Dedication and Acknowledgements

I would like to dedicate this thesis to my family including my husband George, daughter Zoe, and mother Eftihia who have stood by me and supported me during my training and studies in order that I may accomplish my dreams of becoming an Academic and Clinical Endocrinologist.

I would like to acknowledge all the people who have helped me during the culmination of my thesis and my T32 training grant including my mentors Dr. Lynne Wagenknecht and Dr. David Goff, without whom this would not have been made possible.

As Eleanor Roosevelt famously once said: “Do one thing every day that scares you.”
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LIST OF ABBREVIATIONS

AC-aortic calcification
ALT-alanine aminotransferase
aPWV-aortic pulse wave velocity
AST-aspartate aminotransferase
ATP-III-Adult Treatment Panel III
BMI-body mass index
BP-blood pressure
CAC-coronary artery calcification
CCA-IMT-common carotid artery-intima media thickness
CVD-cardiovascular disease
CM-cardiometabolic
CT-computed tomography
DBP-diastolic blood pressure
GGT-gamma-glutamyltransferase
HOMA-IR-homeostasis model assessment of insulin resistance
HDL-C-high density lipoprotein cholesterol
HS-CRP-high sensitivity c reactive protein
HU-Hounsfield units
IL-6-interleukin 6
IRAS-Insulin Resistance Atherosclerosis Study
LDL-low density lipoprotein
LSR-liver to spleen ratio
MA-metabolically abnormal
MAO-metabolically abnormal obese
MAOW-metabolically abnormal overweight
MH-metabolically healthy
MHO-metabolically healthy obese
MHOW-metabolically healthy overweight
NAFLD-nonalcoholic fatty liver disease
NHANES-National Health and Nutrition Examination Study
NHLBI-National Heart, Lung and Blood institute
OR-odds ratio
RBP-4-retinol binding protein 4
PAI-1-plasminogen activator inhibitor type 1
RR-relative risk
SAT-subcutaneous adipose tissue
SBP-systolic blood pressure
SI-fasting insulin
TG-triglycerides
TNF-tumor necrosis factor
WC-waist circumference
WHO-world health organization
WHR-waist to hip ratio
VAT-visceral adipose tissue
VSR-visceral to subcutaneous tissue area ratio
ABSTRACT

Some obese individuals appear to be protected from developing type 2 diabetes mellitus and cardiovascular disease (CVD). This has led to characterizing different body size phenotypes based on cardiometabolic risk factors. Thus, individuals can be classified as being metabolically healthy obese (MHO) versus metabolically abnormal obese (MAO). Although there have been several studies that have tried to characterize and estimate the prevalence of MHO, these studies have examined homogeneous populations consisting of primarily Northern European Caucasian participants. Recent epidemiologic evidence suggests that body fat distribution and several biochemical markers may distinguish the MHO from MAO. The goal of this study is to better understand the characteristics and risk factors associated with these body size phenotypes. Specific aims for this study will be to measure the prevalence and describe fat distribution across these different phenotypes in a minority population. The IRAS-Family cohort was examined that consists of families (N=1611) from two minority groups (African American and Hispanic). Findings suggest that lower levels of visceral and liver fat, despite overall increased total body fat, may be a defining feature of MHO in Hispanic and African Americans. Further, obesity as defined by BMI may not have the same clinical significance for every individual.
INTRODUCTION: CHAPTER ONE

Epidemiology of Obesity:

The obesity epidemic has not only reached epidemic proportions nationwide but is now receiving global attention. For thousands of years obesity was considered a rare phenomenon, and it was not until the twentieth century that it became so common, that in 1997 the World Health Organization (WHO) formally recognized obesity as a global epidemic (1). Globally, there are more than 1.5 billion overweight (i.e., BMI 25.0-29.9 kg/m²) adults, with at least 500 million (200 million men and 300 million women) adults 20 years and older considered obese (i.e., BMI ≥ 30 kg/m²) (2). The only remaining areas of the world where obesity is not common is sub-Saharan Africa and South Asia (Bangladesh) (2). The United States has the second highest obesity rate in the developed world with Mexico having the highest rate of obesity (3). From 1985 to present the obesity rates in the United States have increased dramatically, reaching the current rate of 33% of the adult population (4). Further, rates of obesity vary between socioeconomic strata and minority populations, with minorities and low-income individuals more likely to be overweight and obese. In fact, obesity rates are as high as 50% among African American women (5). Geography also plays a major factor in the prevalence of obesity in the United States, as the American South has been nicknamed the “Obesity Belt” to reflect the fact that residents of this region have higher obesity rates when compared to people of the same race/ethnicity elsewhere in the country. Most concerning is the observation that the prevalence of class III obesity (BMI ≥40 kg/m²) in the United States has increased the most dramatically, from 1.3% in the late 1970s, to 2.9% in the 1980-
1990’s, 4.7% in 2000, to 5.7% in 2008 (6). Among African American women, its prevalence is estimated to be as high as 14% (7).

**Public Health Implications of Obesity:**

Coincident to the observation of a high obesity prevalence rate, is the finding that obesity is an important and independent risk factor in the development and increasing incidence of metabolic syndrome, type 2 diabetes mellitus and CVD (8). It is estimated that about 300,000 excess deaths per year in the United States are due to obesity (9). In addition to the impact on morbidity and mortality, the health-care costs incurred from these conditions are tremendous. The national cost of diabetes in the U.S. in 2007 exceeded $174 billion according to the American Diabetes Association (10). The cost of cardiovascular diseases and stroke in the United States in 2009 was estimated to be $475.3 billion, according to the American Heart Association and the National Heart, Lung, and Blood Institute (NHLBI) (11). Further, people who are obese spend almost $1,500 more each year on health care, which is on average approximately 41 percent more than a normal weight person (12). Therefore, given these public health implications, the necessity for intervention and perhaps policy implementation is paramount as obesity is considered a modifiable risk factor to target for primary prevention.

**Definition of Metabolically Healthy Obesity:**

Mounting evidence suggests that not all obese individuals are contributing to the public health crisis referred to as the obesity epidemic. That is, although obesity remains an important risk factor for the development of metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease, it has become apparent over the last ten to fifteen years that subsets of obese individuals seem to be protected against the development of
these subsequent complications (13). This theory is based on a series of observational studies (primarily cross-sectional) that have shown a significant variation in diabetes and cardiovascular disease markers in individuals with similar body mass index (BMI) and waist circumference (WC) (14-15); most notably some individuals with high BMI and WC do not develop diabetes and cardiovascular disease. Further, it is these observations that have led to the characterization of two distinct (obese) body size phenotypes.

Obese individuals can be characterized with one of two different phenotypes: metabolically abnormal obese (MAO) versus metabolically healthy obese (MHO) (13). The MAO phenotype is typically defined as a BMI ≥ 30 kg/m² and having ≥ 2 cardiometabolic abnormalities, whereas the MHO phenotype is defined as a BMI ≥ 30 kg/m² and having 0 or 1 cardiometabolic abnormalities (16). This most commonly used body size phenotype definition is partially based on the National Cholesterol Education Program’s Adult Treatment Panel III (ATP-III) report for metabolic syndrome that has been modified to include subclinical inflammation and insulin resistance. Specifically, six possible metabolic abnormalities are used to distinguish between the body size phenotypes including: (i) elevated blood pressure, defined by systolic/diastolic blood pressure ≥ 130/85 mmHg or the documented use of antihypertensive medications; (ii) elevated triglycerides defined by a fasting triglyceride level ≥ 150 mg/dL; (iii) decreased HDL-C levels defined by gender-specific criteria (i.e., HDL-C < 40 mg/dL in men and < 50 mg/dL in women) or the documented use of a lipid-lowering medication; (iv) elevated glucose levels defined by a fasting glucose level ≥ 100 mg/dL, or the documented use of an antidiabetic medication; (v) insulin resistance, defined by homeostasis model assessment (HOMA-IR) > 5.13; and (vi) subclinical inflammation,
defined by high sensitivity c-reactive protein (hs-CRP) levels $\geq$ 3 mg/L (14). In general, the metabolically healthy individual is characterized by BMI $\geq$ 30 kg/m$^2$ in the absence of pre-diabetes (i.e., impaired fasting glucose or impaired glucose tolerance) or type 2 diabetes, dyslipidemia, and hypertension (17). More specifically, these individuals are characterized by having favorable metabolic profiles including being normotensive, having high insulin sensitivity, low triglyceride levels, high HDL-C levels, low levels of subclinical inflammation (measured by hs-CRP) despite high overall total body mass relative to height (13). Of note, these same body size definitions can be applied to individuals who are overweight or normal weight based on BMI criteria.

Although the body size phenotype definition as described above is the most commonly used criteria, it has yet to be standardized. This has resulted in several definitions currently in use to describe metabolic health with different inclusion criteria and/or cut-offs to make a distinction between metabolically healthy from metabolically abnormal (16). For example, one definition in use defines metabolic health solely on insulin resistance disregarding other cardiometabolic risk factors (18). Alternatively, other metabolic health definitions may not include insulin resistance in their definition, and/or blood pressure, lipids, or fasting glucose (16). Although there is not yet a uniform definition of these body size phenotypes, Wildman et al. performed sensitivity analyses using definitions with more stringent (i.e., metabolically healthy = 0 cardiometabolic abnormalities; metabolically abnormal = $\geq$1 cardiometabolic abnormalities) and less stringent criteria (i.e., using strictly Adult Treatment Panel III criteria for metabolic syndrome, with metabolically healthy classified as $\leq$ 2 cardiometabolic abnormalities and metabolically abnormal as $\geq$ 3 cardiometabolic abnormalities) (19). Wildman et al. found
the greatest correlation for the definition using the expanded version of the ATP-III criteria for metabolic syndrome, as the prevalence of normal weight individuals with cardiometabolic clustering (i.e., $\geq 2$ cardiometabolic abnormalities) was lower, whereas the prevalence of overweight and obese individuals without cardiometabolic clustering was higher (19).

**Prevalence Studies:**

Despite the lack of a standardized definition of metabolic health, several studies have tried to identify, characterize, and estimate the prevalence of metabolically healthy obesity (8, 16, 18-24). Not surprising, however, the results have been inconsistent, as the prevalence of MHO depends on the definition being used by the study authors. In a study of 314 German participants the prevalence of MHO was measured to be approximately twenty-five percent (25%) when using only insulin resistance to discriminate between MHO and MAO (8). A cross-sectional study of 5440 participants of the NHANES 1999-2004 conducted to not only determine the prevalence of these body size phenotypes but also to examine the demographic and behavioral characteristics associated with being MHO, utilized the expanded version of the ATP-III criteria for metabolic syndrome (i.e., including HOMA-IR and hs-CRP criteria) as their definition of metabolic health (18). In this study, among US adults 20 years and older, 31.7% (approximately 19.5 million adults) of obese adults were metabolically healthy and 51.3% (approximately 35.9 million adults) of overweight adults were metabolically healthy (18). Further, the independent variables associated with being metabolically healthy in this sample population were younger age, non-Hispanic black race/ethnicity, higher physical activity levels, and smaller waist circumference despite similar BMI (18). In a separate study, a
Swiss population-based sample of 2,803 women and 2,557 men aged 35-75 years was examined to estimate the prevalence of metabolic health among obese and non-obese subjects defined by using six different definitions of metabolic health and by using BMI, abdominal obesity (defined by waist circumference $>102$ cm for men and $>88$ cm for women), and body fat percentage derived from bioelectrical impedance analysis to define obesity (16). Among the obese participants ($\text{BMI} \geq 30$ kg/m$^2$), the prevalence of MHO ranged from 3.3 and to 32.1% in men and between 11.4 and 43.3% in women depending on which of the six definitions of metabolic health was used (16). Higher MHO estimates were obtained using abdominal obesity (5.7 to 36.7% in men and between 12.1 and 57.5% in women) and body fat percentage (6.4 to 43.1% in men and between 12.0 and 55.5% in women) to define obesity (16). In a smaller Korean study of 186 male obese subjects the prevalence of MHO again varied based on the definition of metabolic health (20). For instance, MHO ranged from 24.2% when using HOMA-IR as the defining feature of metabolic health, to 59.7% when using the expanded version of the ATP-III criteria, and to 70.4% when using the strict definition of the ATP-III (20). A larger study examined MHO prevalence in obese and non-obese men and women from a population of 2,047 middle-aged Irish men and women using different definitions (21). Again, the prevalence of MHO varied considerably between definitions (2.2% to 11.9%), was higher among females, and increased with age (21). Alternatively, in a recent study that combined data from ten different cohorts in seven European countries (Estonia, Finland, Germany, Italy, Netherlands, Norway, and United Kingdom), the prevalence of MHO was determined using a strict definition to define metabolic health as having no cardiometabolic risk factors (per the 2001 ATP-III criteria for metabolic syndrome) and
no previous diagnosis of cardiovascular disease (22). Despite using unified criteria to classify the MHO phenotype, the authors observed considerable variability in the prevalence of MHO across these seven countries. For instance, the prevalence of MHO in men ranged from 42.7% in the Italian population study to 78.2% in the Finnish population study, and for women 24.0% in the Italians and 64.8% in the Finnish (22). This study illustrates that the discrepancy noted for the prevalence of MHO is not only due to the inconsistent definition of metabolic health being used between studies but may also be a reflection of ethnic, genetic, and/or epigenetic differences. However, if one attempts to make a composite of all prevalence studies, it appears that on average up to 30% of obese people may be metabolically healthy (13).

**Prevalence Studies Based on Ethnicity:**

In addition to the limitations in this field of research as mentioned above, most prevalence studies examining MHO have been conducted in predominantly Northern and Southern European, Caucasian populations (e.g., Caucasians either from or residing in Finland, Norway, Sweden, Germany, Ireland, United Kingdom, Canada, Italy, Spain). In fact, only five studies to date have examined the prevalence of MHO in US minority populations representative of the general population. The first study mentioned earlier by Wildman et al. examined NHANES data of 5440 US adults including women, non-Hispanic blacks and Mexican Americans and found that among overweight or obese individuals, non-Hispanic blacks were 18% more likely to be metabolically healthy compared with non-Hispanic whites (19). The second study by our own research team measured the prevalence of MHO in Hispanic participants (N=1054) included in the IRAS Family Study (23). In this study, seventy percent of the Hispanic cohort was found
to be overweight (32%) or obese (38%). Forty-one percent (n=138) of overweight participants and 19% (n=74) of obese participants met criteria for MH (metabolically healthy) (23). A third study using 822 unrelated African American participants from the Howard University Family Study showed 28% MHO prevalence (24). Finally, two other subsequent studies looking at African American populations showed similar MHO prevalence averaging between 28.5% to 33% (25-26). Further, there is one study to date that has examined the prevalence of MHO in Black South African women. Using HOMA-IR as their definition for MHO, the study authors described that 38% of 122 obese black SA women were insulin sensitive (27). In addition, there have been a few recently published studies that have also examined Asian populations finding strikingly different prevalence rates. In a recent study by Zhang et al, only 3.0% (0.8% of men and 4.5% of women) of 2530 Mongolian Chinese adults were found to be MHO (28). In contrast, using a similar definition of metabolic health as in the Chinese study, a cross-sectional analysis of 16,190 Korean adults, using data from the Fourth Korean National Health and Nutrition Examination Survey, demonstrated the prevalence of MHO in this population to be 47.7% (29). In yet another study looking at 1547 Taiwanese adult men and women (using the strict definition of ATP-III), 28.5% prevalence of MHO was seen (30). Finally, a study by Geetha et al. studied the prevalence of MHO in South Asian Indians. Using data of 2350 participants from the Chennai Urban Rural Epidemiology Study, the authors found 13.3% prevalence of MHO (31). These differences between Caucasian, US minority, and Asian populations may be partly explained by regional differences in the definition of obesity (i.e., different cut-offs for obesity for example in Asian populations compared to Caucasians) in addition to ethnic or epigenetic
differences. Important to this research will be to continue to examine the prevalence of MHO in different ethnic groups from various geographic regions.

