EPIDEMIOLOGY OF GENERAL, REGIONAL AND ECTOPIC SKELETAL MUSCLE

FAT IN AGING AFRICAN ANCESTRY MEN

by

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Submitted to the Graduate Faculty of

Department of Epidemiology

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2016

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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It was defended on

July 27th, 2016

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University of Pittsburgh, 2016

ABSTRACT

OBJECTIVE: We aim to study on the epidemiology of general, regional and ectopic skeletal muscle fat in aging African ancestry men. To be specific, we described the natural changes and identified correlates to the longitudinal changes of these adiposity measures, and evaluated their association with hypertension and mortality.

METHOD: We use Tobago Health Study to achieve the objectives. The demographic characteristics, lifestyle factors, medical history and medication use were self-reported and collected with interviewer-administered questionnaire. Total body fat percentage, trunk fat percentage and leg fat percentage were assessed with Dual-Energy X-ray Absorptiometry (DXA). Ectopic skeletal muscle fat (inter- and intra- muscular fat) was examined by Quantitative Computed Tomography (QCT). The hypertension status was defined as diastolic blood pressure over 90 mmHg or systolic blood pressure over 140 mmHg or having antihypertensive treatment. The date of death was obtained from death certificate and/or proxy. Linear regression was used to identify the potential correlates for the changes in these adiposity measures. Logistic regression was performed to evaluate the association with newly developed hypertension. Cox hazard proportional model was used to assess the association with mortality risks.

RESULTS: Baseline hypertension was associated with greater decline in the muscle attenuation and leg fat. Furthermore, intramuscular fat (reflecting by decreased muscle attenuation) was

associated with newly developed hypertension after adjustment for the baseline and the change in BMI (OR (95% CI) per SD: 1.32 (1.06,1.64)) or WC measurements (OR (95% CI) per SD: 1.35 (1.08, 1.68)). Both intermuscular fat (HR (95% CI) per SD: 1.29 (1.06-1.57)) and intramuscular fat (HR (95% CI) per SD: 1.37 (1.08-1.75)) were significant associated with elevated morality risk in fully adjusted models. None of the other adiposity measures were associated with newly developed hypertension or elevated morality risks.

CONCLUSION: These novel findings confirmed that ectopic fat, though with a small amount, may be more crucial in driving cardiometabolic diseases than general body fat per se, highlighted the importance of maintaining muscle attenuation in healthy aging, and was with great public health significance. Further studies are needed to establish if these associations are independent of inflammation, visceral or other ectopic fat depots, to identify possible biological mechanisms underlying theses relationship, and to replicate our findings in other populations.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

PREFACE

I truly appreciate my academic advisor, Dr. Iva Miljkovic, for the personal and professional assistance she gave during my PhD program, for her encouragement and for her enthusiasm to this study. I am grateful to all my committee members, Dr. Joseph M Zmuda, Dr. Clareann H Bunker and Dr. Ada O Youk, for the inspiring suggestions to this dissertation. My gratitude also goes to Dr. Allison L Kuipers for all her help during the program. They help me to build up a solid background in both theoretical and applied epidemiology, which would benefit my future career.

Besides, I would also like to express my gratitude to my family and friends for supporting me through the difficulties.

1.0   INTRODUCTION

1.1   BACKGROUND

Obesity is increasing alarmingly worldwide [1]. Many studies have linked obesity to metabolic disorders and mortality risk [1, 2]. However, issues such as "metabolically healthy obesity"[3] and "metabolically unhealthy normal weight"[4] indicate that obesity is a complex condition with different phenotypes. Recently, it was suggested that the distribution of body fat and not general obesity per se, might be more important for the longevity and development of metabolic diseases. Body fat distribution can be classified based on the location of body part (i.e. upper and lower body fat), and based on the anatomical location and function (i.e. subcutaneous fat (SAT), visceral (VAT), and ectopic fat).

African ancestry individuals have greater risk of having and dying from cardiovascular disease (CVD) and type 2 diabetes (T2D) [5]. African Americans have higher prevalence of overweight and obesity [6] than Caucasians; however, they have lower total body fat percentage [7], lower VAT [8], lower ectopic liver fat accumulation [9], but greater ectopic intermuscular fat (IMAT) [10] at a given BMI, compared to Caucasians. Thus, the different pattern of fat distribution by race/ethnicity might explain the greater risk of metabolic disease and mortality among Africans ancestry populations.

Fat distributions change during the aging process and gender, physical activity, sedentary lifestyle and other factors serve as modifiers. However, most research focuses predominantly on Caucasians or a small sample of diverse populations, and the effects of these modifiers on longitudinal changes in fat distribution in African ancestry populations are not well established. Furthermore, the impact of different fat depots on incident cardiometabolic disease and mortality are largely unknown, especially in African ancestry population. We have recently reported that ectopic skeletal muscle fat accumulation contributes to development of T2D among African ancestry men [11], but studies focusing on ectopic fat and incident hypertension are lacking. Large, well-designed, prospective studies conducted among African ancestry populations are critically needed in order to better understand the pathophysiology and clinical consequences of different fat depots, to identify high-risk populations, and to provide novel and effective interventions.

1.2   RESEARCH AIMS

In view of the major gaps in our understanding of the epidemiology of epidemiology of general, regional and ectopic fat among African ancestry men, we are proposing to address the following aims in African ancestry men from the Tobago Health Study:

Aim 1: <u>Describe the natural history and changes in body fat distribution with aging among African ancestry men.</u> We will estimate the effect of aging on the longitudinal changes in body fat distribution. We will also identify lifestyle, behavioral, and medical related risk factors for changes in body fat distribution with aging.

Hypothesis 1:

a) Increasing age is associated with an increased rate in total body fat, trunk fat, leg fat, and ectopic skeletal muscle fat accumulation.

b) Excess alcohol consumption, current smoking, sedentary lifestyle, and medications which affect metabolism are associated with greater increase in total body fat, trunk fat, leg fat, and ectopic skeletal muscle fat accumulation.

Aim 2: <u>Evaluate the association of mortality with total body fat, trunk fat, leg fat, and ectopic skeletal muscle fat accumulation.</u>

Hypothesis 2: Greater trunk fat, leg body fat, and ectopic skeletal muscle fat accumulation are positively associated with morality risk in both middle-aged and elderly men, independent of demographic characteristics lifestyle factors and general body fat.

Aim 3: <u>Determine if change in total body fat, trunk fat, leg fat, and ectopic skeletal muscle fat accumulation predicts subsequent development of hypertension.</u>

Hypothesis 3: Greater trunk fat, leg fat, and ectopic skeletal muscle fat accumulation are positively associated with newly developed hypertension, independent of demographic characteristics, lifestyle factors and general body fat.

Overall impact: Estimating the association of general, regional and ectopic skeletal muscle with potential correlates, and cardiometabolic disease and mortality risks will improve our understanding of the heterogeneity of obesity, and could identify novel risk factors of cardiometabolic disease in African ancestry men. Thus, the study has great public health significance.

2.0   LITERATURE REVIEW

2.1   INTRODUCTION

The alarming increase of obesity and overweight prevalence worldwide is a global health issue, affecting both developed and developing countries. Obesity is estimated to cost between 0.7% [12] and 2.8% [13] of a country's total healthcare expenditure. In 2014, over 1.9 trillion adults were overweight, and of these 600 million were obese [14]. The prevalence of overweight and obesity among adults were 39% and 13%, respectively [14]. Worth noticing is that there are significant black-white differences in prevalence of obesity. Obesity is more prevalent in non-Hispanic blacks than in non-Hispanic whites for both men and women (37.3% vs. 31.9% for men and 49.6% vs. 33.0% for women) [15].

Obesity is a complex condition. Although obesity is a well-established risk factor for many diseases, including CVD, T2D, musculoskeletal disorders, and some cancers, not all individuals with excess body fat have metabolic disorders, and these individuals are known as "metabolic healthy obese". Interestingly, some individuals with normal weight have an adverse metabolic risk profile, and this refers to a term "metabolic obese". These phenomena suggests that general body fat per se may not be the key to induce metabolic diseases or other adverse outcomes, whereas some regional fat depots, even with a small amount of fat, may play a more important role in the pathophysiology of obesity.

More emphasis has recently been given to African ancestry populations given their high risk of metabolic disorders, including obesity, heart disease, stroke, hypertension and diabetes [16]. Furthermore, African Americans have greater risk of dying from T2D and CVD compared with

the Whites. Due to the increasing proportion of African Americans in the U.S. population, studying the epidemiology of obesity in African Ancestry populations is of great public health significance. Compared with Caucasians, African ancestry populations have lower percentage of body fat for the same BMI. Thus, it has been hypothesized that the ethnic-specific fat distribution might, at least in part, explain the "general fat independent" risk of metabolic diseases. Tobago is a part of twin-island nation Trinidad and Tobago located in the Caribbean. The population is of West African ancestry with less than 6% admixture [17]. The 1990 census reported that 5,100 males aged 40-79 inhabit on the island of Tobago [18]. Study in Tobago could improve our understanding of the epidemiology of general, regional and ectopic skeletal muscle fat in high-risk African Ancestry populations, an understudied population segment.

2.2   GENERAL, REGIONAL AND ECTOPIC SKELETAL MUSCLE FAT

Adipose tissue is a specialized connective tissue and serves as the largest storage site for triglycerides and an endocrine organ for energy hemostasis [2]. According to structural organization, cellular size and biological function, adipose tissue is categorized into different subtypes [3]. Subcutaneous adipose tissue (SAT) is located directly beneath the skin. Based on the anatomical region, SAT can further be subdivided into superficial subcutaneous fat (SSAT), deep subcutaneous fat (DSAT), and the gluteal-femoral adipose tissue, a SAT located in the lower body parts, such as in the thigh. SAT functions as a lipid storage site, which could uptake and buffer free fat acids (FFAs). The dysfunction of SAT could result in the accumulation of excess fat in the liver, heart, muscle, and other non-adipose tissue and organs depots [4].

Ectopic fat is defined as adipose tissue that accumulates in non-adipose tissues and organs other than the subcutaneous depots. Ectopic fat depots could be subdivided according to their anatomical location and their potential systemic or local effect. Ectopic fat depots with predominantly systematic effects are visceral adipose tissue (VAT, or intra-abdominal adiposity), liver fat, skeletal muscle fat and pancreatic fat. Ectopic fat depots with local effects are perivascular fat, pericardial fat, renal fat, etc [5, 6]. In our study we are focusing on the ectopic fat infiltration beneath the fascia, and within and between muscle fibers, which could also be referred to as myosteatosis.

