolic characterization of individuals with NWO and CF. Gold standard measurements, including dual energy x-ray absorptiometry for body composition assessment, ensure high quality data collection and good internal validity. The CHD cohort is a large cohort of working adults and those studies provide important new information on the pathophysiology and risk factors for NWO that can be generalized to a large proportion of individuals in the U.S.

A limitation of the included studies is the nature of the study design. All relationships described in this dissertation were cross-sectional, and conducting longitudinal studies is an

important next step to evaluate the temporality of these relationships. In particular, the relationships of VAT and low lean mass with clinical outcomes in adults with CF over time is integral to study to inform clinical evaluation and to inform the next level of dietary intervention studies. Another limitation is the use of self-reported dietary intake data. Self-reported dietary data is subject to recall bias, which compromises data accuracy, and social desirability bias where individuals may report or remove foods that seem healthy or unhealthy, respectively (74, 75). Finally, the study using HRM in body composition subtypes and the study in adults with CF have moderately sized groups, and larger, well-powered studies are needed to confirm our results.

Conclusions

In conclusion, we found low levels of physical fitness associated with NWO in adults, and metabolic dysfunction in adults with NWO that was similar to adults with overweight-obesity. We also report increased VAT in adults with CF, which was related to poor diet quality. Although individuals with NWO and CF are typically considered lean by BMI, we found dysregulated metabolism and adverse clinical outcomes in these populations related to adiposity and increased VAT. These results support inclusion of body composition and fat distribution assessment in clinical settings to evaluate individuals for health and disease risk as well as promotion of increased diet quality. In NWO participants, relationships between lean mass and metabolic health should be investigated. Studies conducted in female cohorts may be especially warranted due to the high prevalence of NWO in females. Longitudinal studies are needed to explore these associations for causal relationships and if tools such as HRM have prognostic potential to identify individuals at high risk for developing metabolic disease.

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