**Metabolic Health and Body Composition:**

Following the recognition of the existence of body size phenotypes based on these initial prevalence studies, further research has been undertaken to describe how body composition and body fat distribution may be associated with a favorable metabolic profile in MHO individuals. Although the metabolic profiles of MHO are almost indistinguishable from those of normal weight individuals, the role of fat location in regards to central adiposity in the MHO phenotype is still poorly understood (14). In the study of 314 German individuals by Stefan et al., total body, visceral, and subcutaneous fat with magnetic resonance (MR) tomography was measured (8). Total body and visceral fat were overall higher in the obese groups, compared with the normal-weight group (p<0.05), however, no differences were observed between the MHO and MAO groups (8). Fat in the liver and skeletal muscle was also measured with proton MR spectroscopy. Interestingly, despite the lack of significant difference in visceral fat, ectopic fat in skeletal muscle and liver were much lower in the MHO participants compared to the MAO participants (p<0.05) (8). Despite a lack of significant difference in visceral adiposity between body size phenotypes in this study, multiple subsequent studies have shown that the MHO phenotype is consistently characterized by significantly lower visceral adipose tissue levels despite similar subcutaneous adipose tissue levels when compared to the MAO phenotype (32-36). For instance, in a study of 43 obese, sedentary postmenopausal women, subjects were classified as MHO or MAO and body composition (fat mass and lean body mass) and body fat distribution
(abdominal visceral and subcutaneous adipose tissue areas, midthigh subcutaneous adipose tissue and muscle attenuation) were measured (32). Despite comparable total body fatness between groups (45.2 +/- 5.3% vs. 44.8 +/- 6.6%; P >0.05), MHO individuals had 49% less visceral adipose tissue than MAO subjects (141 +/- 53 vs. 211 +/- 85 cm (2); P: < 0.01). No difference was noted between groups for abdominal subcutaneous adipose tissue (453 +/- 126 vs. 442 +/- 144 cm (2); P: = NS), total fat mass (38.1 +/- 10.6 vs. 40.0 +/- 11.8 kg), and muscle attenuation (42.2 +/- 2.6 vs. 43.6 +/- 4.8 Hounsfield units) (32). In a study of 113 obese sedentary postmenopausal women, body composition was measured by dual-energy x-ray absorptiometry and body fat distribution was measured by computed tomography scan (33). When comparing MHO to MAO groups, no differences were observed for subcutaneous adipose tissue. However, MHO individuals had significantly less visceral adipose tissue than MAO individuals (p<0.05) (33). In a cross-sectional pilot study of 39 obese women (BMI: 31-67 kg/m²), individuals were defined as MHO using the strict definition of ATP-III (34). Women with the MHO phenotype were found to have lower intra-abdominal fat volume despite no difference in abdominal subcutaneous fat volume (34). In a study of 22 adult women using insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp technique as the definition of metabolic health, the MHO participants displayed significantly lower levels of visceral fat (p<0.05) despite comparable total body fatness between groups (35). In a recent study of 395 obese adults from the Pennington Center Longitudinal Study, whole-body bone mineral density and content, percent body fat, fat mass, lean mass and trunk adipose tissue mass was measured using dual-energy x-ray absorptiometry and visceral, subcutaneous, and total abdominal adipose tissue was measured using computed
tomography (36). They examined gender differences and found that in men, MHO had lower fat mass, trunk adipose tissue, visceral, subcutaneous, and total abdominal adipose tissue compared to MAO; whereas women MHO had lower fat mass, lean mass, trunk adipose tissue, visceral, and total abdominal adipose tissue when compared to MAO women (36). Using metabolic syndrome as their definition of metabolic health, Koster et al. examined data from 729 obese men and women from the Health ABC Study and found that the MHO participants had lower abdominal visceral fat and greater thigh subcutaneous fat when compared to the MAO using computed tomography (37). Thus, it appears that regardless of the radiologic mechanism used to measure adiposity or the definition used to define metabolic health, the MHO phenotype appears to be consistently associated with lower visceral and ectopic adipose tissue levels when compared to the MAO counterpart. Further, there is some indication that at the cellular level there are differences in the size and numerical density of adipocytes, as well as volume density of blood vessels in subcutaneous and visceral adipose tissue between body size phenotypes (38).

**Metabolic Health and Ectopic Fat Distribution:**

As mentioned above, coincident to differences in body composition and body fat distribution has been the finding that MHO individuals have lower levels of ectopic liver fat. Given this observation, it has been examined whether this may translate into a lower risk of nonalcoholic fatty liver disease (NAFLD). In a study of 82 obese Italian females, MAO participants had significantly greater levels of hepatic steatosis (p =0.005) as compared to MHO participants (39). In another study of 104 obese, sedentary, postmenopausal women who were classified based on body size phenotype, MHO
participants had significantly lower concentrations of hepatic enzymes (aspartate aminotransferase [AST], Alanine aminotransferase [ALT], and gamma-glutamyltransferase [GGT]) as well as a lower fatty liver index compared with MHO participants (p<0.05) (40). In a study of 16,190 adults from the Fourth Korean National Health and Nutrition Examination Study, a cross sectional analysis revealed that their MHO individuals had significantly lower concentrations of AST and ALT compared to their MAO subjects (41). On the other hand, in a recent study of a similar population (14,384 South Koreans) from an occupational cohort study, the authors found an increase in the odds of fatty liver using liver ultrasound in the obese participants regardless of metabolic health (MHO OR=3.63 [95% CI 3.06, 4.31], p<0.001 and MAO OR=5.89 [95% CI 5.18, 6.70], p<0.001) (42). Indeed there are several experts in the field that suggest that ectopic liver fat may be more important than visceral fat in the determination of healthy obesity (8, 43-44). However, given the scarcity of data and lack of prospective studies, it has yet to be elucidated whether an increase in liver fat is an independent predictor of metabolic health in obese individuals apart from the other observed body distribution differences. Further, these findings have not been replicated in all minority populations.

**Metabolic Health and Inflammatory Markers:**

Because subclinical, chronic inflammation is a recognized, potential mechanism linking obesity and cardiometabolic disease, inflammatory status between body size phenotypes has been studied. That is, in addition to the traditional cardiometabolic risk factors used to define metabolic health, biomarkers customarily associated with obesity and insulin resistance have been examined as possible predictors of MHO versus MAO.
In one of the earliest studies investigating the inflammatory state in obesity in 88 obese, sedentary postmenopausal women, the authors found that the MHO phenotype was associated with a “favorable” inflammation profile with significantly lower levels of hs-CRP and alpha-1 antitrypsin levels in the MHO subjects compared to the MAO subjects (35). In a more recent cross-sectional study of 2047 adult men and women, the MHO participants were found to have significantly lower levels of inflammatory markers including complement component 3, hs-CRP, tumor necrosis factor alpha, interleukin-6, plasminogen activator inhibitor-1, and higher levels of adiponectin compared to the MAO participants (45). In another study of 160 Italian subjects, obese participants were characterized as being MHO versus MAO and several biochemical markers including several serum adipokines and gastrointestinal hormones were measured (46). The MHO group was found to have significantly higher levels of leptin and higher levels of adiponectin compared to the MAO group (p<0.05) (46). In a similar cross-sectional study of 55 obese postmenopausal women, MHO individuals had significantly higher adiponectin levels than the MAO individuals (p<0.05) (47). In a larger study of 1,889 postmenopausal women from the Women’s Health Initiative Observational Study nested case-control stroke study, significantly higher levels of acute-phase reactants (i.e. hs-CRP) as well as other inflammatory marker categories (i.e. adhesion molecules and coagulation products) were independently associated with the MAO group compared to the MHO group (48). In a study of 60 morbidly obese German men and women circulating adipokines were compared between the MHO versus MAO participants (49). The MHO participants had significantly lower markers of inflammation including CRP and IL-6. Further, they had less insulin resistance including lower RBP-4 (retinol binding
protein-4) levels and significantly higher adiponectin levels (49). In the study of 2530 Mongolian Chinese adults mentioned earlier, the MHO phenotype was associated with lower levels of hs-CRP and soluble intercellular adhesion molecule-1 compared to MAO and comparable to the normal weight healthy phenotype (28). At the cellular level, in a cross-sectional study of 29 morbidly obese patients undergoing bariatric surgery, the MHO phenotype (12 of 29 subjects) was associated with lower levels of preadipocyte factor-1 in both the subjects’ subcutaneous as well as omental adipose tissue (50). This is of interest as preadipocyte factor-1 has been shown to inhibit differentiation in adipocyte size and inflammatory profile. In fact, the MHO phenotype in this study was also associated with lower number of macrophages, tumor-necrosis factor alpha, monocyte chemotactic protein-1, granulocyte colony-stimulating factor, and higher levels of adiponectin (50). Another study examining the fatty acid profile as a correlate for inflammation found that the levels of fatty acids (specifically saturated fatty acids), hs-CRP, interleukin-6, and platelet derived growth factor-beta in the MHO resembled that of normal weight healthy individuals (51).

However, there have been conflicting findings in studying inflammation as a potential underlying mechanism for defining metabolic health. In a study of 200 obese subjects, although MHO subjects had lower hs-CRP levels than the MAO subjects, the absolute differences were actually very small and not clinically significant (52). Despite finding lower levels of inflammation in the MHO participants compared to the MAO, Wildman’s et al study using data from the Women’s Health Initiative still showed markedly abnormal inflammatory markers in the MHO groups compared to non-obese groups (48). These inconsistent findings may be a result of ethnic, gender, and age
differences between studies. In addition, the biomarkers being studied as surrogates for subclinical inflammation are quite diverse among these studies making it difficult to interpret the data as a whole. Further, while obesity is characterized by chronic inflammation associated with neutrophil and macrophage infiltration into adipose tissue, the cellular and molecular mechanisms underlying this process remain largely unknown (53). As with the prevalence data, these inconsistencies may also be the result of different criteria being used to define metabolic health between studies. A recent comparative study investigating the circulation of pro-inflammatory cytokines (hs-CRP, interlekin-6, interleukin-1-beta, and tumor necrosis factor-alpha) according to different definitions of MHO concluded that the association between inflammatory markers and body size phenotype are definition dependent (54). That is, some definitions of MHO led to significantly lower levels of interleukin-6, tumor necrosis factor-alpha, and hs-CRP when compared to MAO subjects, but when other definitions of metabolic health were used the differences became non-significant (54). Although intriguing, current evidence supporting these biomarkers as independent predictors of metabolic health is limited and inconclusive at present. As with body fat distribution and body composition, these studies have not been replicated in large minority cohorts representative of the general population. Further, there is an emerging interest in the study of the underlying epigenetic and genetic factors (e.g., Brd2 gene disruption; altered gene expression of peroxisome proliferator-activated receptor) as a potential link between inflammatory status and cardiometabolic risk (55-57).

**Metabolic Health and Subclinical Cardiovascular Disease Risk:**
Although current evidence points to a unique subset of obese individuals who have few cardiometabolic risk factors (i.e. possibly a 10-year CVD risk comparable to healthy normal weight individuals), few longitudinal studies have been specifically designed at present to describe whether MHO individuals have decreased cardiovascular event outcomes compared to MAO individuals. In other words, does the MHO person having a favorable metabolic profile remain like this permanently or is this just part of the natural course of obesity with the evolution of the MHO into the MAO over time? Initial studies have looked at the association between these body size phenotypes and subclinical CVD using surrogate endpoints with conflicting results. In a cross-sectional analysis of 475 postmenopausal women, common carotid artery intima media thickness (CCA-IMT), aortic pulse wave velocity (aPWV), and coronary (CAC) and aortic calcification (AC) was compared among normal weight, MHO, and MAO groups (58). While these surrogate markers of subclinical CVD were significantly lower in the MHO versus the MAO groups (p<0.001), the MHO group still had a significantly higher burden of subclinical CVD compared to the normal weight group (p<0.001) (58). On the other hand, in a separate study of 2540 healthy participants, CCA-IMT and aPWV values were not significantly different between the MHO individuals and normal weight individuals (59). In a subsequent study examining the association between heart rate variability and body size phenotypes in 47 obese, postmenopausal women, MHO individuals had a significantly lower resting heart rate (p<0.05) but higher heart rate variability (p<0.05) when compared to their MAO counterparts (60). In a six year study of 550 individuals without diabetes or macrovascular complications at baseline, the MHO phenotype (again defined based on insulin resistance) was associated with a decrease in the risk of heart
failure examining left ventricular functional capacity, myocardial structure, and performance with echocardiogram when compared to the normal weight unhealthy phenotype (61). In the study by Sung et al mentioned earlier, 14,384 South Koreans from an occupational cohort underwent cardiac computed tomography estimation of coronary artery calcification (42). In this case, the MHO participants did not differ from the normal weight on CAC score (OR=0.93, [95% CI 0.67, 1.31], p=0.68) while CAC score was increased in MAO compared to normal weight (OR=1.64, [95% CI 1.36, 1.98], p<0.001) with the conclusion that in this population the MHO phenotype was associated with a decreased risk of pre-clinical cardiovascular disease relative to MAO (42).