It is important to determine the risk factors relating to the development of obesity and different phenotypes of fat distribution, which could be helpful in identifying the populations with high risk of obesity, especially metabolically unhealthy obesity, and to better understand the pathophysiology of obesity. Moreover, it is also important to identify individuals who will get the most benefit from weight management [19] and from new intervention methods.

2.2.1 Measurements of Body Fat Distribution-Methodology

2.2.1.1 Anthropometric Measurements

Body mass index (BMI) is the anthropometric measurement used in numerous epidemiological studies for body fat, which is calculated as the body weight in kilograms divided by squared height in meters. The World Health Organization (WHO) defines the cutoff points of BMI as underweight (<18.5 kg/m^2), normal weight (from 18.5 to 25 kg/m^2), overweight (from 25 to 30 kg/m^2), obese class I (from 30 to 35 kg/m^2), obese class II (from 35 to 40 kg/m^2), and obese class III (over 40 kg/m^2).

BMI as a simple measurement has some limitations. A meta-analysis showed that the cutoff values of BMI have high pooled specificity (90%) but low pooled sensitivity (50%) in diagnosing obesity [20], which demonstrate that the half of the obese population were misclassified. Besides, BMI cannot distinguish the lean mass from fat mass. Third, the height shrinks with aging process, which makes BMI a less reliable indicator of body fat in the elderly. Fourth, BMI is incapable to identify the age-related body fat redistribution. Thus, BMI per se is not the most appropriate predictor of morbidity and mortality in the elderly [21].

Waist circumference (WC) is an indicator for central fat, which is well correlated with abdominal fat obtained through imaging, especially visceral fat, but costs much less and is easier to perform. WC is measured with inelastic tape on standing participants wearing light clothes at the end of several consecutive natural breaths. However, it is challenging to unify the measurement locations. The common measured locations were summarized as the following: 1) midpoint between the lowest rib and the iliac crest; 2) at minimal circumference; 3) just above the iliac crest; 4) umbilicus; 5) 1 inch above the umbilicus; 6) 1 cm above the umbilicus; 7) at the lowest rib and 8) at the largest circumference [22]. The variations in the measured locations of WC may cause problem. The WHO recommends the use of the midpoints, whereas the National Institutes of Health and National Heart, Lung, and Blood institute recommends the use of the iliac crest. Iliac crest is a bone landmark, which is more stable with weight changes and more reproducible in longitudinal studies, although using this location is less precise and requires more training. It has been reported that different measured locations have a gender-specific relation to VAT, SAT and cardiovascular risk profiles [23]. In men, the association between WC with VAT, SAT and cardiovascular risks was similar across different locations [23]. However, in women, the rib location was associated with decreased VAT induced by weight loss, whereas the iliac crest

location had the lowest association with VAT. Furthermore, in women each WC measured location was strongly associated with SAT compared to VAT [23].

The cutoff points of WC are established corresponding to a BMI of 25 kg/m^2 and 30 kg/m^2, which is 80 cm and 88 cm for women, and 94 cm and 102 cm for men, respectively [24]. However, the cutoff points may differ across ethnicities. The third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) [25] reported that in men, the WC corresponding to a BMI of 25 and 30 kg/m2 is highest in white (91.3 cm and 104.1 cm, respectively), followed by the Mexican American (88.7 cm and 101.2 cm, respectively) and the Black (86.4 cm and 98.8 cm, respectively), whereas in women, the WC cutoff points are not very different across ethnicities. It is worthy noticing that the cutoff points are not based on the predictive effect of WC on CVD risks or mortality. The cutoff points that have clear clinical meaning may be needed.

Waist-to-hip ratio (WHR) is defined as the ratio of the waist circumference to hips circumference. As the WHO recommends, the circumference of the waist should be measured at the midpoint and the hip circumference is measured at the largest circumference of the buttocks with a level parallel to the floor, both are measured while the participant is standing [26]. It has been reported that a WHR of 0.7 for women and 0.9 for men favors health related outcomes. However WHR is difficult to explain biologically since it reflects multiple anatomical entities. Furthermore, two sites need to be measured for WHR, which increase the error of the results.

2.2.1.2 Measurements Using Imaging Techniques

Compared to anthropometric measurements, the development of imaging techniques provides a more precise and sophisticated measurement of regional body fat distribution. The imaging measurements used in studies include Dual-Energy X-ray Absorptiometry (DXA or DEXA), Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Magnetic Resonance

8

Spectroscopy (MRS) and Bioelectric Impedance (BIA). Here we will briefly introduce the DXA and CT measurements which were used in our study.

DXA uses the attenuation of radiation at 2 energy levels to determine different body tissues, which is widely used in assessing bone mass density (BMD) and also considered as one of the "gold standards" for body fat measurement. DXA could also be applied to measure regional body fat distribution. The radiation exposure of DXA is relatively low [27]. However, DXA is reported to underestimate body fat in individuals with low body fat and overestimate body fat in individuals with high body fat in both adults [28] and children [29]. Moreover, DXA is not able to delineate VAT from SAT. Although DXA underestimates the abdominal fat compared to CT measured abdominal fat, the results from DXA and CT are still highly correlated [30]. The use of DXA is not as costly as CT or MRI, and is more feasible and easy applied. Further studies are still needed to support the clinical use of DXA, though the use of DXA for research purposes is well justified.

CT provides the sliced image of any given body area from which the surface and volume can be determined. An attenuation value express as Housfield units (HU) could be read from each pixel of the sliced image, from, which is range from -190 to -30 HU for adipose tissue [19]. CT scan is able to distinguish various adipose tissues in the body, such as intra-abdominal VAT, DSAT and SSAT, and also allows measuring ectopic fat, such as in the liver and muscle [31]. The advantage of CT also includes high reliability and reproducibility [32]. However, the use of CT is limited in large studies due to its radiation exposure and high costs.

2.2.2 Risk Factors for Body Fat Distribution

2.2.2.1 Demographic Characteristics

Advanced age

In general, general body fat increases over adult lifespan [33-36] and might experience a decrease in extreme old age [37]. Cross-sectional studies indicate that total fat or percent body fat decline in individuals aged above 70 years [38]. Longitudinal studies confirmed that general body fat increases with aging, is independent of the body weight change [34, 39], given the offset of total body lean mass loss [40]. The patterns of age-related body weight change and body fat change are different across ethnicities. To be specific, the ages of the peak body weight and the ages of the peak body fat are ethnicity-specific, suggested by cross-sectional studies.

Gender may modify the aging-dependent general body fat changes. Longitudinal studies reported that in men, fat mass increases throughout the lifespan in an approximate rate of 1% [38], whereas in women, fat mass increases until at least age 60-70 and then decreases [34]. Furthermore, the total body fat change may also differ across ethnicities. In a study with a 4-year follow-up, the age-dependent increase of total body fat was greater in normal weight premenopausal African American women than in white women, for a given similar baseline total body fat [41].

During aging process, the fat redistributes to central part of body. Both cross-sectional and longitudinal studies suggest WC increases during aging. The increase of WC is greater in women than in men within the same age and ethnicity. A study has shown that during a 9-year follow-up period [39], WC increases by 4.0 cm in white women aged over 60 and by 0.8 cm in white men. Another study has shown that during a 5-year follow-up [42] WC increases by 4.2 cm and 2.8 cm in black women and men, and by 3.1 cm and 0.2 cm in Hispanic women and men, respectively.

Waist circumference increasing with aging may result from the increase in VAT rather than that of abdominal SAT [43]. Longitudinal study on Caucasians suggests that during aging, SAT (measured with skinfold thickness) declines in both genders [44]

Numerous cross-sectional studies showed that VAT increase throughout lifespan in both genders of all ages and ethnicities. However, the increase of VAT is neither corresponding to the increase of body weight nor to the increase of abdominal fat [42]. The patterns of increases of VAT differ by gender. Cross-sectional study suggested that the increase rate of VAT in middle-aged men is higher than in pre-menopausal women (0.39% per year vs. 0.15% per year) [45]. The increase of VAT accelerates in post-menopausal women and is similar as the men [45]. It is reasonable to hypothesize that hormonal change may explain the transition of VAT increase during menopause. The preventive effect of hormone replacement therapy has been observed in short-term study (12 months) but not in long-term study (2 years) [46].

The accumulation of VAT may be attributable to the progressive inability of storing excess lipid in SAT during aging. The skeletal muscle fat accumulation, which is referred as inter- (IMAT) and intra-muscular fat, accumulates during the aging process with the increasing range of 9 grams/year [47] to 70 grams/year [48], and independent of the change of total weight or SAT [49]. However, most of the current studies are of cross-sectional design. Health, Aging and Body Composition (Health ABC)study was the first longitudinal study to confirm a constant increase of skeletal muscle fat infiltration (inter-muscular fat) with aging after a 5-year follow-up，increase which was similar between ethnicities (Black vs. White) [49]. However, the participants of the Health ABC were recruited to be older, healthy and very high-functioning (aged 70-79 at the baseline), and similar studies are needed in a large, prospective study among African ancestry individuals recruited across a wide age range and without regard to their health status. Physical

activity and sedentary lifestyle may modify the relation between skeletal muscle fat infiltration and aging, which needs to be accounted in the future study.

Gender

Generally, women have a greater total body fat percentage than men [50]. Normally, the range of body fat for women is 20-25%, and that for men is 10-15% [51]. Although the cutoff points of BMI are not gender-specific, WC and WHR both have different cutoff points for each gender.

The regional distribution of fat is different by gender. Before menopause, women have more gluteal-femoral fat, and 50% less VAT fat, compared to men [52]. Despite of fat free mass (FFM), women have less DSAT and more SSAT than men [53]. Women also have less ectopic liver fat accumulation compared with men. The gender difference in the impact of liver fat accumulation may due to the greater accumulation of VAT in men. The gender difference might due to the difference in basal fatty acid oxidation, in regional regulation of lipolysis (mediated by catecholamine and insulin) and in postprandial fatty acid storage [50]. Furthermore, the impact of gender on epicardial fat is consistent. Cross-sectional study showed that epicardial fat is greater in men [14, 54] or otherwise [55]. Recently, none of the study has reported that gender difference on the accumulation of ectopic skeletal muscle fat.