**Metabolic Health and Incident Cardiovascular Disease Risk and Mortality:**

Despite the lack of well-designed prospective studies to examine whether MHO truly exists as a ‘benign’ condition, there have been a few studies using existing data from several observational studies as well as meta-analyses/systematic reviews published within the past year examining incident cardiovascular disease risk and/or mortality. Looking at mortality, in a study reviewing fifteen year all-cause mortality data obtained through the Regional Health Registry for 2,011 Italian participants of the Cremona Study, MHO participants (defined based on the absence of insulin resistance) did not show an increase in all-cause, cancer, or cardiovascular disease mortality risks when compared to normal weight healthy individuals (62). A similar seven year follow-up observational study of 22,203 English and Scottish men and women without known history of cardiovascular disease at baseline demonstrated that the MHO phenotype (defined using the expanded and modified ATP III criteria) were not at increased risk for cardiovascular disease (i.e., physician diagnosed stroke, angina, or myocardial infarction) and all-cause
mortality when compared to the normal weight healthy phenotype (63). To further investigate whether the MHO phenotype persists over time, the Bogalusa Heart Study (a study of 1,098 subjects who participated both as children between 5-17 years and adults between 24-43 years) was examined and it was found that the MHO phenotype persisted from childhood to adulthood (64). That is, in the MHO defined group (described in 4.2% of the study’s children); adults maintained a cardiometabolic profile consistent with the MHO phenotype despite an increase in the level of obesity from childhood to adulthood (64). However, in a study of 2352 adult participants (aged 40-69 at baseline) from the Korean Genome Epidemiology Study, participants with normal blood pressure were subdivided into body size phenotypes and the incidence of hypertension was identified by biennial examinations during an eight year follow-up period (65). In this case, an increased risk of developing hypertension despite adjustment for potential confounders was observed similarly in the MHO, MH overweight, and MAO compared to the normal weight healthy participants (65). Using data of 4,056 adults from the North West Adelaide Health Studies, the MHO phenotype was defined by having two or less International Diabetes Federation metabolic syndrome criteria (66). In this study, only two-thirds (67%) of the MHO participants remained MHO over a period of 10 years follow-up. Interestingly, the participants who transitioned from MHO to MAO were found to have higher waist circumferences compared to the ones that remained persistently “healthy” and were more likely to develop incident diabetes (OR 2.09 [95% CI 0.87, 5.03]) but not cardiovascular disease/stroke (OR 1.16 [95% CI 0.58, 2.29]) when compared to normal weight healthy participants (66). In a recently published study of 3700 participants from the San Antonio Heart Study, the MHO phenotype (defined using
the expanded ATP-III criteria) was associated with an increased risk in both incident diabetes (OR=3.9 [95% CI 2.0, 7.4], p<0.05) and cardiovascular disease (OR=3.9 [95% CI 1.9, 7.8], p<0.05) compared to the normal weight healthy phenotype after 7.4 years of follow-up (67). Incident DM was defined according to the plasma glucose cut-points of the 2003 American Diabetes Association (fasting glucose ≥126 mg/dL and/or 2-h glucose ≥200 mg/dL), and incident CVD was defined as self-reported myocardial infarction, stroke, or coronary revascularization procedure at follow-up or any mention of cardiovascular death on a death certificate (67). However, the MHO phenotype did convey a decreased risk in both incident diabetes (9.9 % versus 30.5 %) and cardiovascular disease (4.7% versus 6.3%) when compared to the MAO phenotype, respectively. These results were noted to be consistent across gender and ethnic categories (Caucasian and Mexican Americans) with the conclusion being that MHO is not a ‘benign’ condition (67). However, in a study of 61,299 adult Norwegian men and women free from cardiovascular disease at baseline (the HUNT Study), the MHO phenotype was not found to be associated with an increased risk of cardiovascular disease (using acute myocardial infarction as endpoint) at 12 years of follow-up [adjusted hazard ratio of 1.1 comparable to the normal weight healthy phenotype] (68). In contrast, they did find a small increase in the risk for the development of heart failure regardless of metabolic status in the obese participants versus normal weight subjects (68). Similarly, in an analysis of 4373 adult men and women from the NHANES-III study, the MHO phenotype was not at increased risk for all-cause mortality when compared to the normal weight healthy phenotype (69). Conversely, in an analysis of 22,654 adults from the EPIC-MORGEN study, the MHO phenotype (defined by ATP-III criteria for metabolic
syndrome) was noted to have an all-cause mortality risk approximately 40% higher (hazard ratio=1.43 [95% CI 1.00, 2.04]) when compared to the normal weight healthy phenotype (70). In an attempt to help bring all this information together, a meta-analysis of fourteen studies with a total of 299,059 participants, 12,215 cases of cardiovascular death, and 7071 cases of all-cause death was conducted (71). Compared with normal weight healthy individuals, the MH overweight (RR 1.47 [95% CI 1.37, 1.58], p<0.05) and MHO (RR 2.00 [95% CI 1.79, 2.24], p<0.05) individuals showed increased risk for cardiovascular events, especially in studies that had follow-up greater than fifteen years (71). Despite showing an increase in cardiovascular events, the study authors did not find an increased risk for all-cause mortality between the metabolically healthy phenotypes (71). MAO/MAOW individuals were at the highest risk for CVD and mortality when compared to the MHO/MHOW (71). In a similar meta-analysis of eight studies with a total of 61,386 participants and 3988 events, MHO individuals had increased risk (RR 1.24 [95% CI 1.02, 1.55], p<0.05) for cardiovascular events and all-cause mortality when compared to normal weight healthy individuals only in studies that had ten or more years of follow-up (4/8 studies) (72). That is, in the studies with less than ten years follow-up, MHO had a similar risk for all-cause mortality and cardiovascular events compared to normal weight healthy individuals (72). As demonstrated in prior studies, MAO had an increased risk for all-cause mortality and cardiovascular events compared to MHO and normal weight healthy individuals (72).

In light of these conflicting reports, it remains very difficult to interpret the above findings. Furthermore, there are many limitations of these studies worth mentioning, especially that of the meta-analyses. First, as the inconsistencies in the prevalence of
MHO suggest, the lack of a standard definition to identify these body size phenotypes may partly account for between-study heterogeneity. It is difficult to determine whether the contradictory findings are partly a result of methodological issues (i.e., variable follow-up duration, small sample size, non-standardized comparison groups), so this indicates the need for the creation of a standardized definition (73). Second, it is difficult to compare studies that are from different regions of the world as for example the BMI cut-off for obesity is different in Asia (BMI ≥ 27.5 kg/m²) versus Europe (BMI ≥ 30 kg/m²) due to ethnic/racial differences noted in proportion of body fat and subsequent risk of cardiometabolic disease despite similar BMI. Third, many of the above studies did not differentiate metabolic health in their normal weight comparison groups, which may than lead to crossover bias in an analysis, making true differences between groups more difficult to detect (73). Fourth, the majority of studies designed to examine subclinical disease and MHO were cross-sectional in design and does not allow for the evaluation of progression of disease. It is possible that the MHO phenotype is not a static one and potentially an individual may transition between MHO and MAO over time. A recent study by Soriguer et al., reported that a substantial proportion of MHO subjects from the Pizarra Study were no longer metabolically healthy after a six year follow-up, with 37% of the MHO reclassified as MAO (74). Fifth, the handful of studies that examined cardiovascular disease outcomes and all-cause mortality were not specifically designed to evaluate body size phenotypes (especially the ones included in the two meta-analyses mentioned). Prospective studies will need to be performed to determine whether MHO is truly a ‘benign’ condition regarding future cardiovascular risk. Further, these studies will
need to include socioeconomically diverse, multi-ethnic populations to be able to make generalizable conclusions.

**Metabolic Health and Weight Loss Interventions:**

Despite increasing recognition in the scientific community that a significant proportion of the obese population may be metabolically healthy, current obesity treatment guidelines do not separate MAO from MHO phenotypes and recommend treatment for all obese individuals, with the first-line approach being lifestyle interventions (75). As chronic disease adds to national health care costs, so do weight loss efforts. Weight loss is estimated to be a billion dollar-a-year industry in the US with the cost of losing thirty pounds averaging at $1200 (this cost does not include surgical weight loss interventions) (12). In order to maximize public health efforts regarding the obesity epidemic, the question arises as to whether all individuals with a BMI \( \geq 30 \text{ kg/m}^2 \) should be advised to lose weight. Rather, should the focus be specifically on MAO individuals, which would not only save the healthcare system valuable time and money but likely have greater impact on a high-risk population? In light of all the contradictory data within this field of research, these questions remain unanswered at the present time. However, a few weight loss intervention trials have been conducted to compare the change and impact on cardiometabolic risk factors in the metabolically healthy versus metabolically abnormal population. In a study by Karelis et al, a small sample of obese post-menopausal women was placed on a six month energy-restricted diet to assess change in insulin sensitivity following weight loss (75). While the MAO participants exhibited significant improvement in insulin sensitivity, the MHO participants had significantly worsened insulin sensitivity following the six month diet (75). In another
study of 129 Korean women who participated in a 12 week weight loss intervention study involving a 300 kcal/day reduction in diet, the MAO participants had significant reductions in blood lipids and inflammatory markers (76). However, there was no significant effect of weight loss on lipid profiles and inflammation in the MHO participants (76). Examining the effects of physical activity, another study took 267 sedentary, post-menopausal MH overweight and MHO women and had them undergo a six month exercise intervention program (3-4 times per week at a targeted heart rate corresponding to 50% of maximal oxygen consumption) while measuring lipid profiles, inflammatory markers, and other cardiometabolic risk factors (77). Surprisingly, despite a significant improvement in their overall cardio-respiratory fitness and weight loss (as measured by kg lost as well as mean reduction in waist circumference), there were no significant improvements in their cardiometabolic risk profile (including blood pressure, lipid profile, hs-CRP, interleukin-6, tumor necrosis factor alpha, and adiponectin levels) (77). In contrast, an intervention trial of 63 MHO and 43 MAO adults (3-6 months of exercise or diet weight loss intervention), demonstrated not only an improvement in fitness and reduction in size (measured by body weight, waist circumference, and total abdominal and visceral adipose tissue levels), but also an improvement in cardiometabolic risk factors especially that of insulin sensitivity in the MHO participants (78). A separate study of 53 MAO and 25 MHO premenopausal women undergoing a 12-week energy restricted diet intervention showed not only a reduction in body weight, waist circumference, and total fat mass in both groups, but also a reduction in cardiometabolic risk factors (including a reduction in fasting insulin, insulin resistance, hepatic enzymes, fatty liver index, and leptin levels, p <0.001) in both groups equally
Conversely, a study of 262 non-diabetic individuals undergoing a 9 month lifestyle intervention weight loss program did not show an improvement in cardiometabolic risk (defined by insulin sensitivity) in the MHO group despite a reduction in body weight and visceral adipose tissue (80). Once again, it is difficult to make any scientifically valid conclusions from these studies in regards to causality. These were all non-randomized trials with various degrees of biased outcomes as some studies did not have pre-specified aims or objectives, some had no comparison group, all were relatively under-powered due to small sample size, and all examined fairly homogeneous populations (i.e. mainly Northern European, Caucasians). Based on the current review of the literature, it appears that no one has conducted a large scale randomized clinical trial to evaluate the impact of weight loss on clinical outcomes comparing metabolically healthy versus metabolically abnormal individuals, so questions remain largely unanswered at this time regarding need for secondary prevention of obesity in the metabolically healthy obese population.

**Metabolic Health and Lifestyle Behaviors:**

Finally, important to the understanding of what determines an individual’s metabolic health status in the context of obesity is the association that dietary and lifestyle behaviors have in the perspective of being labeled MHO versus MAO. It is apparent that both dietary and lifestyle factors are important to the obesity epidemic, but whether these are determinants of MHO are unclear given the inadequate data available at present. Several observational studies have attempted to address this question, but, once again, the results have been inconsistent. In the early study by Brochu et al. mentioned earlier, obese participants (post-menopausal women) displayed the same level of physical activity (measured by doubly labeled water in conjunction with indirect
calorimetry to report physical activity energy expenditure) regardless of their metabolic health status (32). However, in the prevalence study of black South African women, the MHO participants were significantly more physically active (defined by self-reported leisure and vigorous physical activity energy expenditure using the Global Physical Activity Questionnaire) than the MAO participants (27). Further, in the NHANES study by Wildman et al. as well as in the Swiss study by Velho et al., higher physical activity levels were independent correlates of being overweight or obese and having only 0 or 1 cardiometabolic abnormalities, respectively (16,19). In the cross-sectional pilot study of 39 obese women by Hayes et al., there were noted differences in levels of physical activity between body size phenotypes (70% MHO versus 25% MAO), but the authors noted that this only accounted partially for the differences in metabolic health seen among these obese participants (34). Another study examined differences in functional capacity (defined by walking distance and walking speed by the six minute walk test) between body size phenotypes (defined by the ATP-III criteria) in 86 obese premenopausal and postmenopausal women (81). Interestingly, although the levels of physical activity were not different between body size phenotypes, the MHO women performed significantly better on the six minute walk test (p<0.01) compared to the MAO women (81). Looking at dietary factors and physical activity, a recent study examined 775 obese American adults ages 40-59 years from the INTERMAP (International Population Study on Macro/Micronutrients and Blood Pressure) cohort and did not show any differences in diet composition or activity behaviors between body size phenotypes (82). A total of 83 nutrient variables, including total energy and macro/micronutrient intakes, as well as sixteen food groups and 42 food based subgroups including meat, fish,
dairy, eggs, fruits, vegetables, and grains were examined with no significant differences noted (82). In a study of 184 Chinese postmenopausal women, physical activity was assessed by self-reported questionnaire and cardio-respiratory fitness was assessed by maximal oxygen consumption (using a symptom limited maximal exercise test on a cycle ergometer) (83). Although there was a higher proportion of MHO women being more physically active and having higher cardio-respiratory fitness than MAO women, these differences were not statistically significant (83). Similarly, looking at a younger population of overweight and obese youth (13-18 years old) cardio-respiratory fitness was assessed (using a graded maximal cycle ergometer test to determine peak oxygen uptake) and there were no differences noted between body size phenotypes (84). In contrast, a study of 10 MHO and 10 MAO (age and weight matched women) found significant differences in cardio-respiratory fitness (17% higher maximal oxygen consumption and greater energy expenditure in the MHO group) between the two groups (85). On the flip side, a recent study examined whether sedentary behavior defined by television viewing time differed between body size phenotypes (86). In a nationally representative sample of 4931 English adults from the English Longitudinal Study of aging, there were no differences in leisure-time sedentary behavior between the obese phenotypes, although the MHO subjects’ television viewing times was 5.8 times higher (p<0.01) than normal weight healthy participants (86). Finally, a recent cross-sectional study of 1,008 Irish men and 1,039 Irish women investigated to what extent differences between MHO and MAO are explained by dietary composition, dietary quality, and food pyramid compliance using several definitions of metabolic health (87). The study’s authors found that total calorie intake, dietary macronutrient composition and dietary
quality were similar regardless of BMI (87). However, some differences were noted between number of daily servings of fruit and vegetables, diary, meats, fats, and high fat/sugar food and drinks between MHO and MAO subjects, depending on the definition used to denote metabolic health (87). This study also examined physical activity duration, intensity and compliance with Irish physical activity guidelines and did not find any significant differences between body size phenotypes (although MHO was associated with higher physical activity if insulin resistance was the only criteria used to define metabolic health) (87). Evidence supporting the role of diet and physical activity in MHO has been inconsistent to date, which may reflect the range of definitions across studies used to define metabolic health. Nevertheless, it is well recognized that good dietary habits and physical activity exerts many benefits including decreased risk of obesity, metabolic syndrome/type 2 diabetes, NAFLD, cardiovascular disease, cancer, arthritis, and all-cause mortality. Thus, it is important to continue examining what dietary factors and other lifestyle habits contribute to the MHO phenotype, which may then assist in developing targeted interventions for the metabolically abnormal phenotype.