Ethnic difference

Based on the cutoff points of BMI, the prevalence of generalized obesity and overweight were higher among African Americans [6] than white Americans. Furthermore, within the same level of BMI, age and gender, black women have a lower total body fat percentage compared to the white women [7]. Since the degree of body fatness matters for the disease development in

physiological view, it has been long debated whether ethnicity-specific BMI cutoff points for metabolic diseases are needed. Furthermore, different ethnicities had different fat distribution with the increases of total fat (e.g. the accumulation of VAT, SSAT, DSAT and liver fat varies) [56]. African American women have lower SAT compared with the white women [6], whereas, African American men have higher SAT [6]. Compared to white men, black men have less visceral [8] and liver fat [9, 57], independent of their obese status. However, black men have greater accumulation of skeletal muscle fat [10].

2.2.2.2 Lifestyle Factors

Diet

The excess storage of body fat could be attributed to the positive energy balance, which may due to the over intake of calorie from diet. However, the dietary factors are very complicated. The food groups, the macronutrients and eating pattern interact together in driving health related issues. The dietary factors, including but not limited to fiber intake, high fat diet, protein intake and sugar sweetened beverage, directly or indirectly contribute to changes in body weight and regional body fat accumulation and distribution.

General calories restriction induced weight loss is related to the reduction of VAT and SAT, preferentially VAT in both men [58, 59] and women [60]. However, energy-reduced diets based on restriction of fat, protein or carbohydrate also induce similar changes in body weight [61], abdominal fat, VAT, SAT or hepatic fat [62].

High fat diet is positively associated with obesity and body fat, especially VAT [63]. The positive association between dietary fat intake and weight gain seems to differ by age, gender, health status, activity levels and genetic predisposition [64]. Furthermore, the type of dietary fats

contributes differently to the structure and function of fat [65]. Higher intake of saturated fatty acids (SFA) is associated with higher percent whole body fat mass and trunk fat in cross-sectional study of population-based sample aged over 65 [66]. In contrary, higher intake of mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) is reported to be associated with more favorable insulin sensitivity and inflammatory profiles. There is scarce evidence on the impact of MUFA and PUFA on the body fat distribution.

The greater consumption of carbohydrate-containing food is associated with obesity and cardiometabolic diseases. Recent studies indicate that via moderate low-carbohydrate diet (M-LCD) both men and women experience greater reduction of VAT than that of SAT [67, 68]. After adjusting for age and energy intake, the reduction of VAT in men induced by M-LCD was only observed during the early stage (3 months) [67].

Traditionally, studies focusing on the macronutrient components: protein, carbohydrates and fat, suffer from many methodological issues. For observational studies, the measurement errors and residual confounding are inevitable. For clinical trial, the results are limited from short duration of follow-ups, small sample size and inadequate adherence rate. Currently, studies on the dietary pattern, foods and food groups are making progress. There is a possible link between the sugar-sweetened beverages consumption and obesity. Longitudinal data supports the positive association of increased consumption of sugar-sweetened beverages and weight gain in younger and middle-aged women [69], Black women [70], Caucasian men [71], and offspring of diabetic individuals [72] even after adjusting for other lifestyle factors.

Cereal fibers are reported to prevent metabolic disorders, such as T2D and CVD, which may be related to its impact on body fat and fat distribution. Cross-sectional study in elder Caucasians [73] indicates that higher consumption of whole-grain sources is negatively associated

with BMI, total body fat and trunk fat among the elderly, independent of other healthy lifestyle factors. Health Professional study, a longitudinal study among middle-aged and elder men [74] suggests that whole-grain may be inversely associated with weight gain. A 12-weeks intervention study [75] showed that cereal fibers may alter the distribution of body fat, especially decrease the percentage abdominal fat, independent of total body weight change. Recently, Framingham Heart Study [76] showed a cross-sectional association of increased intake of whole grain with lower VAT and SAT, whereas increased intake of refined-grain is associated with greater VAT. Several questions need to be addressed in the future: the potential mechanisms for the protective effect of cereal fibers, the health impact of different cereal fiber types, and the effect of cereal fibers on the longitudinal changes in body fat distribution.

Alcohol Consumption

Alcohol consumption is an important risk factor for obesity. Ethanol is the macronutrient with the highest energy density (7.1 kcal per gram) beside fat. Ethanol is metabolized immediately after digested. Ethanol has pharmacological impact on nervous system, primarily by activating γ-aminobutyric acid receptors, which is associated with the regulation of appetite [77]. The effect of ethanol on body fat distribution is controversial. Many factors may confound or modify the association of alcohol consumption with body weight and body fat distribution, including food intake, physical activity, drinking behaviors, gender, and type of drinks. Thus, this is a complicated topic that needs further investigation.

A systematic review [78] on the alcohol consumption and body weight revealed the contradictory results from the cross-sectional, longitudinal and experimental studies. Weight gain or increased abdominal obesity is often observed in heavy drinkers. Weight loss or lower body fat

mass is often related to chronic alcohol drinkers. Light-to-moderate alcohol intake, especially wine, protects weight gain.

Physical Activity

Physical activity is a main source of energy expenditure and plays an important role in energy balance. The impact of physical activity on fat mass could be both acute and long-termed, including increased blood flow, fat mobilization, and reduced fat storage. However, the observational studies on the association of physical activity with general, regional or ectopic fat suffer from the possible bias due to the common use of self-reported data. Furthermore, the physical activity is multifaceted by intensity, type, duration, and so forth, which increase the difficulty in measurement.

Experimental studies indicate that physical activity interventions induce weight loss, though with a relatively small effect size, which may be attributable to the intensity of activity. However, when we take regional fat depots into account, even a slight reduction of some of the significant fat depots may play an important role in disease prevention. It is reported that VAT and/or SAT reduce preferentially by physical activity, which is without concomitant with total weight loss [79], which may be an explanation of the "weight independent" effect of physical activity on metabolic disorders. In contrast , emerging evidence suggests that this preferential or selective effect should be attributed to different or even less accurate measurement across studies [80, 81].

The reduction rate of VAT may depend on the intensity of exercise. It was [82] suggested that 10 MET hours per week is the minimum requirement for effective loss of VAT in non-metabolic disordered participants. An alternative explanation is that the threshold of physical

activity can induce detectable VAT loss. Mechanically, VAT is sensitive to the adrenal-driven adipocyte lipolysis that occurs with vigorous exercise [83].

As to abdominal SAT, the greater loss is observed among men [59, 84-86], whereas as to thigh SAT, the greater loss is observed among women [86]. Furthermore, the regional fat loss in overweight men may be different by age groups [87]. In the elderly, the greater loss is observed in abdominal subcutaneous fat, whereas in the younger men, the greater loss is observed in thigh subcutaneous fat.

Evidence also suggests that physical activity is associated with IMAT. In a 4 week study [88] where one leg was immobilized in healthy participants, IMAT increased in the immobilized limb independent of reduction in whole body mass. In a 1-year intervention study [89], physical activity intervention protected the elderly from IMAT increase. However, the effect of physical activity on IMAT may depend on the intensity, which may need to be high enough to cause detectable change of IMAT. Indeed, a moderate physical activity intervention showed no association with IMAT in experimental [80] or observational [90] studies. However, experimental studies suffer from short duration of intervention and limited adherence. Thus, longitudinal studies with more precise physical activity assessment may be more suitable to address these issues.

Sedentary Lifestyle

Sedentary lifestyle is associated with the development of obesity, and is an independent risk factor for cardiovascular disease, hypertension, diabetes, metabolic syndromes, coronary heart diseases and mortality, even after adjustment for physical activity. Interestingly, BMI could only partly explain the association between sedentary lifestyle and cardiovascular factors, which suggests that the regional fat distribution may also be a crucial mediator for the adverse impact of sedentary lifestyle on health outcomes.

Longitudinal studies indicate that increased television watching time is associated with the increase of WC, after accounting for physical activity and other covariates [91]. However, these findings may not be attributed to VAT since studies showed an insignificant association between sedentary lifestyle and abdominal VAT in middle-aged [92] and elder [90] populations.

Sedentary lifestyle might be associated with other ectopic fat depots than visceral fat. A study [67] of 539 diverse elderly showed that greater time spent in leisure sitting is associated with the greater pericardial fat, independent of physical activity, demographic characteristics, CVD risk factors, BMI, and inflammatory markers. Contrary to these findings, another study showed that the association between pericardial fat and sedentary lifestyle became insignificant after adjusting for moderate-vigorous physical activity [93]. Thus, the association between sedentary lifestyle and ectopic fat might be different by the intense of physical activity. . A threshold of the physical activity might exist to effectively prevent the adverse impact of sedentary lifestyle.

No association of SAT and other ectopic fat, with leisure sitting time were observed in cross-sectional studies [67]. The causal link between ectopic fat and sedentary lifestyle has not been established. Current studies are limited by use of self-reported measurements and relatively small sample size. Thus, a well-designed longitudinal study, with objective measurements on sedentary lifestyle and physical activity is warranted.

2.3 ASSOCIATION WITH MORTALITY

Obesity is not only a major risk factor for metabolic disorders, but also plays an important role in premature mortality. Numerous studies have reported a positive association between increased

weight and elevated risk of mortality. However, emerging studies have observed that mild overweight, even obese in some sub-population favors survival, which is referred as "obesity paradox". Evidence also suggests that 30% overweight or obese subjects are "metabolically healthy". The phenomenon indicates that instead of general adiposity per se, the distribution of fat also contributes to the development of metabolic diseases and mortality. Several important questions remain unanswered. In particular, the independent impact of general, central and ectopic fat on mortality risk and the mechanisms that can explain the increased risk of mortality induced by excess weight and fat distribution are largely unknown. With the aging of the U.S. population, it is crucial to identify the high-risk populations, and furthermore, to discover the mitigating factors. We will review the existing literature on this topic to describe the main findings, methodological issues, and possible mechanisms.

2.3.1 Methodological Issues

Here we introduce the methodological issues related to the studies on the association of BMI with mortality, which could also be observed in studies on mortality risks using other fat measurements (eg. WC, WHR, imaged measured fat depots).

2.3.1.1 Reverse Causation and Obesity Paradox

Reverse causation is a phenomenon that obesity-induced diseases lead to both lower BMI and higher risk of mortality, and lead to an underestimation of the true association between BMI and mortality. In this case, low BMI is a result of disease rather than the cause. Specifically, the weight loss is either caused by disease per se or by intentional behavior changes given the awareness of disease. Thus, the study on the effect of obesity on mortality is complicated by the

19

high heterogeneity of the lean population, which includes healthy active population, smokers, and population with conditions leading to unintentional or intentional weight loss. Reverse causation has stronger impact when the study population is older because of the accumulation of chronic conditions and high prevalence of comorbidities.

Reverse causation may bias the association study between obesity and mortality among populations with wasting conditions, including congestive heart failure, end-stage renal disease [94], advanced malignancies, COPD, cancer [95], AIDS and the elderly [96-99]. In such conditions, moderate over-weight, and even obesity favors the survival, which is referred as "obesity paradox". In addition, traditional cardiometabolic risk factors favor survival among populations with such wasting conditions. One possible explanation is that adipose tissue is a reserve depot of nutrition and energy and protects individuals during exposure to acute indults or chronic wasting, that people in low-normal range of BMI might not be benefit from [100]. Another explanation is survival bias, which means that the survived individuals with obesity or overweight may have characteristics to resist the adverse impact of excess adiposity.

"Obesity paradox" might not apply to certain conditions or cause-specific mortality, such as among patients with cardiometabolic related disease or mortality [101, 102], which means that among these individuals greater BMI level is associated with higher risk of mortality. Paradoxical BMI-mortality risk association may interact with fitness level since among high-fit individuals lower BMI levels are not associated with higher risk of premature death [103]. Whether "obesity paradox" is an artificial association caused by methodological issues or a real relation reflecting biological mechanisms is still unclear.

Previous studies presented several ways to minimize the bias caused by reverse causation, including depletion of the early deaths within the first few years of follow-up, or exclusion of those

who have cardiovascular disease and cancer at baseline. However, neither of the methods is perfect in solving reverse causation. Many of the conditions that lead to weight loss may take years to the final mortality, such as cirrhosis, COPD, and some neurodegenerative diseases. Moreover, individuals may have undiagnosed diseases or preclinical conditions, which could not be detected and eliminated from baseline. Allison et al suggested that excluding early death has a minimal magnitude in effect, and has a questionable and ambiguous clinical meaning (ref).In addition, it will induce bias because of decreased overall age of participants and reduced statistical power. Thus, there is still no conclusive way to solve the system error caused by reverse causation. However, a longer follow-up may always be a better method. In the study conducted by Berrington et al [104], the association between underweight and increased mortality weakened after 15 years of follow-up in comparison with 5-years of follow-up (HR 1.21 vs. 1.73), since preexisting disease may be under-diagnosed or subclinical in the early year of follow-up.

2.3.1.2 Smoking Habits as Confounders

In the past, smoking was considered as a major confounder since smokers tend to have lower weight, but much higher mortality rate, which leads to an artificial association between lower weight and elevated mortality risk. Given the variations of smoking duration, amount and degrees of inhalation, statistical adjustment for smoking status is inadequate to reduce the confounding induced by smoking. Past smoking is also a concern since the quitting duration impacts the mortality risk. The association between lower range of BMI and mortality was stronger among former smokers who quit smoke in less than 20 years than among current smokers [14]. Some evidence suggest the U-shaped relation between BMI and mortality could be partly attributed to confounders, such as smoking [105]. Studies that are restricted to never-smokers

found a stronger association with high BMI levels and an attenuated association with low BMI levels than do studies that include the current- and past- smokers [105-109].

However, a meta-analysis recently [110] opposes that smoking is an important source of bias since many studies that excluded or adjusted for smoking had little or no effect on the association between BMI and mortality, especially in the overweight category. The study also claims that over-adjustment of smoking or other factors in the causal pathway attenuates the association of mortality with obesity, but not with overweight. The Prospective Studies Collaboration [111] suggested that the negative association between BMI and mortality in the lower range is mainly due to smoking-related respiratory disease and lung cancer, which partly explained the necessity of adjusting for smoking in the lower range of BMI.

2.3.1.3 Over-Controlling for Intermediate Variables in the Causal Pathway

Incident hypertension, diabetes and dyslipidemia are known to be biological intermediates in the pathway of obesity to mortality. Controlling for these factors usually reduces the strength of association between obesity and mortality and underestimates the overall effect of obesity on mortality. Thus, studies that adjust for incident hypertension, diabetes and dyslipidemia should be used to estimate the mediation degree of these intermediates, but not to reflect the overall effect of obesity on mortality.

2.3.1.4 Imperfection of Adiposity Measurements

Many epidemiologic studies use self-reported height and weight to calculate BMI values, which may induce systematic bias [110], since the prevalence of obesity may be underestimated by over-reported height and under-reported weight. The misclassification of subjects into incorrect BMI categories based on self-reported data substantially resulted in systematic errors, which are

difficult to correct. The errors caused by self-reported data were different by demographic characteristics (e.g., age, sex, race), measured values and data collection method [110].Meta-analysis showed that studies using self-reported data is more heterogeneous and have higher summary, compared to studies using measured data [110].

It is widely accepted that BMI is not a good proxy of adiposity, since it fails to delineate fat-free mass from fat mass. For instance, with a BMI of 30 kg/m^2, total body fat of men varies from 23% to 41%, and that of women varies from 30% to 51% [112]. The sensitivity of classifying body fat mass with BMI is poor and decreases with aging [113]. For the higher range of BMI, the sensitivity of classifying a higher body fat mass is only 20-50% [113]. The wide variance leads to misclassification of muscular subjects with more lean mass as overweight [114].

The reliability of BMI as an indicator of general adiposity of body decreases with aging process. Height shrinkage during aging is one possible reason that leads to an over-estimation of the BMI. Moreover, lean mass decreases gradually and adiposity mass increases with centralization during aging, whereas BMI is incapable to reflect such changes and redistribution in body composition. Some fat depots are considered to be more deleterious for metabolic diseases compared to others, such as intra-abdominal fat or liver fat. Thus, same BMI in middle-aged or older population may have different clinical importance. .

2.3.1.5 Survival Bias

In the study of the elderly, the depletion of the susceptible individuals may lead to survival bias, since those who have pre-existing disease, obesity-related disease or illness which may cause weight loss are more predisposed to die before reaching the age of 65 and naturally be excluded from the study. Those who are included in the study of obesity-related mortality may have characteristics that could resist the adverse impact of excess adiposity. Thus, the association

between obesity and mortality among the elderly may be underestimated because of the exclusion of the premature death form the cohort.

2.3.2 Anthropometric Measurements and Mortality Risk

The relation between BMI and mortality is reported to be U-shaped, J-shaped, or linear. The largest proportion of deaths (over 80%) occurred in persons with BMI of at least 30 [115]. Many studies are in agreement of an optimal range of BMI from 20 to 25 kg/m^2 for lowest mortality risk in population free of smoking or preexisting diseases [14, 116], or after eliminating of the first 5 years follow-up [54]. The discrepancy of the curve shape may be attributed to methodological issues or different cause of death.

The Prospective Studies Collaboration [54] further examined the association between BMI and cause-specific mortality. In the upper range of BMI (BMI>25 kg/m^2), after adjustment for smoking status (additive effect), higher BMI was associated with 30% greater risk of overall mortality, 120% for diabetic mortality, 82% for hepatic mortality, 60% for renal mortality, 40% for vascular mortality, 20% for respiratory mortality and 10% for neoplastic mortality. In the lower range (BMI<22.5 kg/m^2), BMI was negatively associated with overall mortality, which may attribute to smoking-related respiratory disease and lung cancer. These partly explained the necessity of adjustment for smoking in the lower range of BMI.

Physical activity is reported to improve obesity-related risk factors, such hypertension, lipid profiles and blood glucose and is a predictor for mortality independent of body composition [55, 71]. However, evidence from a meta-analysis suggests that the association between BMI and increased risk of mortality is independent of physical activity. Comparing the highest BMI category with reference group, the summarized RR of all-cause mortality is 1.23 (1.18-1.29) with

adjustment for physical activity, and 1.24 (1.21-1.28) without adjustment for physical activity [72]. The variation of physical activity measurements and duration across studies makes the independent association between BMI and mortality still controversial.

Age is the most important modifier on the association between BMI and mortality. Cancer Prevention Study II [117] revealed that with increasing age, the relative increase of mortality risk in higher BMI range declines. The aging related attenuation could be explained by reverse causation and survival bias. Furthermore, BMI is less reliable in measuring adiposity among the elderly. Besides, high prevalence of baseline risk factors for mortality dilutes the impact of individual risk factors. The association between a high BMI and mediators in the pathway to mortality, such as dyslipidemia, hypertension and insulin resistance [96], also weakens during aging process.

Despite the attenuation, obesity is still a crucial risk factor for mortality in the elderly, since the absolute increases in death rates with increasing BMI was greatest in the elderly men and women. Thus, it is with great importance to detect the optimal BMI ranges that favor survival for the elderly, which may be different than the younger and middle aged population. Among younger and middle-aged adults, studies are in agreement that mortality risk increases at low and high BMI ranges with the optimal BMI is 18.5-24.9 kg/m^2 [118, 119]

Though the adverse effect of lower BMI is clear among the elderly (age>65 years), the relationship between high end of BMI and mortality is inconclusive [120]. The optimal range of BMI which favors mortality is less clearly defined. Most studies reported a U-shaped with higher optimal BMI values compared with younger population or J-shaped associations [121]. Other studies showed inverse linear or no excess risk of mortality with the increase of BMI [114, 122-124], which means BMI may only have lower cutoff points for survival. Evidence supports that

25

BMI of 25-27 kg/m^2 is not a risk factor for all-cause mortality or cardiovascular mortality among population aged over 65 [125]. The optimal BMI among older subjects is suggested to be shifted upwards, with ranges from 27-31 kg/m^2 [116, 126, 127]. Thus, the recommendation of a relatively lower BMI for survival benefit is not appropriate for the elderly [124].

It is controversial whether gender is a modifier on the association between BMI and mortality. Individual studies revealed that the association between BMI and all-cause mortality differs by gender. Among the elderly, being overweight favors survival in men, but not in women [128, 129]. However, results from a recent meta-analysis [110] including 97 studies suggest otherwise, that both genders, of all age or only age over 65 showed similar association between all-cause mortality and overweight or obesity (measured or self-reported).

The association between BMI and mortality may also be race/ethnic-specific. However, most of the studies have focused on the U.S or European Caucasians. The association between BMI and mortality in other ethnicities, especially African ancestry populations, is less well defined. It is suggested that increased BMI has lower impact on African Americans than Caucasians [117], which means that moderate overweight may not increase mortality risk in African ancestry populations as it does in Caucasian populations. In addition, severe obesity was associated with elevated risk of mortality to a less extent than in Caucasians.