In conclusion, primary and secondary prevention of obesity remains important in tackling the future of the current obesity epidemic and subsequent comorbid conditions that accompany obesity. However, for secondary prevention, identifying obese patients with this potential protective profile of metabolically healthy obesity could assist in the determination of which subset of the obese population needs to be only periodically observed versus which groups needs to have intensive and early therapeutic interventions. Our current understanding of these body size phenotypes and their determinants are quite limited and is confounded by contradicting results across studies in this field of research.
This has made it difficult to recommend individualized approaches to treat obesity based on metabolic health. Important to this field of research is the need to standardize the definition of metabolic health when comparing body size phenotypes in order to improve comparability between studies, generate more accurate prevalence estimates, and define the phenotype that has the most reduced risk of future clinical disease (73). Further, there is a need for well-designed longitudinal studies with socioeconomically, multi-ethnic populations that examine how timing and duration of obesity affect metabolic health, and whether MHO is a static or dynamic state throughout life and the aging process in regards to the risk of the development of cardiometabolic disease and all-cause mortality(88). Critical unanswered questions remain as to why and how the difference exists between MHO and MAO regarding body composition, body fat distribution, hepatic steatosis, and markers of inflammation and insulin resistance. Future research should continue to investigate what underlying molecular, cellular, genetic, and epigenetic factors play a role in the difference between these body size phenotypes and thereby increase risk of clinical disease. Nevertheless, from a clinical as well as public health standpoint, distinguishing between metabolically healthy and metabolically abnormal obesity could help identify which individual at the greatest cardiometabolic risk and could benefit the most from intensive weight loss specific interventions (89). Future research on body size phenotypes will assist in developing approaches on the detection, treatment, and prevention of disease tailored to the individual patient (89).

**Rationale for Chapter 2 and 3:**

As mentioned, the role of fat location in regards to central adiposity in the MHO phenotype is still poorly understood. Although there have been several studies that have
tried to identify, characterize, and estimate the prevalence of MHO, these studies have examined relatively homogeneous populations consisting of primarily Northern European Caucasian participants. Further, recent epidemiologic evidence suggests that several biochemical markers may distinguish the MHO from the MAO.

The goal of this study is to better understand the characteristics and risk factors associated with the MHO phenotype versus the MAO phenotype. In addition to the obese population, we would also like to examine the overweight population. Thus, we will apply the MH (metabolically healthy) and MA (metabolically abnormal) phenotypes separately to an obese as well as an overweight population as the definition of cardiometabolic health can be applied to individuals regardless of BMI. We will also compare our MH population to a normal weight healthy population defined by the same criteria of cardiometabolic health used to describe our obese and overweight population. To achieve this goal I will use data collected from IRAS-Family an extension of IRAS (Insulin Resistance Atherosclerosis Study), an epidemiologic cohort study of 1625 men and women designed to study the genetics of insulin resistance and visceral adiposity (90). The IRAS-Family cohort consists of families (N=1611) from two minority groups (African American and Hispanic) recruited from three clinical sites from the original IRAS study. I will examine both the Hispanic (Chapter 2) and African American (Chapter 3) cohorts. The major strengths and unique aspects of this study include a large sample of comprehensively phenotyped Hispanic Americans and African Americans with detailed measures of body composition by abdominal CT scans and a panel of and inflammatory markers (90).
Specific Aims & Hypotheses of Chapter 2 and 3:

I propose the following specific aims that will allow for testing of the following hypotheses:

1. To assess the prevalence of metabolically healthy obesity (MHO) and metabolically healthy overweight (MHOW) in the Hispanic and African-American cohort of IRAS-Family and to contrast the prevalence across gender and age.

**H1.** The MHO and MHOW phenotype will be more common in the Hispanic and African-American cohorts than in the predominately Caucasian cohorts of prior published epidemiologic studies. Healthy overweight/obesity will be more common in women than in men, and in younger than in older based on prior published prevalence studies.

2. To characterize the clinical and metabolic factors associated with MHO and MHOW including location of fat in the visceral, subcutaneous and liver depots using abdominal CT-derived measurements, levels of physical activity, and prevalence of NAFLD.

**H2a.** Fat stores in the MHO and MHOW phenotype of the IRAS Family Study participants will be similar in SAT (subcutaneous adipose tissue), lower in VAT (visceral adipose tissue), lower in ectopic fat tissue (i.e., liver), and especially lower VAT/SAT ratio when compared to the contrasting metabolically abnormal obese (MAO) and metabolically abnormal overweight (MAOW) phenotype.

**H2b.** Visceral and ectopic fat in MHO phenotype will not differ from the normal weight healthy phenotype.
H2c. Healthy overweight/obesity will be more common in individuals with higher levels of physical activity.

H2d. Prevalence of NAFLD will be lower in the MHO and MHOW phenotype compared to the MAO and MAOW phenotype.

3. To identify distinctive biochemical markers associated with MHO and MHOW versus MAO and MAOW in the African American cohort.

H3a. Biochemical marker concentrations including IL-6, adiponectin, retinol-binding protein-4 (RBP4), PAI-1, TNF-alpha receptors 1 and 2, will be significantly different in the two phenotypes. Specifically, the MHO and MHOW phenotype will have higher circulating levels of adiponectin and lower levels of IL-6, RBP4, PAI-1, and TNF compared to the MAO and MAOW.

H3b. Biochemical marker concentrations in MHO and MHOW phenotypes will not differ from the normal weight healthy phenotype.

These studies will provide critical information regarding the heterogeneity of cardiometabolic risk that has been observed among obese individuals.
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CHAPTER 2: A METABOLICALLY HEALTHY OBESE PHENOTYPE IN HISPANIC PARTICIPANTS IN THE IRAS FAMILY STUDY

INTRODUCTION

Recent evidence suggests that not all obese individuals contribute to the epidemic of type 2 diabetes, cardiovascular disease (CVD) and other co-morbid conditions (1-2). Although obesity remains an important risk factor in the development of these conditions, it has become apparent over the last decade that a subset of obese individuals seems to be protected against any subsequent complications. That is, significant variation exists in cardiometabolic risk factors among individuals of similar body mass index (BMI) and waist circumference (WC), while a person's CVD risk may depend jointly on body size and metabolic profile (3-7). Since BMI and WC are imprecise measures of obesity, investigators have identified a relatively new phenomenon of body size phenotypes (3-7). Specifically, obese individuals can be characterized as either being phenotypically metabolically abnormal (MA) or metabolically healthy (MH) (2). The MA obese phenotype is typically defined as BMI $\geq 30$ kg/m$^2$ and having $\geq 2$ cardiometabolic abnormalities, whereas the MH obese phenotype is defined as BMI $\geq 30$ kg/m$^2$ and having no or one cardiometabolic abnormality (3). The definition of cardiometabolic abnormalities is partially based on the National Cholesterol Education Program’s Adult Treatment Panel III report for metabolic syndrome (7). In general, the MH obese individual is characterized by BMI $\geq 30$ kg/m$^2$ in the absence of pre-diabetes/type 2 diabetes, dyslipidemia, and hypertension (6). More specifically, these individuals are characterized by having favorable metabolic profiles including being normotensive,
having high insulin sensitivity, low triglyceride levels, high HDL-C levels, and low levels of subclinical inflammation (2).

Several studies using primarily Caucasian/Northern European populations have tried to identify, characterize, and estimate the prevalence of MH obesity (1, 5, 6). In a recent study of 314 German participants the prevalence of MH obesity was measured to be approximately twenty-five percent (25%). (1). In a separate study, the prevalence of MH obesity ranged from 3.3 and to 32.1% in men and between 11.4 and 43.3% in women in a population based sample of 2803 Swiss women and 2557 Swiss men (5). In an attempt to examine a more heterogeneous population, a cross-sectional study of 5440 participants of the NHANES 1999-2004 was conducted to determine the prevalence of body size phenotypes in the US (7). On average, among US adults 20 years and older, 31.7% (approximately 19.5 million adults) of obese adults were metabolically healthy and 51.3% (approximately 35.9 million adults) of overweight adults were metabolically healthy (7). Similar prevalence patterns were noted for all races/ethnicities sampled including non-Hispanic whites, non-Hispanic blacks, and Mexican Americans (7). No other studies to date have examined the prevalence of MH obesity in minority populations representative of the general US population.

Following the recognition of the existence of body size phenotypes based on initial prevalence studies, further research has been undertaken to describe how body composition and/or body fat distribution may be related to metabolic health. Several studies have shown that the MH obese phenotype is characterized by significantly lower visceral adipose tissue (VAT) levels despite similar subcutaneous adipose tissue (SAT)
levels when compared to their MA obese counterparts (8-11). Coincident to differences in body composition and body fat distribution, it has been observed that MH obese individuals may have lower levels of ectopic liver fat and possibly a lower risk of NAFLD (1, 12-16). However, these studies have been performed in Caucasian/Northern European populations and have yet to be replicated in minority US populations.

Given the sparse data and poor understanding of the role that body size phenotypes have in minority US populations, the primary aims of this study are to assess the prevalence of different body size phenotypes in a Hispanic cohort and to characterize the clinical and metabolic factors associated with MH obesity or MH overweight including fat distribution in visceral, subcutaneous, and liver depots as measured by computed tomography (CT) across these different body size phenotypes. To accomplish these goals, we used data from the IRAS Family Study which contains detailed and extensive standardized phenotypes of all our measures of interest (17). To date, it is the only existing large multi-center trial that encompasses multi-generational pedigrees from individuals of Hispanic ethnicity in the United States.

METHODS AND PROCEDURES

Study Population

The IRAS Family Study is an epidemiologic cohort study of men and women specifically designed to investigate the genetics of insulin resistance and visceral adiposity (17). Multi-generational families of Hispanic background were enrolled using probands of the original IRAS study supplemented from the general population (18). Briefly, two sites recruited and examined family members of Hispanic ethnicity (San
Antonio, TX, and San Luis Valley, CO) from 1999-2002. Insulin resistance was measured using the intravenous glucose tolerance test, and abdominal obesity was measured using CT. Cardiometabolic disease risk factors were also assessed. Follow-up examinations were conducted in 2005-2006; liver density scans were done during this period. Eligibility criteria included (i) self-reported Hispanic ethnicity, (ii) 18 years of age or older, (iii) under 350 pounds (because of CT size limitations), and (iv) not having conditions that interfere with measurement of insulin resistance or any cardiometabolic (CM) risk factor (19). This current study included all Hispanic individuals for whom we had the data necessary to estimate the MH phenotype (n=1054). All studies were conducted using protocols approved by the Institutional Review Boards at each participating institution, and all participants provided informed consent.

Measurement of Baseline Characteristics

**Self-Reported Data:** Age, ethnicity, and physical activity were assessed by self-report. An estimate of usual frequency of vigorous leisure-time physical activity was reported, using a defined response set ranging from “rarely or never” to “5 or more times per week.” The use of antihypertensive, lipid-lowering and antidiabetic medications were also assessed by self-report.

**Anthropometric Measurements:** Height and weight were measured in duplicate to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight/height squared (kg/m²). WC was measured to nearest 0.1 cm at the level of the iliac crest at the end of normal respiration. The waist to hip ratio (WHR) was measured by dividing WC by hip circumference (measured at the level of the greater trochanters with legs closed). Seated
systolic/diastolic blood pressure was measured three times using a mercury manometer, after a five minute rest by centrally trained technicians using identical equipment. The mean of the last two measurements was used to calculate blood pressure.

CT-Derived Measurements: CT imaging for abdominal fat distribution was obtained under a standardized protocol and scans were read centrally (19). Participants received a scout view of the abdomen and pelvis followed by three axial images, all while participants had suspended respiration. Three 10-mm-thick images were obtained through the L2–L3, L4–L5 and T11–T12 disc spaces. If the T11–T12 image did not include liver and spleen, a fourth image was obtained using a scout to determine an appropriate intervertebral disc location. Liver and spleen density were quantified in Hounsfield units (HU) in the entire liver and spleen as visualized in the slice, excluding any visible vasculature. The image obtained at the L4–L5 disc space was used for determination of VAT area and SAT area; bowel fat was excluded. A bimodal histogram of adipose and muscle tissue was generated for each subject image and used to determine the adipose tissue area. Thus each subject is used as its own control to determine the range of adipose tissue HU. Adipose tissue was highlighted and computed with an average attenuation range of −138 to −40 HU for VAT and -154 to -42 HU for SAT. The ratio of liver to spleen density (LSR) was calculated; LSR <1.0 is an accepted cut point for a discrete outcome of NAFLD (20). Unadjusted liver density is also accepted as a continuous outcome (21). All CT images were coded for pathology and image quality; poor-quality studies were excluded from analysis (19). Percent total body fat and lean mass were obtained using dual-energy X-ray absorptiometry (DXA) scan. Liver density and DXA
studies were obtained only at follow-up examination five years following the baseline examination (i.e. no baseline measurements available).

*Laboratory Measurements:* Plasma triglyceride (TG) concentrations, high-density lipoprotein (HDL-C) cholesterol concentrations, alanine transaminase (ALT), aspartate transaminase (AST), and \( \gamma \)-glutamyl transpeptidase (GGT) levels were determined by enzymatic colorimetric assays using a Chemistry Analyzer Model ATAC 8000 (Elan Diagnostics, Smithfield, RI). Plasma glucose was measured using the glucose oxidase technique on an automated autoanalyzer (YSI, Yellow Springs, OH). Insulin was measured by radioimmunoassay (Linco Research, St Charles, MO). HOMA (Homeostasis model assessment) was used to evaluate insulin resistance using the formula: fasting plasma insulin level (microunits per milliliter) \( \times \) fasting plasma glucose level (millimoles per liter)/22.5. Plasma adiponectin concentration was quantified using radioimmunoassay (Linco Research, St Charles, MO). High-sensitivity C-reactive protein (hs-CRP) was measured using an ultrasensitive enzyme-linked immunosorbent assay (Calbiochem, La Jolla, CA).

**Definition of Cardiometabolic Risk Factors**

Six possible metabolic abnormalities were used to distinguish between MH and MA phenotypes (7). These included (i) elevated blood pressure, defined by systolic/diastolic blood pressure \( \geq 130/85 \) mmHg or documented use of antihypertensive drugs; (ii) elevated TG defined by fasting TG level \( \geq 150 \) mg/dL, (iii) decreased HDL-C level defined by gender-specific criteria (i.e. HDL-C \( < 40 \) mg/dL in men and \( < 50 \) mg/dL in women) or documented use of a lipid-lowering medication; (iv) elevated glucose level
defined by fasting glucose level ≥ 100mg/dL or documented use of antidiabetic medication; (v) insulin resistance, defined by HOMA-IR > 5.13; and (vi) subclinical inflammation, defined by hs-CRP levels ≥3 mg/L.

**Body Size Phenotype Definitions**

We employed a previously developed and rigorously evaluated definition of MH and MA (7) to overweight (BMI 25.0-29.9 kg/m²) and obese participants (BMI ≥ 30.0 kg/m²). Participants with BMI < 25.0 kg/m² were defined as normal weight and not further sub-divided as MH or MA. The MH overweight phenotype was defined as BMI 25.0-29.9 kg/m², and having no or one metabolic abnormality. The MH obese phenotype was defined as BMI ≥ 30.0 kg/m² and having no or one metabolic abnormality. The MA overweight phenotype was defined as BMI 25.0-29.9 kg/m², and having 2 or more metabolic abnormalities. The MA obese phenotype was defined as BMI ≥ 30.0 kg/m² and having 2 or more metabolic abnormalities. We also included a comparison group comprised of normal weight participants defined as BMI ≤ 25.0 kg/m² with a healthy metabolic profile based on our definition of MH (N=234). Further, we excluded 26.4% (N=84) of the normal weight participants from the analyses who were deemed to be MA based on our definition of metabolic health.

**Statistical Analysis**

Quantitative variables were expressed as means and standard deviations and qualitative variables as number of participants and percentages. The IRAS Family Study consists of correlated data between family members (17). Thus, all family relationships
were examined using the generalized estimating equation approach using the SAS (Cary, NC) PROC GENMOD procedure. The models account for familial correlation using a sandwich estimator of variance under exchangeable correlation. The α-level for testing significance of main effects was set a priori at p<0.05. General linear mixed models were used to calculate adjusted beta estimates to examine whether body size phenotype (MH vs. MA) was associated with VAT, SAT, or liver density. Logistic regression was used to calculate odds ratio of NAFLD across body size phenotypes. All analyses were repeated after exclusion of 118 persons with type 2 diabetes (not shown).

RESULTS

Seventy percent (70%) of the Hispanic cohort were overweight (32%) or obese (38%). Forty-one percent of overweight participants (n=138) and 19% of obese participants (n=74) met criteria for MH (Table I). In addition to expected differences in metabolic factors used to define these groups (Table I), MH individuals were, on average, younger than MA groups, more physically active, less likely to be on medications, had smaller WC and a lower WHR, and had higher levels of circulating adiponectin. Liver enzymes which may serve as a biochemical marker for NAFLD were not consistently associated with phenotype. AST was lower in MH obese and GGT was lower in MH overweight participants. ALT did not differ in either obese or overweight phenotypes. VAT areas were lower in MH (p<0.0001); however, SAT areas did not differ between MH and MA groups (p=0.3221; p=0.171). Consequently, visceral to subcutaneous tissue area ratio (VSR) was lower in MH compared to MA individuals (p=0.0096; p<0.0001). Liver density was higher in the MH obese group compared to MA obese individuals,
indicating lower levels of fat in liver (p=0.0022). Similarly, NAFLD prevalence was reduced in MH groups (22.6% versus 44.1%, in MH and MA obese groups, and 17.9% versus 27.3% in MH and MA overweight groups, respectively). Percent fat and lean mass as measured by DXA did not differ between MH and MA phenotypes.
**Table I. Baseline Characteristics of IRAS-Family Hispanic Participants.**

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Obese Phenotype</th>
<th>Overweight Phenotype</th>
<th>Normal Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Abnormal</td>
<td>P-value</td>
</tr>
<tr>
<td>N</td>
<td>74</td>
<td>323</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.7(12.1)</td>
<td>44.4(13.4)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Female, N (%)</td>
<td>41(55.4)</td>
<td>206(63.8)</td>
<td>0.3623</td>
</tr>
<tr>
<td>Center, N (%)</td>
<td>SA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44(59.5)</td>
<td>212(65.6)</td>
<td>0.8301</td>
</tr>
<tr>
<td></td>
<td>SLV</td>
<td>30(40.5)</td>
<td>111(34.4)</td>
</tr>
<tr>
<td>Physical Activity, N (%)</td>
<td>Rarely/never</td>
<td>16(21.6)</td>
<td>114(35.5)</td>
</tr>
<tr>
<td></td>
<td>1-3/mth</td>
<td>20(27.0)</td>
<td>82(25.5)</td>
</tr>
<tr>
<td></td>
<td>1/week</td>
<td>11(14.9)</td>
<td>37(11.5)</td>
</tr>
<tr>
<td></td>
<td>2-4/week</td>
<td>18(24.3)</td>
<td>72(22.4)</td>
</tr>
<tr>
<td></td>
<td>5+/week</td>
<td>9(12.2)</td>
<td>16(5.0)</td>
</tr>
<tr>
<td>BP meds, N(%)</td>
<td>3(4.1)</td>
<td>68(21.1)</td>
<td>3(2.2)</td>
</tr>
<tr>
<td>Lipid meds, N (%)</td>
<td>0(0.0)</td>
<td>23(7.1)</td>
<td>2(1.5)</td>
</tr>
<tr>
<td>Diabetes meds, N (%)</td>
<td>2(2.7)</td>
<td>49(15.2)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>114.6(13.6)</td>
<td>124(16.9)</td>
<td>112.6(13.3)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>75.1(8.4)</td>
<td>79.2(10.1)</td>
<td>74(8.5)</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>46.2(9.1)</td>
<td>38.6(10.9)</td>
<td>48.6(12.5)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>95.4(41)</td>
<td>149.9(90.5)</td>
<td>82.2(30.8)</td>
</tr>
<tr>
<td>Fasting Glucose, mg/dL</td>
<td>90.3(6.4)</td>
<td>113.7(38.1)</td>
<td>91.6(6.8)</td>
</tr>
<tr>
<td>S</td>
<td>1.8(1.2)</td>
<td>0.8(0.8)</td>
<td><strong>&lt;.0001</strong></td>
</tr>
<tr>
<td>HOMA</td>
<td>3.1(1.4)</td>
<td>7(5.8)</td>
<td>2.5(1.2)</td>
</tr>
<tr>
<td>Hs-CRP, mg/L</td>
<td>2.7(3.8)</td>
<td>5.7(5)</td>
<td>1.7(1.8)</td>
</tr>
<tr>
<td></td>
<td>MH Mean (SD)</td>
<td>MA Mean (SD)</td>
<td>p-value</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td>Adiponectin, µg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>11.4(10.8)</td>
<td>12.6(10.1)</td>
<td>0.3164</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>18.2(7.6)</td>
<td>21.1(10.4)</td>
<td>0.0173</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>40.1(37.8)</td>
<td>45.9(44.0)</td>
<td>0.3294</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.2(2.6)</td>
<td>35.7(4.7)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Waist Circumference, cm</td>
<td>97.5(8.6)</td>
<td>103.9(11.1)</td>
<td>0.0007</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86(0.09)</td>
<td>0.88(0.08)</td>
<td>0.0638</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>115.3(47.8)</td>
<td>160.4(60.2)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>SAT, cm²</td>
<td>466.8(119.6)</td>
<td>487.1(131.3)</td>
<td>0.3221</td>
</tr>
<tr>
<td>VSR</td>
<td>0.28(0.17)</td>
<td>0.36(0.18)</td>
<td>0.0096</td>
</tr>
<tr>
<td>Liver Density*, (HU)</td>
<td>50.8(10.8)</td>
<td>44.8(13.2)</td>
<td>0.0022</td>
</tr>
<tr>
<td>Liver to Spleen Ratio* (LSR)</td>
<td>1.10(0.23)</td>
<td>1.00(0.30)</td>
<td>0.0091</td>
</tr>
<tr>
<td>NAFLD (LSR &lt;1), N (%)</td>
<td>12(22.6)</td>
<td>105(44.1)</td>
<td>0.0025</td>
</tr>
<tr>
<td>% Fat from DXA*</td>
<td>36.4(8.7)</td>
<td>38.9(7.2)</td>
<td>0.1025</td>
</tr>
<tr>
<td>% Lean from DXA*</td>
<td>61(8.4)</td>
<td>58.8(7.0)</td>
<td>0.1167</td>
</tr>
</tbody>
</table>

*N (%) or mean (SD); p<0.05 indicates significant differences between metabolically healthy (MH) and metabolically abnormal (MA) obese or overweight adjusted for familial correlation.

**Normal Weight metabolically healthy (MH) participants only.

For abbreviations : ALT indicates alanine transaminase, AST aspartate transaminase, BMI body mass index, BP blood pressure, DBP diastolic blood pressure, DXA dual X-ray absorptiometry, GGT gamma-glutamyl transpeptidase, HDL-C high-density lipoprotein
cholesterol, HOMA, homeostasis model assessment, Hs-CRP high-sensitivity C-reactive protein, HU Hounsfield units, LSR liver to spleen ratio, NAFLD non-alcoholic fatty liver disease, S$_I$ fasting insulin, SA San Antonio, SAT subcutaneous adipose tissue, SBP systolic blood pressure, SLV San Luis Valley, WHR waist-hip ratio, VAT visceral adipose tissue, and VSR visceral to subcutaneous tissue area ratio.

*Statistical testing not performed because these variables define the MH and MA phenotype.

†Measures obtained at follow-up, sample sizes reduced by approximately 20%
With adjustment for age, gender, geographic location, family relationships, liver enzymes, and BMI, MH obese participants still had lower VAT (p=0.0005), VSR (0.02), and higher liver density (p=0.0002), than MA obese participants, and MH overweight participants had lower VAT (p=0.008) and VSR (p=0.004) than MA overweight participants (Table II and Figures 1-3). Odds of NAFLD were reduced in MH (OR= 0.34, P=0.0007 for obese and OR=0.60, P=0.1031 for overweight) compared to MA (not shown). As in unadjusted comparisons, no differences were observed for SAT, percent fat and percent lean mass. When we excluded participants with diabetes from these analyses, despite a reduction in precision, the interpretation did not change (not shown).

The normal weight comparison group was compared to both MH obese and overweight groups (Table II) adjusted for age, gender, geographic location, family relationships, liver enzymes, and BMI. VAT areas and VSR did not differ between normal weight and MH obese or overweight participants. LSR did not differ between normal weight and MH overweight participants. In contrast, both MH obese and overweight participants had lower liver density, higher SAT, higher percent fat, and lower percent lean mass than normal weight participants.
Table II. Measures of Fat distribution Among Metabolically Healthy and Metabolically Abnormal Hispanics in the IRAS Family Study.*

<table>
<thead>
<tr>
<th></th>
<th>Obese Phenotype</th>
<th>Overweight Phenotype</th>
<th>Normal Weight** (n=234)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metabolically healthy (n=74)</td>
<td>Metabolically abnormal (n=323)</td>
<td>Metabolically healthy (n=138)</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>107.7 (5.9)</td>
<td>127.7 (4.7)</td>
<td>106.3 (3.1)</td>
</tr>
<tr>
<td></td>
<td>0.0005</td>
<td>0.0205</td>
<td>0.0003</td>
</tr>
<tr>
<td>SAT, cm²</td>
<td>458.9 (13.4)</td>
<td>476.0 (7.0)</td>
<td>293.7 (6.7)</td>
</tr>
<tr>
<td></td>
<td>0.2546</td>
<td>0.0256</td>
<td>0.3186</td>
</tr>
<tr>
<td>VSR</td>
<td>0.33 (0.02)</td>
<td>0.37 (0.02)</td>
<td>0.35 (0.01)</td>
</tr>
<tr>
<td>Liver Density+ (HU)</td>
<td>51.1 (1.7)</td>
<td>44.9 (1.4)</td>
<td>53.6 (1.3)</td>
</tr>
<tr>
<td></td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.0004</td>
</tr>
<tr>
<td>LSR+</td>
<td>1.10 (0.03)</td>
<td>0.98 (0.03)</td>
<td>1.17 (0.03)</td>
</tr>
<tr>
<td>% Fat from DXA+</td>
<td>33.3 (0.7)</td>
<td>33.6 (0.4)</td>
<td>32.7 (0.5)</td>
</tr>
<tr>
<td></td>
<td>0.6445</td>
<td>0.6445</td>
<td>0.6988</td>
</tr>
<tr>
<td>% Lean from DXA+</td>
<td>64.1 (0.7)</td>
<td>63.8 (0.3)</td>
<td>64.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td>0.6898</td>
<td>0.6898</td>
<td>0.7097</td>
</tr>
</tbody>
</table>

*Adjusted for age, gender, family, clinic, liver enzymes, and BMI (Adjusted mean (SE) values with p-values indicating obese and overweight group differences; p <0.05 indicates significant differences between MH and MA groups).

**Normal Weight metabolically healthy (MH) participants only.

+Measures obtained at follow-up, sample sizes reduced by approximately 20%

SAT not adjusted for BMI

1 p-values comparing metabolically healthy obese to metabolically abnormal obese

2 p-values comparing metabolically healthy overweight to metabolically abnormal overweight

3 p-values comparing normal weight to metabolically healthy obese

4 p-values comparing normal weight to metabolically healthy overweight
Figure 1. Visceral Adipose Tissue Areas for Obese, Overweight, and Normal Weight Hispanic Americans in the IRAS Family Study.

* p-values <0.05 comparing VAT between MH phenotypes to MA phenotypes.

VAT was not different between normal weight phenotype and MH obese or MH overweight phenotypes. Adjusted for age, gender, and clinic site. MA indicates metabolically abnormal and MH metabolically healthy.
Figure 2. Liver Density Areas for Obese, Overweight, and Normal Weight Hispanic Americans in the IRAS Family Study.

* p-values <0.05 comparing VAT between MH phenotypes and MA phenotypes. Adjusted for age, gender, and clinic site.

+ p-values <0.05 comparing VAT between MH phenotypes to normal weight phenotype. Adjusted for age, gender, and clinic site.
**Figure 3.** Visceral Adipose Tissue to Subcutaneous Adipose Tissue Ratio for Obese, Overweight, and Normal Weight Hispanic Americans in the IRAS Family Study. 

* p-values <0.05 comparing metabolically healthy phenotypes to metabolically abnormal phenotypes. Adjusted for age, gender, and clinic site.