In summary, the U-shaped association of BMI with mortality may be due to methodological issues. The effective way to adjust for smoking and preexisting diseases is still controversial. It is important to evaluate whether the association of BMI with mortality is independent of physical activity. Also, the distribution of body fat and ectopic fat should also be taken into consideration. Thus, a well-designed, longitudinal study is warranted with multi time measured points of BMI and other adiposity measurements.

Since BMI simply reflects the general body fat, WC and WHR are convenient and economical way to measure central fat. Compared to general body fat, central body fat is more deleterious to health related outcomes and mortality [123]. The deleterious impact could be independent of general body fat [129]. The studies on the association of mortality with WC and WHR also suffer from the methodological issues mentioned above.

When WC is treated as a continuous variable, a strong linear association with mortality is shown in both genders [130, 131]. The association between WC and mortality persist in the very high end [132]. However, the optimal cutoff points of WC for mortality are still unclear. Studies using different arbitrary cutoff points showed different results. A pooled analysis in 650,000 adults showed 52% increased risk of mortality in men (HR (95% CI): 1.52 (1.45-1.59)) for WC of $\geq$110 vs <90 cm) and 80% increased risk in women (HR (95% CI): 1.80 (1.70-1.89) for WC of $\geq$95 vs <70 cm) after adjusted for BMI [130]. In contrary, when using clinical cutoff points (<94 cm, <102 cm and $\geq$102 cm for men, <80 cm, <88cm and $\geq$88 cm for women), no association is observed between greater WC and increased mortality risk in the older adults (aged over 55 years) [121, 133].

Furthermore, the optimal cutoff points of WC on mortality risk might be gender specific, which could be different than clinical cutoff points. Study [134] showed that with clinical cutoff points, the positive association between WC and mortality only existed in nonsmoking men, but not in women. The difference in the association between WC and mortality by gender might due to the late onset of abdominal obesity among women compared to men. The redistribution of fat from the depots around the hips and buttocks to the abdominal depots usually be observed after the menopause among women [135]. The gender difference might also be attributed to the visceral fat, which are greater preserved in men than in women.

27

Intendent of gender, age may also modify the association between WC and mortality. Studies showed that in the elderly, greater WC was associated with lower mortality risk in both genders [131], or only in men [128]. The inconsistent association in the elderly compared with that in younger and mid-aged population may due to the survival bias. The individuals who survive to an older age and are included in the cohort might resist the adverse impact of central obesity. Being mild-grade central obesity may even be favorable to individuals with certain health condition [129], since the individuals with lower WC may suffer from wasting conditions, yet have an increased risk of mortality. Naturally, the WC increases with aging process. In the elderly, WC corresponding to BMI of 25 and 30 kg/m^2 is higher compared with that in the younger population. Again, the association between central fat and mortality might be complicated among the elderly.

Ethnicity may be associated with different distribution of fat and predisposition to obesity related disorders. Previous studies are predominately focused non-Hispanic Whites. Studies in the minorities are scarce. A 9-year longitudinal study [136] showed no difference in WC associated mortality risk across ethnicities, which needs further confirmation in future studies.

The studies on association between WC and cause-specific mortality are inconclusive. Some studies reported insignificant association between WC and circulatory mortality [121], coronary heart disease related mortality [101] or cancer mortality [101]. In contrast, the Canadian heart health survey concluded that WC best predicted CVD risk factors [137] and CVD mortality [138]. WC was also associated with cancer mortality [138], though in much weaker degree compared with CVD and respiratory disease [130].

Smoking and preexisting diseases are confounding the association between WC and mortality. Smokers tend to have a metabolically more adverse fat-distribution profile, such as greater central adiposity. If smoking is not taken into account, the role of WC on mortality might

be underestimated [134]. Likewise, the positive association between WC and mortality were mainly observed in those with worse health conditions and functional limitation [139]. Thus, same methodological issue is that whether and how to effectively adjust for smoking and preexisting diseases.

Another issue in WC studies is the high correlation with BMI. Many studies reported underpowered results on the association between WC and mortality risk independent of BMI. However, with enough sample size, adjustment for BMI could increase the linearity and strengthen the association of WC and mortality by simultaneously controlling preexisting diseases, pathologic conditions, or general frailty [130]. The studies on the association between WC and mortality within BMI categories reported that in Caucasians, increased WC was associated with elevated risk of mortality at all levels of BMI from 20-50 kg/m^2 [130, 132]. In women, the strongest association between WC and mortality was observed in the category with normal BMI [132]. Generally, the joint of BMI and WC provide better prediction on the risk of morality.

In summary, the association between VAT and mortality is a relatively novel area of research in obesity field with only a few studies conducted for far. The impact of age, gender, and ethnicity on this association has not been fully addressed. The risk factors and modifiers for the accumulation of VAT should also be studied.

Liver Fat

The population with increased liver fat accumulation are considered either a 'healthy obese' or 'metabolically unhealthy obese' [140-142]. Mechanically, the liver fat accumulation might induce insulin resistance through hepatic diacyglycerol, which results in impaired activation of downstream insulin signaling and increased reactive oxygen species and increased production of pro-inflammatory cytokines and very-low-density lipoprotein [143]. Unlike the certain

associations of Non-Alcohol Fat Liver Disease (NAFLD) with the development of hepatocellular carcinoma and cardiovascular risk factors, the association of NAFLD with mortality is still controversial and inadequately studied. The recent studies were limited by their small sample size, selected patient population (for instance, patients receiving liver biopsy), use of only surrogate markers of NAFLD such as liver enzymes, and a lack of other fat depots. The comparison across studies is difficult since the definitions and measurements of liver fat accumulation are inconsistent.

A longitudinal study using the NHANES III data [144] found that NALFD (with and without liver enzyme increase) was not associated with all-cause, CVD mortality, and cancer mortality in US population aged 18-74 years. NALFD was defined as the presence of moderated to severe hepatic steatosis (measured with ultrasound) with normal liver enzymes level.

In contrary, a community-based study among 3,543 Chinese adults [145] revealed that the NAFLD patients had higher annual all-cause mortality (0.54% VS. 0.19%) and CVD mortality (0.54% VS. 0.17%) after a 4-year of follow up. The NAFLD was defined based on the guidelines of the Chinese Liver Disease Association, with the combination of abdominal ultrasonic findings with medical history, clinical symptoms, and laboratory. Similarly, another longitudinal study among Caucasians [146] reveled that NAFLD was independently associated with all-cause (HR (95% CI): 1.98 (1.21-3.27) and CVD mortality 2.41 (1.05-5.55) only among men. The study used elevated gamma-glutamyl-transferase (GGT) levels (>80%) and the presence of hyper echogenic liver ultrasound to define NAFLD

In conclusion, in the studies published thus far, the results on the association between NAFLD and mortality risk have been inconsistent. There are many unsolved questions on the association between NAFLD and mortality risk, such as the prevalence of NAFLD in different

populations by the consensus definition, and the impact of liver fat on mortality independent of other fat depots by age, gender and ethnicities.

Pericardial Fat

Pericardial fat is associated with CVD risk profiles and prevalent CVD disease. Thus, it is biologically plausible to assume that pericardial fat may be associated with mortality risk. However, only two studies have focused on this topic. The Framingham study [147] revealed that pericardial fat was not associated all-cause mortality after 5 years of follow-up after adjusting for important covariates such as age, sex, comorbidities, smoking status, and BMI. In contrast, the Rancho Bernardo Study [148] showed that the highest tertile of pericardial fat is significantly associated with increased all-cause mortality risks after 12 years of follow-up. The association between pericardial fat and mortality was not different between genders.

The studies above mentioned are limited due to the small number of outcome events (71 and 49 deaths by each study, respectively), which may undermine the power to detect a real association. Furthermore, the weight change during the follow-up was not taken into consideration. Given the fact that the findings are based on predominately Caucasians, the association of pericardial fat with mortality risk should also be addressed in in other ethnicities. .

Skeletal Muscle Fat Accumulation

The studies on the association between the ectopic skeletal muscle fat accumulation and mortality risk are scarce with inconclusive results. Walking and Leg Circulation Study II (WALCS II) [149] recruited 434 patients with lower extremity peripheral arterial disease (PAD) who were followed up for 47.6 months. Lower calf muscle density (indicative of greater intra-muscular fat) was significantly associated with higher all-cause mortality (HR (95% CI) for lowest tertile: 1.80

(1.07-3.03), 2nd tertile: 0.91 (0.51-1.62), highest tertile: 1.00, P-trend=0.020), independent of age, sex, race, comorbidities, smoking, BMI, physical activity and ankle-brachial index (ABI). Similarly, the Osteoporotic Fractures in Men (MrOS) Study [150] among 1138 community dwelling older men with a mean age of 77 years also revealed that lower calf skeletal muscle density significantly increased the risk of all-cause mortality by 17% during a 7.2 years follow-up. Recently, The AGES-Reykjavik study on Icelandic individuals aged 66 to 96 years revealed that the greater thigh inter-muscular fat was independently associated with elevated mortality risk in men, but not in women [151].

Contrary to these studies, no association between greater calf muscle density with all-cause mortality was found after adjusting for height, weight, age, gender, comorbidities and other covariates in the "Invecchiare in Chianti" (InCHIANTI) study, a prospective population-based study among Italians (N=934) older than 65 years after 6 years of follow-up [152].

This novel topic has not been adequately studied and the results are limited to the highly selective populations and relatively small sample size. Furthermore, African American populations which have a greater inter-muscular fat [153] and a higher risk of T2D related mortality compared with Caucasians should be given more attention, as recent studies were predominantly focused on Caucasians and Asians. In addition, age is a major modifier in the association between inter-muscular fat and mortality, since inter-muscular fat increases with aging [154]. However, the existing studies are focusing on the elderly and the results may not be applied to the younger population. Finally, the association between inter-muscular fat and mortality risk may be gender-specific, which needs confirmation in future studies.

The studies on the body fat distribution and mortality risk are warranted to clarify and reinforce primary targets for obesity reduction and promote the development of public health policy and clinical recommendation [155].