// VSR was not different between normal weight phenotype and MH obese or MH overweight phenotypes. Adjusted for age, gender, and clinic site.
DISCUSSION

In this cohort of Hispanic individuals, MH obese and overweight participants had lower CT-determined measures of VAT, VSR, liver density, and decreased odds of NAFLD compared to MA groups, despite similar body size based on BMI. These findings persisted with adjustment for age, gender, geographic location, family relationships, liver enzymes, and BMI. Second, VAT areas did not differ between normal weight and MH obese or overweight individuals, despite differences in BMI. Similarly, LSR did not differ between normal weight and MH overweight individuals, despite differences in BMI. Finally, VSR did not differ in MH obese and overweight compared to normal weight individuals. Taken together, these findings suggest that the term "metabolically healthy" may be useful to identify obese and overweight individuals who, despite their higher BMI, may not be at any greater risk of type 2 diabetes and CVD compared to individuals with a normal BMI.

This is the first study in a large Hispanic cohort that has evaluated abdominal fat distribution in different body size phenotypes. Several small studies have shown the MH obese phenotype, compared to the MA obese phenotype, is associated with lower VAT despite similar amounts of SAT (8-11; 22-24). For example, in a study of 113 obese, sedentary postmenopausal women, body composition was measured by dual-energy x-ray absorptiometry and body fat distribution was measured by computed tomography scan (8). When comparing MH obese to MA obese groups, no differences were observed for subcutaneous adipose tissue. However, MH obese individuals had significantly less visceral adipose tissue than MA obese individuals (p<0.05) (8). In a separate study of 43 obese, sedentary postmenopausal women, subjects were classified as MH obese or MA
obese and body composition (fat mass and lean body mass) and body fat distribution (abdominal visceral and subcutaneous adipose tissue areas, mid-thigh subcutaneous adipose tissue and muscle attenuation) were measured (22). Despite comparable total body fatness between groups (45.2 +/- 5.3% vs. 44.8 +/- 6.6%; P >0.05), MH obese individuals had 49% less visceral adipose tissue than MA obese subjects (141 +/- 53 vs. 211 +/- 85 cm²; P: < 0.01). No difference was noted between groups for abdominal subcutaneous adipose tissue (453 +/- 126 vs. 442 +/- 144 cm²; P: = NS), total fat mass (38.1 +/- 10.6 vs. 40.0 +/- 11.8 kg), and muscle attenuation (42.2 +/- 2.6 vs. 43.6 +/- 4.8 HU) (22).

In addition to the observation that MH obese individuals have lower VAT levels compared to their MA obese counterparts, several studies have found that MH obese individuals have lower levels of ectopic liver fat and potentially a decreased risk of NAFLD (1, 12-16). In a study of 314 obese Germans, MH obese participants had significantly less ectopic fat (i.e. liver) compared to MA obese participants (1). In a study of 82 Italian women screened for MH obesity, MA individuals had significantly greater evidence of fatty liver (i.e. hepatic steatosis) and thus higher concentrations of hepatic enzymes (i.e. AST, ALT, and GGT) than their MH counterparts (12). Finally, in a study of 104 obese postmenopausal women, those with the MH obesity phenotype had a lower fatty liver index compared to the MA obese subjects. (13). However, given the limited data available, it has yet to be elucidated whether an increase in liver fat is an independent predictor of metabolic health in obese individuals apart from the other observed body distribution differences. The present study extends these earlier findings to a large cohort of Hispanic individuals at significant risk for overweight, obesity, and
NAFLD. Our data suggest that a less fatty liver and smaller VAT areas may be important defining features of cardiometabolic health in obese and overweight individuals.

The primary strength of the present study is that data were derived from a large sample of comprehensively phenotyped Hispanics using direct measures of insulin resistance, inflammatory markers, and CT-derived adipose tissue distribution (19). To our knowledge, this is the first report to describe similarities in fat depot location between normal weight and the MH obese or overweight phenotype, and this is only the second report characterizing MH obesity in a Hispanic cohort (7). Finally, the IRAS Family data in this study also possesses unique, precise information regarding NAFLD.

Our study has several limitations. First, although the concept of describing obese and overweight individuals based on cardiometabolic risk is becoming more recognized in the scientific community, the definition of body size phenotype has not been standardized. We chose our criteria based on procedural rigor for selecting cut-points (7, 25). Second, our study population came from two distinct regions of the US, which may limit generalizability of our findings to other Hispanics (26). Third, 5 years elapsed between collection of baseline measurements (including cardiometabolic risk factors defining MH and MA), liver density measurement, NAFLD assessment, and total body fat measurements. However, despite the time difference between measures, we found clear evidence of decreased VAT, decreased liver density, and reduced odds of NAFLD in MH overweight or obese individuals compared to MA participants. Finally, we are unable to evaluate the association between the MH phenotype and CVD risk given the limitations of our data collection from IRAS-Family. Several recent studies have examined the association between MH obesity and subclinical CVD with surrogate
endpoints such as carotid artery intima media thickness, aortic pulse wave velocity, coronary calcification, and heart rate variability with conflicting results (25, 27-28).

This study is noteworthy because it provides specific and sensitive markers describing fat distribution in visceral, subcutaneous, and ectopic (i.e. liver) adipose tissue. CT-derived measures in our study indicated that visceral and liver fat depots are defining features of MH obesity and overweight: these groups tend to have similar visceral and liver fat depots to normal weight counterparts, but lower visceral and liver fat depots compared to MA participants. Several intervention trials have indicated that weight loss in MH obese individuals may be ineffective or paradoxically harmful regarding cardiometabolic risk factors, whereas other trials have shown significant improvement in these risk factors in MH obese participants (29-33). These discrepancies likely reflect inconsistent criteria for MH versus MA obesity, and as this study demonstrates, if distinctions between body size phenotypes are to be adopted more widely, they must be validated in a range of settings and different patient populations.

In conclusion, the present study indicates that the MH overweight and obesity phenotypes are relatively common in our Hispanic participants, who live in San Antonio, TX and San Luis Valley, CO. Previously, MH or MA obesity has been distinguished by specific cardiometabolic risk factors such as blood pressure. However, we found that fat stored in visceral and liver depots can also be used to differentiate the presence of the MH and MA phenotype. These findings are significant as they suggest that obesity as defined by BMI may not have the same physiologic importance for every individual. As the obesity crisis has reached global epidemic proportions, the necessity for alternative approaches to primary and secondary prevention and perhaps policy implementation is
paramount. From a clinical as well as public health standpoint, distinguishing MH and MA obesity could help identify which individuals are at increased risk and would benefit the most from intensive weight loss specific intervention (34). Future research on body size phenotypes (including subsequent risk of CVD and type 2 diabetes) will assist in developing approaches for the detection, treatment, and prevention of disease that are more tailored to individual patients (34).
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CHAPTER 3: BODY FAT DISTRIBUTION AND BIOMARKERS IN METABOLICALLY HEALTHY OBESITY IN AN AFRICAN AMERICAN POPULATION: THE IRAS FAMILY STUDY

INTRODUCTION

The obesity epidemic has tremendous public health implications in regard to risk of type 2 diabetes, cardiovascular disease (CVD) and other co-morbid conditions (1). However, recent evidence suggests some obese individuals do not contribute to this public health crisis (2). Specifically, significant variation exists in cardiometabolic (CM) risk factors among individuals of similar BMI and waist circumference, while an individuals’ CVD risk may depend jointly on body size and metabolic profile (3-7). Since BMI and waist circumference (WC) are imprecise measures of obesity, investigators have identified a relatively new phenomenon of body size phenotypes (3-7). Obese individuals can be characterized as either Metabolically Abnormal (MA) Obese or Metabolically Healthy (MH) Obese (2). The MA obese (MAO) phenotype is typically defined as BMI ≥ 30 kg/m² and having ≥ 2 CM abnormalities, whereas the MH obese (MHO) phenotype is defined as BMI ≥ 30kg/m² and having 0-1 CM abnormality(3). Most studies that have estimated the prevalence of MHO have looked primarily at Caucasian/Northern European populations (1, 5). A recent cross-sectional analysis of the general US population estimated the prevalence of MH obesity to be approximately thirty percent (7). Based on current epidemiologic evidence, obesity prevalence also varies by racial and ethnic group for both men and women. For instance, relative to US non-Hispanic whites, the likelihood of being obese has been found to be significantly greater among US non-
Hispanic blacks (OR for men, 1.13 [95% CI, 1.01-1.27]; OR for women, 2.26 [95% CI, 2.02-2.51]) and for Mexican American women (OR, 1.53; 95% CI, 1.31-1.78) (8).

At present, the location of fat in MH obesity has been inadequately studied, and body size phenotypes in minority US populations are also poorly understood (3). As mentioned, although there have been several studies that have tried to identify, characterize, and estimate the prevalence of MHO, these studies have primarily examined relatively homogenous populations. Further, recent epidemiologic evidence suggests that several biochemical markers may distinguish the MHO from the MAO (9-16). The primary goal of this study is to assess the prevalence of different body size phenotypes in an African American cohort including obese, overweight, and normal weight participants. Secondary goals are to better understand the characteristics and risk factors associated with the MHO phenotype versus the MAO phenotype including fat distribution in visceral, subcutaneous, and liver depots as measured by computed tomography (CT) in African Americans representative of the US general population. The final goal of this study is to attempt to identify distinctive biochemical markers typically associated with obesity and insulin resistance among these body size phenotypes. This study will provide critical information regarding the heterogeneity of cardiometabolic risk that has been observed among obese individuals. Furthermore, this will be the fifth known study to date to describe the phenotypic variation in an African-American population and the first study to describe fat distribution using sensitive and specific imaging techniques.
METHODS

Study Population

The IRAS Family Study is an epidemiologic cohort study of men and women designed to investigate the genetics of insulin resistance and visceral adiposity (17). Multi-generational families of African American background were enrolled using probands of the original IRAS study supplemented from the general population (18). Briefly, one site recruited and examined family members of African American ethnicity (Los Angeles, California) over a 2.5-year period, 1999-2002. Insulin resistance was measured using the intravenous glucose tolerance test, and abdominal obesity was measured using CT. Cardiometabolic disease risk factors were also assessed. Follow-up examinations were conducted between years 2005-2006 at which time liver density was obtained. Eligibility criteria included (i) self-reported African American ethnicity, (ii) 18 years of age or older, (iii) under 350 pounds for CT examination, and (iv) not having conditions that interfere with measurement of insulin resistance or any CM risk factor (19). This current study included the African American cohort, which had data necessary to estimate the MH phenotype (n=526). All studies were conducted using protocols approved by the Institutional Review Boards at each participating institution and all participants provided informed consent.

Measurement of Baseline Characteristics

Self-Reported Data: Age, ethnicity, and physical activity were assessed by self-report. An estimate of usual frequency in vigorous leisure-time physical activity was
assessed with a defined response set ranging from “rarely or never” to “5 or more times per week.” The use of antihypertensive, lipid-lowering and antidiabetic medications were also assessed by self-report.

*Anthropometric Measurements:* Height and weight were measured in duplicate to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight/height squared (kg/m²). Waist circumference (WC) was measured to nearest 0.1 cm at the level of the iliac crest at the end of normal respiration. The Waist to Hip Ratio (WHR) was measured by dividing WC by hip circumference (measured at the level of the greater trochanters with legs closed). Seated systolic/diastolic blood pressure was measured three times using a mercury manometer, after a five minute rest by centrally trained technicians using identical equipment. The mean of the last two measurements was used to calculate blood pressure.

*CT-Derived Measurements:* CT imaging for abdominal fat distribution was obtained under standardized protocol and scans were read centrally. Participants received a scout view of the abdomen and pelvis followed by three axial images all during suspended respiration. Three 10-mm-thick images were obtained through the L2–L3, L4–L5 and T11–T12 disc spaces. If the T11–T12 image did not include liver and spleen, a fourth image was obtained using scout to determine an appropriate intervertebral disc location. Liver and spleen density were quantified in Hounsfield Units in the entire liver and spleen as visualized in the slice, excluding any visible vasculature. The image obtained at the L4–L5 disc space was used for determination of visceral adipose tissue (VAT) area and subcutaneous adipose tissue (SAT) areas; bowel fat is excluded from
these measurements. The ratio of liver to spleen density (LSR) was calculated; LSR <1.0 has become an accepted cut point for a discrete outcome of Nonalcoholic Fatty Liver Disease (NAFLD) (19). Unadjusted liver density is also accepted as a continuous outcome (19). All CT images were coded for pathology and image quality; poor-quality studies were excluded from analysis (19). Percent total body fat and lean mass were obtained using dual-energy X-ray absorptiometry (DXA) scan. Liver density and DXA studies were obtained only at follow-up examination.

**Laboratory Measurements:** Plasma triglyceride (TG) concentrations, high-density lipoprotein (HDL) cholesterol, Alanine transaminase (ALT), aspartate transaminase (AST), and γ-glutamyl transpeptidase (GGT) were determined by enzymatic colorimetric assays using a Chemistry Analyzer Model ATAC 8000 (Elan Diagnostics, Smithfield, RI). Plasma glucose was measured using the glucose oxidase technique on an automated autoanalyzer (YSI, Yellow Springs, OH). Insulin was measured by radioimmunoassay (Linco Research, St Charles, MO). HOMA (Homeostasis model assessment) was used to evaluate insulin resistance using the formula: Fasting Plasma Insulin Level (microunits per milliliter) x Fasting Plasma Glucose Level (millimoles per liter)/22.5. Plasma adiponectin concentration was quantified using radioimmunoassay (Linco Research, St Charles, MO). High sensitivity C-reactive protein (hs-CRP) was measured using ultrasensitive ELISA (Calbiochem, La Jolla, CA). Interleukin-6 (IL-6), Retinol Binding Protein 4 (RBP4), Plasminogen Activator Inhibitor Type 1 (PAI-1), and Tumor Necrosis Factor (TNF) Alpha Receptors 1 and 2 were measured using highly sensitive enzyme-linked immunosorbent assays (R&D systems, Minneapolis, MN).
Definition of Cardiometabolic Risk Factors

Six possible metabolic abnormalities were used to distinguish between MH and MA phenotypes (7). These included: (i) elevated blood pressure defined by systolic/diastolic blood pressure ≥ 130/85 mmHg or documented antihypertensive use, (ii) elevated TG defined by fasting TG level ≥ 150 mg/dL, (iii) decreased HDL-C level defined by gender specific criteria (i.e. HDL-C level < 40 mg/dL in men and < 50 mg/dL in women) or documented use of a lipid-lowering medication, (iv) elevated glucose level defined by fasting glucose level ≥ 100mg/dL or documented use of antidiabetic medication, (v) insulin resistance defined by HOMA-IR > 5.13, and (vi) subclinical inflammation defined by measured hs-CRP levels ≥3 mg/L.