2.3.3   Weight Change

Many studies have linked weight change to increased risk of mortality, especially among the elderly. However, several issues have to be noted. First, in non-experimental settings, weight change is usually caused by existing- or preclinical chronic conditions indicating difficulty in maintaining hemostasis. Specifically, weight loss may be caused by wasting or lethal disease, whereas weight gain may be caused by disease such as edema in the heart failure. Thus, a reverse causation is an inevitable limitation in these studies. Smoking status and other residual confounders may also bias the association between weight change and mortality. Second, weight change also leads to redistribution of body fat and to changes in body composition. Studies showed that weight cycling was associated with body fat centralization [156, 157] and net loss of lean tissue, since the lean mass lost during period of weight loss is not completely regained [158, 159]. Third, intentional and unintentional weight change could have different impact on mortality. Fourth, the definition of weight change varies from change of 3% to 5% or 5 kg, which makes the findings incomparable.

In middle-aged population, the long-term impact of weight change on mortality is not clear. The British Regional Heart Study [160] showed that the association of weight loss (defined as ≥ 4% loss) or weight-gain-and-loss (defined as ≥ 4% gain and ≥ 4% loss) with CVD or total 8-years mortality was attenuated after adjusting or excluding the men with pre-existing disease. In contrast, the Erfurt Male Cohort Study (ERFORT study) [161] showed that weight fluctuation during 15

years with 4 times measurements (defined as initial and final BMI differed by <3 kg/m2 and/or the sum of absolute deviation were > 3.49 BMI unit) was an important risk factor for all-cause mortality (HR (95% CI): 1.86 (1.31–2.66)) after adjusting for age, pre-existing cardiovascular disease or diabetes mellitus, smoking and socio-economic status. These recent studies suggested that middle-aged individuals with disease burden suffer more from the adverse impact of weight change.

Among the elderly, the positive association between weight change and mortality is clearer. The Cardiovascular Health Study [162], revealed an increased risk of mortality with weight loss (defined as over 5% loss of body weight) (HR (95% CI): 1.58 (1.33-1.88)) and weight cycling (defined as over 5% loss/gain of body weight followed by over 5% gain/loss of body weight) (HR (95% CI): 1.66 (1.38-2.00)) , but not with weight gain. Similarly, The Dubbo Osteoporosis Epidemiology Study [163] showed that weight loss (defined as annual weight loss at least 1%) ((HR (95% CI) for men: 2.6 (1.9-3.7); for women 2.2 (1.7-2.9)) and weight fluctuation (defined as at least 3% of CV) ((HR (95% CI) for men: 1.5 (1.1-2.0); for women 1.3 (1.0-1.7)) were significantly associated with increased all-cause mortality among both men and women. Though the weight change definitions were different across studies, the results showed a robust adverse impact of weight loss and weight fluctuation on all-cause mortality among the elderly.

Moreover, lack of information on body composition changes and redistribution during weight change poses a major issue in the studies among the elderly. The Osteoporotic Fracture in Men Study (MrOS) study [120] found a higher risk for men with weight loss (HR (95% CI) 1.84 (1.50–2.26), total lean mass loss (1.78 (1.45–2.19)), and total fat mass loss (1.72 (1.34–2.20)) than that for men who were stable for each body composition measure, after accounting for baseline lifestyle factors and medical conditions. Men with total fat mass gain had a slightly greater

34

mortality risk (1.29 (0.99–1.67)) than those who remained stable. These associations were not different by baseline age, obesity, or self-reported health status. However, the results were limited to men and did not reveal how body composition change modifies the association between weight change and mortality.

The Health ABC study [164] further examined the mitigating effect of body composition change on the association between weight change, and mortality among community dwelling, initially well-functioning women (N=1044) and men (N=931) aged 70 to 79. Weight cycling and weight loss significantly predicted mortality, independent of smoking status, comorbidities and other risk factors. However, when percentage change of lean mass or fat mass was taken into account, the association between weight cycling (HR (95% CI): 1.55 (1.07-2.23) for women; 1.15 (0.80-1.65) for men) or weight loss (HR (95% CI):1.67 (1.19-2.34) for women; 1.09 (0.81-1.47) for men) and mortality was attenuated. The results indicate that the change of body composition during the period of weight loss or fluctuation may contribute to the association between weight change and mortality. Furthermore, the mitigating effect of body composition change on weight change induced mortality is more important among men compared with women.

Emerging evidence suggests that intentional and unintentional weight loss have different effects on mortality. The Iowa Women's study [165] showed no association between intentional self-reported weight loss (over 9.1 kg) during adulthood and the risk of overall or cardiovascular disease mortality in postmenopausal women. However, unintentional weight loss (over 9.1 kg) increased total mortality risk by 26-57% and cardiovascular disease mortality risk by 51-114%. The association was more profound among those with health conditions.

The Cancer Prevention Study II Nutrition Cohort [166] confirmed that low levels of weight cycling (intentional loss of 10 or more pounds (≥4.5 kg)) was associated with increased risk of

mortality (1–4 (RR (95% CI): 0.93 (0.89-0.97)), or 5–9 (0.88 (0.81-0.94)) weight cycles in men and 1–4 (0.93 (0.89-0.98)) weight cycles in women) in borderline significance. No association was observed between high numbers of weight cycles (($\geq$20 cycles) and mortality. However, the lack of information on the timing, magnitude, and duration of each phrase of weight cycle limited the findings. Another study [167] focused on middle aged or older women failed to show that mild (RR (95% CI): 0.83 (0.75-0.93)) or severe (RR (95% CI): 0.89 (0.77-1.04)) level of weight cycling initiated by intentional weight loss increase the risk of all-cause mortality.

The impact of weight change, especially intentional weight loss on mortality could not be rigorously established in observational studies. The Arthritis, Diet, and Activity Promotion Trial (ADAPT) [168], as a randomized clinical trial (RCT), reported that individuals assigned to weight-loss intervention (diet only/diet+ exercise) had a lower risk of mortality (HR (95% CI): 0.5 (0.3-1.0) than did those assigned to non-weight-loss intervention (exercise only/ health lifestyle) in overweight/obese elderly with osteoarthritis of the knee. Another RCT [169] showed that dietary weight loss intervention (diet only/ diet + low sodium) was not associated with decreased mortality risk (HR (95% CI): 0.93 (0.63-1.37)).

The protective effect of intentional weight loss may due to weight loss per se or to the interventions. Numerous studies conducted thus far indicate that most health profiles can be improved by changing lifestyles, with or without weight loss. If the association of intentional weight loss with lower rate of mortality were confirmed, it would be helpful in designing weight loss intervention and would have a great clinical importance for recommending weight loss to older patients.

In summary, in old age, weight changes are more common and have different impact than in middle age, when weight regulation is more effective. Several studies indicate that weight loss

or weight fluctuations were associated with mortality among the elderly. Thus, studying and monitoring weight change among the elderly is of great clinical importance. Reverse association is a common issue in weight change studies among the elderly given to the high prevalence of pre-existing diseases. The effect of body composition change and redistribution of fat and lean mass during the weight change on mortality has not been adequately studied thus far. Previous studies suggest that the changes in total body fat mass or lean mass may attenuate the association between weight change and mortality. Future studies should also take into account the regional fat redistribution, such as the accumulation of ectopic fat during weight change. Moreover, well-designed factorial RCT should be performed to further determine whether intentional weight loss is a protective factor for survival.

2.4   ASSOCIATION WITH CARDIOMETABOLIC DISORDERS

Cardiometabolic disorders is a constellation of syndromes, including high blood pressure, increased levels of triglycerides, fasting plasma glucose, and C-reactive protein, insulin resistance, and low high-density lipoprotein cholesterol level [170]. Obesity is a well-established risk factor for cardiometabolic disorders, with insulin resistance as the possible culprit.

Numerous prospective studies on the association between obesity and cardiometabolic diseases, especially Type 2 Diabetes (T2D) have made a remarkable progress in the obesity field. However, several issues remain unclear. For example, although it has been now widely accepted that regional fat distribution is more important in driving cardiometabolic disorders than total adiposity per se [171], the mechanism linking regional fat distribution with cardiometabolic disorders is still unclear. In addition, the relative contribution of various regional fat depots (eg,

abdominal visceral fat, DSAT, SSAT, and other ectopic fat) on incident cardiometabolic disorders (e.g., T2D and hypertension) is largely unknown. Moreover, whether the visceral and ectopic fat in other depots are the causal factors or markers of cardiometabolic disorders is still debatable. Another concern is whether ectopic fat can explain the observed "obesity paradox" among patients with cardiometabolic disorders. Finally, the role of race/ethnicity in the association between visceral/ectopic fat and cardiometabolic disorders remains controversial.

In this chapter, we will briefly introduce the potential etiological mechanisms underlying the association between obesity and cardiometabolic disorders, and discuss recent epidemiological studies on the relative role of total abdominal, and ectopic fat, as well as weight changes in cardiometabolic risks.

2.4.1 Mechanism Linking Obesity to Insulin Resistance

Insulin resistance, with its compensatory hyperinsulinemia and associated lipid abnormalities, is essential in linking obesity to T2D, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Many studies revealed that the tissue sensitivity to insulin declines by ~30%-40% when an individual is over ideal body weight by 35-40% [172]. It is important to note that even normal-glycemic obese individuals are exposed to a persistent state of hyperinsulinemia. There are several possible mechanisms for obesity related insulin resistance, such as endocrine, inflammatory, neutral and cell intrinsic pathways.

2.4.1.1 Endocrine Factors

Fatty Acids (FAs)

Plasma FAs concentration increases among obese population, which may be attributed to the increased release of FAs by excess fat tissue. FAs and glucose serve as an energy source. FAs and glucose compete in the oxidative metabolism process in insulin-response cells, which may substantially lead to obesity related insulin resistance. Recent studies revealed that the FAs and other metabolites, such as acyl-CoAs, ceramides, and diacyglycerol may activate protein kinases and increase the inhibitory serine phosphorylation of insulin receptor substrates (IRS) and substantially impair the uptake of glucose, the rate-limiting step for glucose metabolism.

Adipokines

Also known as an endocrine tissue, adipose tissue secretes many specific hormones, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), adiponectin, resistin, leptin, retinol-binding protein 4 (RBP4) and Plasminogen activator inhibitor-1 (PAI-1). These adipokines mediate the crosstalk between adipose tissue and insulin targeted tissues (e.g. skeletal muscle and liver), and affect energy metabolism and insulin sensitivity.

Inflammatory Mechanisms

Experimental, epidemiological and clinical evidence have suggested that chronic inflammation plays an important role in obesity related insulin resistance. It was reported that concentrations of inflammatory markers, such as IL-6, TNF-α, C-reaction protein (CRP), are elevated in obese and insulin resistant individuals. Furthermore, macrophages accumulate in white adipose tissue in obese condition [173] and cause insulin resistance in animal models [174].