Body Size Phenotype Definitions

We employed a previously developed definition of MH and MA (7) because it has been broadly applied to multiple cohorts, it has been tested for sensitivity, and it encompasses a broad range of risk factors including insulin resistance, and subclinical inflammation. We applied the definitions to both overweight participants (BMI 25.0-29.9 kg/m²) and obese participants (BMI ≥ 30.0 kg/m²). Participants with BMI < 25.0 kg/m² were defined as normal weight and not further sub-divided as MH or MA. The MH overweight phenotype was defined as BMI 25.0-29.9 kg/m², and having no or one metabolic abnormality. The MH obese (MHO) phenotype was defined as BMI ≥ 30.0 kg/m² and having no or one metabolic abnormality. The MA overweight phenotype was defined as BMI 25.0-29.9 kg/m², and having 2 or more metabolic abnormalities. The MA
obese (MAO) phenotype was defined as BMI ≥ 30.0 kg/m² and having 2 or more metabolic abnormalities.

**Statistical Analysis**

Descriptive statistics were computed to characterize the study population. Quantitative variables were expressed as means and standard deviations and qualitative variables as frequencies and percentages. Two group comparisons were performed using Student’s t-test or chi-square test for quantitative and qualitative variables, respectively. Fisher exact test was used for comparison of NAFLD prevalence given small sample sizes. General linear mixed models with a random family effect were fit to examine whether body size phenotype (MH vs. MA) was associated with VAT, SAT, liver density, and levels of specific biomarkers. All mixed model analyses first examined whether gender had an interaction with metabolic group (none found). An alpha level of 0.05 was used to indicate statistical significance. All analyses were performed in SAS Enterprise Guide (version X, Cary, NC).

**RESULTS**

Seventy nine percent (79%) of the African American cohort were overweight (35%) or obese (44%). Of overweight participants, 52% (n=96) met criteria for MH, and of obese participants, 22% (n=51) met criteria for MH (Table I). In addition to expected differences in metabolic factors used to define these groups (Table I), MH individuals were, on average, younger than MA groups, more physically active, less likely to be on medications, had smaller WC and WHR, and had higher insulin sensitivity. Further, the MH groups had higher levels of circulating adiponectin (MHO only), lower levels of
TNF-1, TNF-2, and RBP4 (MH overweight only), and lower levels of PAI-1 (both MHO and MH overweight). Liver enzymes were not significantly associated with any particular phenotype. That is, AST, ALT, and GGT did not differ in either obese or overweight phenotypes. VAT areas were lower in MH obese and overweight (p<0.0001); however, SAT areas were still lower when comparing MHO and MAO (p=0.0024) but did not differ between the MH overweight and MA overweight groups (p=0.0562). Consequently, visceral to subcutaneous tissue area ratio (VSR) was lower in MH compared to MA (p=0.0030; p=0.0002). Liver density was higher in the MH obese group compared to MA obese, which is indicative of lower levels of fat in liver (p=0.0041). NAFLD prevalence was reduced but not significant in MH groups (6.7% versus 12.5%, in MHO and MAO groups; p= 0.3764 and 10.1% versus 15.6% in MH and MA overweight groups; p=0.2974 respectively). Percent fat from DXA did not differ between MH and MA phenotypes, nor did percent lean mass from DXA.
<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Obese Phenotype</th>
<th>Overweight Phenotype</th>
<th>Normal Weight**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Abnormal</td>
<td>P-value</td>
</tr>
<tr>
<td>N</td>
<td>51</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.5 (10.3)</td>
<td>45.7 (11.7)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Female, N (%)</td>
<td>32 (62.8)</td>
<td>122 (66.7)</td>
<td>0.5675</td>
</tr>
<tr>
<td>Physical Activity, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rarely/never</td>
<td>8 (15.7)</td>
<td>54 (29.5)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>1-3/mth</td>
<td>15 (29.4)</td>
<td>35 (19.1)</td>
<td></td>
</tr>
<tr>
<td>1/week</td>
<td>9 (17.7)</td>
<td>26 (14.2)</td>
<td></td>
</tr>
<tr>
<td>2-4/week</td>
<td>16 (31.4)</td>
<td>52 (28.4)</td>
<td></td>
</tr>
<tr>
<td>5+/week</td>
<td>3 (5.9)</td>
<td>16 (8.7)</td>
<td></td>
</tr>
<tr>
<td>BP meds, N (%)</td>
<td>2 (3.9)</td>
<td>75 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Lipid meds, N (%)</td>
<td>0 (0.0)</td>
<td>17 (9.3)</td>
<td></td>
</tr>
<tr>
<td>Diabetes meds, N (%)</td>
<td>0 (0.0)</td>
<td>28 (15.3)</td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>112.7 (11.5)</td>
<td>124.9 (18.1)</td>
<td></td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>73.1 (8.1)</td>
<td>77.6 (10.2)</td>
<td></td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>49.1 (10.9)</td>
<td>42.5 (11.2)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>61.4 (29.6)</td>
<td>95.5 (64.4)</td>
<td></td>
</tr>
<tr>
<td>Fasting Glucose, mg/dL</td>
<td>90.2 (6.6)</td>
<td>116.9 (45.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD) 1</td>
<td>Mean (SD) 2</td>
<td>p</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>S1</td>
<td>1.6(0.8)</td>
<td>0.8(0.7)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HOMA°</td>
<td>2.6(1.3)</td>
<td>5.9(3.1)</td>
<td></td>
</tr>
<tr>
<td>HS-CRP, µg/mL</td>
<td>2.6(3.2)</td>
<td>6.4(5.5)</td>
<td></td>
</tr>
<tr>
<td>Adiponectin, µg/mL</td>
<td>10.0(4.3)</td>
<td>7.4(5.4)</td>
<td>&lt;.0022</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>2.3(1.5)</td>
<td>3.5(5.5)</td>
<td>0.1467</td>
</tr>
<tr>
<td>TNF-1, ng/mL</td>
<td>1.8(0.4)</td>
<td>2.7(4.6)</td>
<td>0.3022</td>
</tr>
<tr>
<td>TNF-2, ng/mL</td>
<td>6.0(1.6)</td>
<td>6.9(4.6)</td>
<td>0.2826</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>26.0(20.8)</td>
<td>42.8(32.2)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>RBP4, µg/mL</td>
<td>26.4(8.0)</td>
<td>25.7(7.5)</td>
<td>0.5525</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>7.9(4.2)</td>
<td>7.7(4.1)</td>
<td>0.7927</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>17.1(4.6)</td>
<td>18.2(5.6)</td>
<td>0.1890</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>32.8(27.4)</td>
<td>41.1(41.0)</td>
<td>0.1760</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.7(3.2)</td>
<td>37.1(5.3)</td>
<td>0.1760</td>
</tr>
<tr>
<td>Waist Circumference, cm</td>
<td>96.4(7.8)</td>
<td>106.3(11.4)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.82(0.07)</td>
<td>0.87(0.08)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>83.6(37.9)</td>
<td>139.6(59.5)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>SAT, cm²</td>
<td>467.8(154.9)</td>
<td>543.8(147.7)</td>
<td>0.0024</td>
</tr>
<tr>
<td>VSR</td>
<td>0.21(0.13)</td>
<td>0.28(0.15)</td>
<td>0.0030</td>
</tr>
<tr>
<td>Liver Density+, (HU)</td>
<td>57.1(5.4)</td>
<td>52.2(8.8)</td>
<td>0.0041</td>
</tr>
<tr>
<td>Liver to Spleen Ratio+ (LSR)</td>
<td>1.25(0.15)</td>
<td>1.15(0.20)</td>
<td>0.0152</td>
</tr>
<tr>
<td>NAFLD (LSR &lt;1)+, N (%)</td>
<td>2(6.7)</td>
<td>15(12.5)</td>
<td>0.3764</td>
</tr>
<tr>
<td>% Fat from DXA+</td>
<td>36.5(10.1)</td>
<td>38.1(8.2)</td>
<td>0.3775</td>
</tr>
<tr>
<td>% Lean from DXA+</td>
<td>61.0(9.8)</td>
<td>59.3(7.9)</td>
<td>0.4002</td>
</tr>
</tbody>
</table>
*N (%) or mean (SD); p<0.05 indicates significant differences between metabolically healthy (MH) and metabolically abnormal (MA) obese or overweight adjusted for familial correlation.

**Normal Weight metabolically healthy (MH) participants only.

For abbreviations : ALT indicates alanine transaminase, AST aspartate transaminase, BMI body mass index, BP blood pressure, DBP diastolic blood pressure, DXA dual X-ray absorptiometry, GGT gamma-glutamyl transpeptidase, HDL-C high-density lipoprotein cholesterol, HOMA homeostasis model assessment, hs-CRP high-sensitivity C-reactive protein, IL-6 interleukin-6, TNF-1 tumor necrosis factor-1, TNF-2 tumor necrosis factor-2, PAI-1 plasminogen activator inhibitor-1, RBP4 retinol binding protein 4, HU Hounsfield units, LSR liver to spleen ratio, NAFLD non-alcoholic fatty liver disease, $S_I$ fasting insulin, SAT subcutaneous adipose tissue, SBP systolic blood pressure, WHR waist-hip ratio, VAT visceral adipose tissue, and VSR visceral to subcutaneous tissue area ratio.

^Statistical testing not performed because these variables define the MH and MA phenotype.

^Measures obtained at follow-up, sample sizes reduced by approximately 20%
With adjustment for age, gender, family relationships, liver enzymes, and BMI, MHO continued to have lower VAT (p=0.0001) but no significant differences for VSR (p=0.3204) or liver density (p=0.0825) compared to the MAO participants. The MH overweight had significantly lower VAT (p=0.0002) and VSR (p=0.0005) than MA overweight but no significant differences in liver density (p=0.6708) (Table II). Similar to unadjusted comparisons, no differences were observed for SAT for both obese and overweight groups.

The normal weight reference group was compared to both MH obese and overweight groups (Table II) adjusted for age, gender, geographic location, family relationships, liver enzymes, and BMI. VAT areas did not differ between normal weight individuals and MH overweight (p=0.4564) or the MHO participants (p=0.2672). Liver density did not differ between normal weight, MHO, or MH overweight. In contrast, both MH obese and overweight had significantly higher SAT levels and lower VSR ratios.
**Table II.** Differences in measures of fat distribution between metabolically healthy (MH) versus metabolically abnormal (MA) African American-adjusted for age, gender, liver enzymes, and BMI\(^*\) (Adjusted mean (SE) values with p-values indicating obese and overweight group differences; p <0.05 indicates significant differences between MH and MA groups).

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Overweight</th>
<th>Normal Weight (n=108)</th>
<th>(\text{p-value})</th>
<th>(\text{p-value})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Metabolically healthy (n=51)</td>
<td>Metabolically abnormal (n=183)</td>
<td>(\text{p-value})</td>
<td>Metabolically healthy (n=96)</td>
<td>Metabolically abnormal (n=88)</td>
</tr>
<tr>
<td>VAT, (\text{cm}^2)</td>
<td>81.2(5.8)</td>
<td>106.3 (4.8)</td>
<td><strong>0.0001</strong></td>
<td>86.2 (4.5)</td>
<td>108.2 (4.7)</td>
</tr>
<tr>
<td>SAT, (\text{cm}^2)</td>
<td>386.0(11.3)</td>
<td>392.1(9.5)</td>
<td>0.6159</td>
<td>341.0(9.0)</td>
<td>347.7(9.4)</td>
</tr>
<tr>
<td>VSR</td>
<td>0.26(0.02)</td>
<td>0.28(0.02)</td>
<td>0.3204</td>
<td>0.30(0.02)</td>
<td>0.37(0.02)</td>
</tr>
<tr>
<td>Liver Density (\ast) (HU)</td>
<td>58.0 (1.6)</td>
<td>55.0(1.2)</td>
<td>0.0825</td>
<td>55.3(1.1)</td>
<td>54.7(1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>57.3(1.6)</td>
<td>0.7504</td>
</tr>
</tbody>
</table>

\(\ast\)Measures obtained at follow-up, sample sizes reduced by approximately 20%

1 \(\text{p-values comparing metabolically healthy obese to metabolically abnormal obese}\)

2 \(\text{p-values comparing metabolically healthy overweight to metabolically abnormal overweight}\)

3 \(\text{p-values comparing normal weight to metabolically healthy obese}\)

4 \(\text{p-values comparing normal weight to metabolically healthy overweight}\)
Finally, with adjustment for age, gender, family relationships, liver enzymes, and BMI (Table III) both MHO and MH overweight participants exhibited significantly lower levels of PAI-1 (p=0.0020; p<0.0001) and higher levels of circulating adiponectin (p.0007; p=0.0075). The other biomarkers measured were not significantly associated with any particular body size phenotype excluding RBP4 which was noted to be lower in the MH overweight group compared to the MA overweight group (p=0.0034). Further, the normal weight reference group as compared to the MHO and MH overweight groups had similar levels of all biomarkers (including PAI-1 and adiponectin).
Table III. Differences in biochemical marker concentrations between metabolically healthy (MH) versus metabolically abnormal (MA) African American-adjusted for age, gender, liver enzymes, and BMI (Adjusted mean (SE) values with p-values indicating obese and overweight group differences; p <0.05 indicates significant differences between MH and MA groups).