Two transcription factor-signaling pathways are linked to the inflammatory effects of obesity and insulin resistance: the NK-κB pathway and the c-Jun NH2-terminal kinase (JNK) pathway. Some pro-inflammatory stimuli including cytokines such as TNF-α, and pattern recognition receptors, such as the toll-like receptor and the receptor for advanced glycation end products, could activate both pathways. In addition, toll-like receptor is the gatekeeper of innate immune system and can be activated by fatty acids, which suggests an association between elevated circulating or tissue lipid concentration and the immune system. The NK-κB target gene products, including IL-6, IL-10, TNF-α, CRP, resistin, PAI-1 could be the mediators of insulin resistance [175].

Neural Mechanisms

Brain takes signals, including leptin and insulin, and sends signals as a response to control eating behaviors and substrate metabolisms to promote homeostasis of energy stores and fuel metabolism. It was also reported that circadian rhythm is associated with metabolic syndromes, including hyperlipidemia, hepatic steatosis and hyperglycemia. Evidence shows that alternating shift work is independently associated with diabetes, rather than day-shift work [176]

2.4.1.2 Cell Intrinsic Mechanisms

Oxidative Stress

Fat accumulation is associated with oxidative stress, a phenomenon described as an excess production of highly reactive molecular species over antioxidant defenses. Many studies reported that the elevated production of reactive oxygen species (ROS) might result from hyperglycemia and increased level of FAs. The oxidative stress and ROS can cause insulin resistance by activating

multiple serine/threonine kinase signal cascades, and by getting involved in the insulin signal pathway, such as IRS.

Mitochondrial Dysfunction

Studies reported that insulin resistance is related to elevated fat accumulation in muscle and liver, which is accompanied with a reduced level of mitochondrial oxidative and phosphorylation in the elderly [177]. Similarly, mitochondrial dysfunction associated with an accumulation of fat in muscle was found in young, lean, insulin resistant offspring of patients with T2D [178]. This may be due to the fact that functional mitochondria participate in FA oxidation and then increase ROS. With the advancing ROS production and development of insulin resistance, the mitochondrial function is impacted, and the intracellular lipid accumulation further increases, which exacerbates the insulin resistance.

Endoplasmic Reticulum (ER) Stress

ER stress may also be associated with insulin resistance. In mice model, ER stress was found to be associated with obesity and unhealthy diet induced metabolic disorders. ER stress could promote a JNK-dependent serine phosphorylation of IRS-1, which subsequently inhibits insulin receptor signaling [179], and may also elevate oxidative stress and contribute to insulin resistance. Ectopic fat accumulation or chronic exposure of FFAs could serve to induce ER stress.

2.4.2   Anthropometric Measurements and Cardiometabolic Disorders

An association between general obesity and cardiometabolic disorders has been shown in numerous studies. However, not all obese or overweight individuals have metabolic disorders

41

[180]. 10-25% obese or overweight individuals are metabolically normal, which refers to "healthy obese" individuals. In contrast, some normal-weight individuals are metabolically abnormal, which refers to "metabolically obese" individuals. The key mechanism that explains the difference between "healthy" and "unhealthy obese" may be the preserved insulin sensitivity.

Rather than fat mass itself, adiposity dysfunction may be even more important in the pathophysiology of metabolic diseases [181]. As a marker of dysfunctional adipose tissue, abdominal obesity is prevalent in cardiometabolic disorders, and reflects the failure in maintaining metabolic homeostasis or a state of inflammation/insulin resistance. Studies have confirmed that WC or WHR, as surrogate of abdominal fat, was predictors for cardiometabolic disorders, such as T2D [182, 183], hypertension [184], and hyperlipidemia [185].

It is still not clear what the best anthropometric predictor for cardiometabolic disorders is. It is believed that WC and WHR add a value to BMI in predicting cardiometabolic disorders risks. With the emerging emphasis on the clinical impact of central obesity, it has been long debatable whether BMI, WC, or WHR could be better predictors of T2D. Emerging evidence suggest that WC is a better predictor than WHR for T2D. The Health Professional's Follow-up Study [186], a 5-year follow-up study including 51,429 U.S men, aged 40-75 years of age, showed that BMI was the strongest predictor of T2D, followed by WC. In the ongoing study [187], the predictive effects of WC and BMI on T2D were similar but better than that of WHR after 13 years of follow-up. However, evidence from a meta-analysis [188] on 32 published studies suggested otherwise, that BMI, WHR and WC were similarly informative in predicting incident diabetes, which may due to their high correlation. The pooled relative risks for incident diabetes were 1.87 (1.67-2.10), 1.87 (1.58-2.20) and 1.88 (1.61-2.19) per standard deviation for BMI, WC and WHR, respectively. In both genders, greater BMI, WC and WHR are associated with T2D development [189]. However,

42

the respectively predictive effects of the anthropometric measurements may be gender- and age-specific. In a prospective population-based cohort study with 3055 men and 2957 women without diabetes at baseline, WC had the greatest relative risk with T2D in older women. BMI was the strongest predictor in younger women. In young men, the predictive effect of BMI, WHR and WC were not different.

Anthropometric measurements are imprecise and limited by nature and provide little information in understanding the mechanism liking different fat distribution pattern to cardiometabolic outcomes. To be specific, BMI is limited in distinguishing body fat from body lean mass, and in controlling for the difference of body fat distribution among individuals. WC and WHR are imprecise for obese population since the body markers are unclear to identify. Furthermore, WC and WHR cannot distinguish VAT from SAT. Thus, the application and improvement of imaging measurements enabled us to better understand the association between regional body fat and cardiometabolic disorders.

2.4.3 Imaging measurements of Body Fat Distribution and Cardiometabolic Disorders

2.4.3.1 SAT

Abdominal SAT

Since SAT is considered to be a "metabolic sink", mechanically greater SAT should be protective for cardiometabolic disorders. However, greater abdominal fat accumulation in subcutaneous depot is not always protective. Results on the association between abdominal SAT and insulin resistance/T2D risk are inconclusive. Some studies have reported that excess

abdominal SAT is a strong indicator of insulin resistance or dyslipidemia [190, 191], whereas others suggested otherwise [151, 192].

Anatomically, SAT can be further divided in to SSAT and DSAT by the Scarpa's fascia [193]. The two sub depots of SAT exhibit opposite metabolic functions. SSAT shows markedly weaker association with metabolic risk profiles in glucose-tolerant volunteers, similar to thigh subcutaneous fat (a depot generally regarded as a weak determinant of insulin resistance, will be fully discussed below) [194]. Both absolute and relative amount of abdominal SSAT explains the "beneficial effect" of abdominal SAT on cardiometabolic profiles [195].

In contrary, DSAT is metabolically and functionally similar to VAT since it reflects more of the VAT expression profile, such as 11β-HSD1, leptin, and resistin, and may related to the development of obesity-related abnormalities [196]. Thaete K et al [194] showed that DSAT (r =−0.64, P < 0.001) and visceral fat (r =−0.61, P < 0.001) were strongly correlated with insulin-stimulated glucose utilization and other key aspects that define insulin resistance (e.g., blood pressure, fasting insulin, and lipids) in glucose-tolerant volunteers. Another case-control study on 365 asymptomatic subjects showed that greater DSAT was significantly associated with increased inflammation and oxidative stress, suggesting that DSAT could be an important determinant for cardiometabolic disorders [197]. Furthermore, the clinical impact of DSAT on IR may differ between genders. In men, DSAT strongly predicted global insulin resistance (IR; homeostatic model assessment of IR) and liver-specific IR (insulin-like growth factor binding protein-1) independent of other obesity measurements [198] . In women, although DSAT was associated with IR, no additional predictive value was provided [198]. The different amounts of SSAT and DSAT compartments in SAT may explain the discrepancy of the association between SAT and cardiometabolic risk profiles.

However, recent studies were limited by their cross-sectional design, and thus, no cause-effect association could be established. Well-designed longitudinal studies are warranted to clarify the mechanism that links DSAT and SSAT to cardiometabolic disorders, to estimate the predictive value on cardiometabolic disorders (e.g., T2D, hypertension, and hyperlipidemia), and identify the mitigating factors that contribute to the different accumulation patterns.

Gluteal-femoral SAT

Peripheral fat mass might be protective for atherogenic metabolic profiles [199]. Mechanically, femoral fat depots are less sensitive to lipolytic stimuli than are abdominal depots [200, 201] and are more likely to take up non-esterified fatty acids (NEFA) from the circulation, which is a long-known adverse fatty acid for glucose metabolism, insulin resistance, beta-cell dysfunction and diabetes. Lowering NEFA levels from circulation protects other organs from fat accumulation, such as the liver, the pancreas and the skeletal muscle [202].

Greater gluteal-femoral SAT is associated with favorable cardiometabolic disorders. Larger hip or thigh circumference has been linked to a favorable glucose and lipid profiles. Greater lower-body fat mass, measured by DXA, is associated with a favorable fasting and post-load glucose level [203] and insulin sensitivity [204], and presents a favorable inflammatory nature [205]. In addition, the association between gluteal-femoral SAT and favorable glucose level might be gender-specific. The Health ABC study [206] showed that, the protective effect of thigh SAT was only observed in older men but in not older women, after adjusting for abdominal VAT, abdominal SAT, and thigh inter-muscular fat area.

Based on previous findings, large longitudinal studies are needed to confirm the protective impact of gluteal-femoral SAT on cardiometabolic disorders, using CT or MRI measurements, among different ethnicities/race groups, with the adjustment for other important fat depots.

2.4.3.2 Visceral Fat

The excess accumulation of visceral fat reflects adipose tissue dysfunction. Visceral fat (VAT) is more metabolically deleterious than is SAT. Its association with insulin resistance and adverse lipid profiles [207] might due to the anatomical location and different secretion of cytokines. VAT and macrophages infiltrated in VAT produce more pro-inflammatory cytokines (e.g., TNF-α and IL-6) and less adiponectin, which act in concert in driving insulin resistance, endothelial dysfunction and subsequent atherosclerosis [199]. Anatomical location of visceral fat may cause systematic or/and local effect via different mechanisms.