<table>
<thead>
<tr>
<th></th>
<th>Normal Weight (n=108)</th>
<th>Obese Metabolically Healthy (n=51)</th>
<th>Overweight Metabolically Healthy (n=96)</th>
<th>Obese Metabolically Abnormal (n=183)</th>
<th>Overweight Metabolically Abnormal (n=88)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6*</td>
<td>0.68(3.7)</td>
<td>2.2(2.6)</td>
<td>2.8(2.5)</td>
<td>9.5(2.6)</td>
<td>0.0546</td>
<td>1.8(3.6)</td>
<td>0.8424</td>
</tr>
<tr>
<td>PAI-1</td>
<td>19.5(3.7)</td>
<td>32.6(2.9)</td>
<td>22.3(2.8)</td>
<td>40.3(3.0)</td>
<td>&lt;.0001</td>
<td>24.9(3.8)</td>
<td>0.3389</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>9.5(0.7)</td>
<td>6.9(0.6)</td>
<td>9.2(0.6)</td>
<td>7.3(0.6)</td>
<td>0.0075</td>
<td>11.3(0.7)</td>
<td>0.0714</td>
</tr>
<tr>
<td>RBP4</td>
<td>26.9 (1.1)</td>
<td>25.7(0.9)</td>
<td>25.9(0.9)</td>
<td>29.4(1.0)</td>
<td>0.0034</td>
<td>25.1(1.1)</td>
<td>0.2573</td>
</tr>
<tr>
<td>TNF1*</td>
<td>2.0(0.5)</td>
<td>2.4(0.4)</td>
<td>1.8(0.4)</td>
<td>1.8(0.4)</td>
<td>0.9724</td>
<td>2.0(0.5)</td>
<td>0.9814</td>
</tr>
<tr>
<td>TNF2*</td>
<td>6.4(0.6)</td>
<td>6.4(0.4)</td>
<td>5.7(0.4)</td>
<td>6.1(0.4)</td>
<td>0.4593</td>
<td>6.2(0.6)</td>
<td>0.8144</td>
</tr>
</tbody>
</table>

*Measures obtained at follow-up, sample sizes reduced by approximately 20%

1 p-values comparing metabolically healthy obese to metabolically abnormal obese

2 p-values comparing metabolically healthy overweight to metabolically abnormal overweight

3 p-values comparing normal weight to metabolically healthy obese

4 p-values comparing normal weight to metabolically healthy overweight
DISCUSSION

Several novel findings emerged from the present classification of MH and MA phenotypes in this cohort of African Americans. First, MH obese and overweight groups had lower CT-determined measures of VAT and VSR, higher liver density (MHO only), lower levels of PAI-1, and higher levels of adiponectin compared to MA groups, despite similar body size based on BMI. The finding of lower visceral fat stores, lower PAI-1, and higher adiponectin persisted with adjustment for age, gender, family relationships, liver enzymes, and BMI. Second, VAT areas and VSR did not differ between normal weight reference groups and the MH obese or overweight groups, despite differences in body size based on BMI. Similarly, liver density did not differ between the normal weight group and MH overweight, despite differences in body size. Finally, biomarkers generally associated with obesity, atherogenesis, and insulin resistance did not differ significantly in MH obese and overweight groups compared to the normal weight group. Taken together, these findings suggest that the term "metabolically healthy" may be a useful way to identify obese and overweight individuals that, despite their BMI, may not differ in their risk profile of type 2 diabetes and CVD compared to individuals with a normal BMI.

In regards to prevalence estimates, we found that approximately one-fourth of the obese cohort was MH and greater than one-half of the overweight participants met criteria for MH. There have been three other studies to date that have examined prevalence of MHO in African American participants (20-22). The first study examined 822 unrelated African American participants from the Howard University Family Study demonstrating 28% MHO prevalence (20). Two other subsequent studies looking at
African American populations showed similar MHO prevalence averaging between 28.5% to 33% (21-22). When compared to the prevalence studies examining primarily non-Hispanic whites, the prevalence of MHO and MHOW appears to be higher on average in African Americans. Interestingly, although US prevalence rates suggest that obesity (based on BMI) is more prevalent in African American women compared to men, our study did not find any gender difference in prevalence of MHO (or MHOW).

This is the first study in a large African American cohort that has evaluated abdominal fat distribution in different body size phenotypes. Based on observational studies, the degree of adiposity associated with a given level of BMI varies by racial and ethnic group (7-8). In our study, when comparing studies of Caucasian populations, relative to non-Hispanic white men and women at the same BMI level, African American men and women tend to have lower fat mass in regards to visceral and ectopic fat levels. The implication of this finding is the cardiometabolic risks associated with a given BMI level may be potentially lower for African Americans than for Caucasians. The present study extends the findings to our previous study of a large cohort of Hispanic individuals who were at significant risk for overweight, obesity, and NAFLD (23).

Our data suggests that smaller VAT areas, higher adiponectin levels, and lower PAI-1 levels may be important defining features of CM health in African American obese and overweight individuals. That is, research indicates that ectopic fat distribution and certain biomarkers may be important predictors of cardiometabolic and vascular risk, in addition to overall obesity itself (9-16, 24-27). For instance, accumulation in the abdominal visceral area, compared with overall obesity, has an equally or more important role in the development of cardiometabolic risk, and fat depots in liver tissue cause
adverse cardiometabolic effects by increasing insulin resistance (24). Adiponectin is a protein hormone produced exclusively in adipose tissue that modulates a number of metabolic processes including glucose regulation and fatty acid oxidation (11-12, 20). In general, obesity is associated with low levels of adiponectin. In fact, a low level of adiponectin is considered to be an independent risk factor for developing metabolic syndrome and type 2 diabetes (11-12, 20). Further, PAI-1 is a serine protease inhibitor that functions as the principal inhibitor of tissue plasminogen activator and urokinase, the activators of plasminogen and fibrinolysis (25). It is mainly produced by the endothelium lining blood vessels but is also secreted by adipose tissue. It has been demonstrated to be at increased levels in certain types of cancer, obesity, and metabolic syndrome and has been linked to increased occurrence of thrombosis and accelerated development of atherosclerosis associated with these conditions (25). Thus, the significant differences in body fat distribution/body composition and biomarker levels noted between body size phenotypes in our cohort may provide a contribution to the current understanding of the link between body composition, inflammation, and cardiometabolic risk.

The primary strength of the present study is that data were derived from a large sample of African Americans that has been comprehensively phenotyped using direct measures of insulin resistance, inflammatory markers, and adipose tissue distribution (19). To our knowledge, this is the first report to describe similarities in fat depot location in African Americans between normal weight and the MH obese or overweight phenotype, and this is only the fifth report characterizing MH obesity in an African American cohort (7; 20-22). Several limitations should be considered when interpreting the study findings. First, although the concept of describing obese and overweight
individuals based on CM risk is becoming more recognized in the scientific community, the definition of body size phenotype has not been standardized. The criteria we used were chosen based on procedural rigor for selecting cut-points (7). Second, our study population of African Americans only comes from one distinct clinic site which may limit generalizability to other African Americans. Finally, there is a five-year time difference between collection of baseline measurements (including CM risk factors defining MH and MA) and liver density measurement, NAFLD assessment, and total body fat measurement. We also did not exclude individuals with chronic liver disease which may have affected our estimates concerning NAFLD prevalence and liver density. Despite the reduced sample size on follow-up visit and the time difference between measures, we have demonstrated decreased VAT, decreased levels of PAI-1, and increased levels of circulating adiponectin in MH overweight/obese compared to MA participants.

This study is noteworthy because it provides specific and sensitive markers describing fat distribution in visceral, subcutaneous, and ectopic (i.e. liver) adipose tissue. CT-derived measures in our study indicate that visceral fat depots are defining features of MH obesity and overweight. These groups tend to have similar visceral fat depots to normal weight counterparts but lower visceral fat depots compared to MA participants. The IRAS Family data in this study also possesses unique, precise information regarding NAFLD; NAFLD has been inadequately described in the literature, particularly concerning disease risk. The present study will aid in the future development of standard classification criteria for MH versus MA obesity. This study demonstrates that if distinctions between body size phenotypes are to be adopted more widely, they must be
validated in a range of settings and different patient populations. That is, there is a need for well-designed longitudinal studies with socioeconomically, multi-ethnic populations that examine how timing and duration of obesity affect metabolic health (26), specifically cardiovascular clinical events.

In conclusion, the present study indicates that MH overweight and obesity are relatively common in African American populations residing in Los Angeles, CA. Previously, MHO and MAO has been distinguished by specific CM risk factors such as blood pressure. However, this study reveals fat stored in visceral and differences in levels of specific biomarkers are novel risk factors that can also be used to differentiate MH and MA individuals. These findings set the stage for a better understanding that obesity does not have the same clinical implication for every individual. From a clinical standpoint, distinguishing MH and MA obesity has implications in medical decision making by identifying which individuals would benefit the most from intensive weight loss specific intervention (27). As breakthroughs in genomics are ushering in a new era of personalized medicine, future research on body size phenotyping will assist in developing approaches for the detection, treatment, and prevention of disease that are more tailored to individual patient needs (27).
REFERENCES


23. Chapter 2


CURRICULUM VITAE:

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EDUCATION

M.S. Wake Forest University, Winston-Salem, NC 12/2014

M.D. Medical College of Virginia, Richmond, VA 05/2007

M.P. H. Virginia Commonwealth University, Richmond, VA 08/2003

M.S. Pharmacology Georgetown University, Washington, DC 11/1999

B.S. Biology/Chemistry Old Dominion University, Norfolk, VA 12/1996

PROFESSIONAL/TRAINING EXPERIENCE

09/2014-Present Assistant Professor: Division of Endocrinology & Metabolism. UT Southwestern Medical Center. Dallas, TX

07/2011-08/2013 Clinical Fellow: Division of Endocrinology & Metabolism. Wake Forest University Medical Center. Winston-Salem, NC

08/2010-06/2011 Adjunct Faculty-Instructor: Internal Medicine-Section on General Internal Medicine. Wake Forest University Medical Center. Winston-Salem, NC

07/2010-07/2012 Research Fellow (T32 Post-Doctoral Fellow in Quality of Care and Outcomes Research for CVD and Stroke): Division of Public Health Sciences, Department of Epidemiology. Wake Forest University Medical Center. Winston-Salem, NC

06/2007-06/2010 House-Staff Officer (Internship and Residency): Internal Medicine. Wake Forest University Medical Center. Winston-Salem, NC


01/1997-06/1997 Tutor. Old Dominion University. Norfolk, VA

RESEARCH EXPERIENCE

2012-2014 M.D. Wake Forest University Graduate School of Arts and Sciences-CPTS (Clinical Population Translational Science) graduate program. M.S. Thesis dissertation involving statistical data analysis using the IRAS-Family database.

2011-2012 M.D. Wake Forest University Medical Center-Clinic Clinical Fellow in Endocrinology & Metabolism. QI project involving creation of a DKA order set for the Emergency Department for improvement in patient safety. Systems improvement project for the diagnostic work-up of hyponatremia as an outpatient including instituting the water load test in our outpatient clinic and creation of patient education guides.

2010-2012 M.D. Wake Forest University Medical Center- T32 Post-Doctoral Fellow in Quality of Care and Outcomes Research for CVD and Stroke-Statistical data analysis using ACCORD trial and IRAS-Family databases.

2009-2010 M.D. Wake Forest University Medical Center-Tinsley Harrison Scholars Program-Statistical data analysis using ACCORD trial database.

2006-2007 M.D. Virginia Commonwealth University-Research Project-Statistical data analysis for clinical research trial evaluating repeat DXA utility in osteopenia.


1997-1999 M.S. Division of Pharmacology at Georgetown University- basic science research concentrated in pharmacogenetics/pharmacokinetics

1994-1996 Undergraduate Research at Old Dominion University-basicscience research emphasis on cell culture and electron microscopy

TEACHING EXPERIENCE

2011-2013 Clinical Fellow: Division of Endocrinology & Metabolism. Wake Forest University Medical Center. Winston-Salem, NC
2010-2011 Adjunct Faculty- Instructors: Internal Medicine-Section on General Internal Medicine. Wake Forest University Medical Center. Winston-Salem, NC

2007-2010 House-Staff Officer: Internal Medicine. Wake Forest University Medical Center. Winston-Salem, NC


1997-Tutor. Old Dominion University. Norfolk, VA

1995-1996-Teaching Assistant. Old Dominion University. Norfolk, VA

PRESENTATIONS AND PUBLICATIONS

Peer Reviewed Journal Articles:


Review Articles:


**Abstract:**


**Poster Presentation:**

Carolinas Chapter/American Association of Clinical Endocrinologists Annual Meeting in Hilton Head Island, SC. A Metabolically Healthy Obese Phenotype in Hispanic Participants in the IRAS Family Study. 08/2012.


NC-ACP Annual Chapter 2009 Scientific Meeting in Durham, NC. Clinical Vignette (Seizures: A Not So Rare Cause). 01/2009.

Second Annual Fellow and Resident Research Day in Winston-Salem, NC. Clinical Vignette (Seizures: A Not So Rare Cause). 05/2008


National Conference of Undergraduate Research at Western Michigan University. Drug X and Its Effect on Sperm Architecture 02/1995

**Oral Presentations:**

Endocrine Grand Rounds in Dallas, TX. **Title:** A Metabolically Healthy Obese Phenotype in Hispanic Participants in the IRAS Family Study. 01/2014.
Endocrine Grand Rounds in San Antonio, TX. Title: A Metabolically Healthy Obese Phenotype in Hispanic Participants in the IRAS Family Study. 04/2014.

DEP (Diabetes Expertise Program) in Winston-Salem, NC. Title: Normal Glucose Metabolism: Classification and Pathophysiology of Diabetes Mellitus. 05/2012 & 09/2012.

Dissertations:

MS in CPTS-Investigating Metabolically Healthy Obesity in an Ethnic population: The IRAS Family Study-2014

MPH-Predicting Fractures using the Osteoporosis Self-Assessment Tool-2003

MS in Pharmacology-Genetic Predisposition to Aminoglycoside Toxicity-1998

AWARDS/VOLUNTEER/LEADERSHIP EXPERIENCE

08/2012-First Place winner for poster session. Presented at Carolinas Chapter/American Association of Clinical Endocrinologists Annual Meeting in Hilton Head Island, SC

2010-2012- T32 Grant in Quality of Care and Outcomes Research for CVD and Stroke. Wake Forest University Medical Center. Winston-Salem, VA

2009-2010-Tinsley Harrison Research Scholarship. Wake Forest University Medical Center. Winston-Salem, VA

05/2008-Silver Award. Presented at Second Annual Resident and Fellow Research Day for Best Resident Clinical Vignette. Wake Forest University Medical Center. Winston-Salem, VA


2004-2005-Unite For Site Chapter MCV/VCU. Medical College of Virginia. Richmond, VA

2003-2005-City of Joy MCV/VCU. Medical College of Virginia. Richmond, VA

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2002-2003-*Meals on Wheels*. Richmond, VA

2002-2003-*MPH Curriculum Committee*. Virginia Commonwealth University. Richmond, VA

2001-2002-*Greater DC Cares*. Washington, DC.

2001-2002-*Zaccheaus Medical Free Clinic*. Washington, DC

EXAMS/LICENSURES

| Texas State Medical License, since January 2014 |
| North Carolina State Medical License, since August 2010 |
| American Board of Internal Medicine Certification: |
| Endocrinology, Diabetes, & Metabolism, since October 2013 |
| Internal Medicine, since August 2010 |
| USMLE Step 3- November 2008 |
| USMLE Step 2 CK-September 2006 |
| USMLE Step 2-June 2006 |
| USMLE Step 1-July 2005 |

PROFESSIONAL MEMBERSHIPS

| American Association of Clinical Endocrinologists (NC Chapter), since 2012 |
| American Association of Clinical Endocrinologists, since 2011 |
| The Endocrine Society, since 2011 |
| North Carolina Medical Society, since 2010 |
| American College of Physicians, since 2003 |
| American Medical Association, since 2003 |
| MPH Alumni Association at VCU, since 2003 |
Sigma Xi Research Society, since 1994
Tribeta Biological Honor Society, since 1994
Golden Key National Honor Society, since 1994
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