Abdominal Visceral Fat

Greater visceral fat is a marker of dysfunctional SAT, and is associated with cardiometabolic risk profiles [199], such as insulin resistance, diabetes, hypertension, hyperlipidemia and metabolic syndromes [171]. The adverse impact of abdominal VAT seems to be stronger than that of abdominal SAT [191, 208]. The Framingham Heart Study [191] showed that abdominal VAT was cross-sectionally associated with multiple cardiometabolic risk factors (e.g., hypertension, triglyceride, diabetes, etc.) among community-based sample (mean age 50 years), and more so among women than among men. These significant associations persisted even after further adjustment for BMI and WC. The Jackson Heart Study [208] among African Americans (mean age 58 years) reported similar cross-sectional findings : VAT was positively associated with a risk of cardiometabolic disorders, including fasting plasma glucose, triglyceride, HDL-C, hypertension, diabetes, and metabolic syndromes, even after accounting for BMI. Likewise, with elevated VAT, women were at higher risk of metabolic disorders compared to men.

Recently, several longitudinal studies have confirmed the adverse impact of VAT on cardiometabolic risk. The Japanese-American Community Diabetes Study first showed that

increased VAT was associated with incident hypertension among 300 Japanese Americans after a 10- to 11-year follow-up. The Dallas Heart Study [209] confirmed this finding with larger sample size and in a mixed population (903 participants, 60% nonwhite) after a 7-year follow-up. The Dallas Heart Study [210] also reported that VAT was independently and robustly associated with incident diabetes and incident prediabetes. The similar association was previously observed among Japanese-American population [211].

Until now, there has been no clear proof whether VAT directly causes or at least plays a necessary part in the development of cardiometabolic disorders. Several studies further examined the impact of VAT reduction on insulin resistance and T2D. In animal models, removal of VAT (omentum) by surgery improved insulin resistance and glucose tolerance [212]. However, omentectomy was not found to benefit insulin sensitivity in morbidly obese and diabetic patients [213]. Other studies suggested that reduction of VAT via lifestyle intervention (such as diet and exercise) and medications (such as thiazolidinedione (TZD) and GLP-1 analogue) may reduce insulin resistance and improve glucose metabolism [214]. It is still unclear whether the reduction of VAT per se or the intervention method itself favors glucose metabolism.

Liver Fat Infiltration

Nonalcoholic fatty liver disease (NAFLD) is defined as ectopic accumulation of triglycerides in more than 5% of hepatocytes in individuals without excessive alcohol consumption and negative viral and autoimmune liver disease [215]. The prevalence of NAFLD is 31% in United States [216]. NAFLD is related to cardiometabolic disorders, such as obesity, T2D, dyslipidemia and insulin resistance.

Numerous studies showed that NAFLD is associated with the cardiovascular risk profiles, including age, male gender, hypertension, smoking, and hyperlipidemia, carotid artery intima-

media thickness, hsCRP, atheroma formation, mediastinal fat pad, endothelial dysfunction and coronary calcium scores [217]. Cardiovascular disease related mortality are the leading cause of death among NAFLD patients, instead of liver complication [218].

Studies have also linked NAFLD to insulin resistance [219, 220]. NAFLD is significantly associated with decrease peripheral glucose disposal via impaired glucose oxidation and glycogen synthesis, instead of affecting endogenous glucose production [221].

NAFLD is commonly found in individuals with increased VAT. It is still unclear whether NAFLD and VAT predict metabolic disorders differently. Many believe that NAFLD was a stronger predictor for T2D than VAT. In insulin-treated diabetic patients, the percent of hepatic fat was more correlated with daily insulin dose and the sensitivity of endogenous glucose production, compared to VAT [222]. Another study [223] tried to determine the independent association of hepatic fat (intrahepatic triglyceride) and VAT to metabolic function. In subjects matched on same hepatic fat, but different VAT, no difference in insulin sensitivity was found. Greater hepatic fat was associated with lower insulin sensitivity in liver, skeletal muscle, and adipose tissue [223].

Pancreatic Fat Infiltration

Pancreatic fat is the fat infiltration in the pancreas with elevated level of triglyceride, FFAs, cholesterol, total fat and cytokines in pancreas [224], and has been suggested to be associated with obesity in several studies [225, 226]. Pancreatic fat is difficult to measure [227]. With the improvement of technology, both MRI and magnetic resonance spectroscopy (MRS) are proved to be suitable to detect pancreatic fat compartment. Recently, the first population level study was conducted using MRI as measurements and defined that the normal range of percent pancreatic fat is between 1.8 % to 10.4 %. The prevalence of fatty pancreas is 16.1% in Hong Kong Chinese

health volunteers, and more prevalent in men and postmenopausal women [227]. However, the "normal range" of pancreatic fat for other populations has not yet been established, along with the prevalence of fatty pancreas in Caucasians, African Ancestry populations and other Asian groups.

Cross-sectional study showed that pancreatic fat measured by MRS was negatively associated with insulin secretion in individuals with impaired fasting glucose and/or glucose tolerance [228]. Pancreatic fat was a stronger predictor of impaired insulin secretion compared to visceral fat, in a stepwise multivariate regression analysis. However, pancreatic fat may not be as strong predictor for pancreatic β cell function, as VAT is [228]. Recent cohort study of Hong Kong [227] confirmed that pancreatic fat could further increase insulin resistance, but was not an important contributor to the impaired pancreatic β cell function.

In the future, more population-based studies of pancreatic fat are warranted. The correlation of pancreatic fat and other ectopic fat, especially liver fat, should be considered when quantifying the relevant contribution of each fat compartment to insulin resistance and T2D.

Ectopic Skeletal Muscle Fat

Ectopic fat infiltration within and around skeletal muscles (also known as myosteatosis), can be divided into two subtypes: inter-muscular fat (IM fat, visible fat beneath the fascia lata) and intra-muscular fat (fat within the muscle cells). Skeletal muscle is a critical site for glucose metabolism, which is in charge of up to 80% glucose disposal via insulin dependent mechanism. More than 90% T2D patents are characterized by irresponsiveness of their myocytes to an stimuli of insulin, which substantially leads to impaired glucose uptake into muscles [229].

Numerous cross-sectional studies have linked myosteatosis to insulin resistance. Intra-muscular fat measured by skeletal muscle biopsy or H-magnetic resonance spectroscopy has been positively associated with insulin resistance in non-diabetic adults [230-232], and offspring of T2D

49

[233, 234]. However, this association was not found among endurance-trained athletes with high intramyocellular lipids, who are highly insulin sensitive [235]. It has been suggested that their high levels of intramyocellular lipids serve as energy source, and might be an adaptive response to high oxidative requirements during exercise ("Athlete's Paradox"). In contrast, increased level of intramyocellular lipids is deleterious in patients with insulin resistance and T2D due to imbalance between increase FFAs and impaired fatty acid oxidation [236]. Thus, whether high intramyocellular lipids is physiological or pathological may depend on the oxidative capacity [237]. Besides the findings from cross-sectional study, the recent Tobago Health Study indicates that intermuscular fat was positively associated with incident diabetes [11].

It has been suggested that intra-muscular fat accumulation may cause decreased mitochondrial oxidative activity, which lowers mitochondrial adenosine triphosphate (ATP) synthesis, and substantially increases intra-muscular fatty acid metabolites (fatty acyl-CoA and diacylglcerol), and subsequently leads to impaired insulin signaling and insulin resistance [238]. The accumulation of intra-muscular fat may also impair the glucose metabolism via insulin receptor substrate 1 (IRS-1)/phosphatidylinositol 3-kinase (PI3K) pathway and growth-factor-regulated protein kinase B (AKt/PKB) pathway[239]. In addition, the accumulation of intra-muscular fat can also increase the production of reactive oxygen species (ROS) by mitochondria and induce oxidative stress, and substantially lead to the activation of multiple serine/threonine kinase signaling cascades [148]. The activated kinase can phosphorylate a number of targets, such as insulin receptor and IRS protein family and result in a decreased extent of insulin-stimulate tyrosine phosphorylation [240]. The decreased association and/or activity of downstream signaling molecules lead to insulin resistance [240]. However, none of these mechanisms have yet been confirmed in humans. .

Even low level of inter-muscular fat is thought to be able to significantly increase insulin resistance. The association between inter-muscular fat and insulin resistance or T2D has been found in obese individuals [241], the elderly of both genders [242], non-diabetic women [243], African Ancestry men [244], and healthy postmenopausal women [245]. Recently, Miljkovic et al [246] reported a novel association of abdominal inter-muscular fat with hyperinsulinemia and insulin resistance among older Caucasian men in The Osteoporotic Fractures in Men (MrOS) study .

The accumulation of inter-muscular fat may impair nutritive blood flow to muscles, and substantially lead to impaired insulin action and insulin diffusion capacity [241, 247]. Inter-muscular fat may also increase the local release of pro-inflammatory cytokines, such as interleukin-6 (IL-6), leptin and C-reactive protein (CRP), which results in local inflammation within the muscle fibers [248]. Endoplasmic reticulum (ER) stress response is another potential mechanism for obesity induced insulin resistance [249].

Myosteatosis is also reported to be associated with hypertension. Few cross-sectional studies linking hypertension to intramuscular fat have been inconclusive. The Health, Aging and Body Composition (Health ABC) Study first revealed that thigh inter-muscular fat was associated with prevalent hypertension in elderly African Americans ,but not elderly Caucasians, and this association was especially evident among elderly African American men (age 70 or above). However, in Health ABC study no such association was found with intra-muscular fat as measured by muscle attenuation [250]. Similarly, the Framingham Study among 2949 middle-aged participants reported a significant association between paraspinous intramuscular fat (as measured by muscle attenuation) and hypertension, though the association was lost after further adjustment for visceral fat or BMI [251]. The discrepancy in results between the previous studies and ours

might be due to the different site of measurements as the association between intramuscular fat and cardiometabolic disorders may be specific for specific muscles [246].

Our research team has recently reported for the first time that ectopic skeletal muscle fat accumulation contributes to development of T2D among African ancestry men. However, there is still a critical need for a large longitudinal study focusing on the ectopic skeletal muscle fat accumulation and incident hypertension among high-risk African ancestry populations. Weight Change

Large population based prospective studies have consistently showed that weight loss is a protective factor, whereas weight gain is a risk factor for insulin resistance and/or T2D [252, 253]. In particular, weight gain in early adulthood is a stronger indicator of higher risk and earlier onset of T2D compared to weight gain between 40-55 [252]. Although, body weight increases with advancing age, a stable weight in adulthood is important for healthy aging and chronic diseases prevention.

There are several ways for weight loss, including exercise, diet change, pharmaceutical therapy and bariatric surgery. A randomized, controlled trial showed that the level of glucose disposal similarly increased by diet-induced weight loss and exercise-induced weight loss, and was significantly greater compared to exercise intervention without weight loss [254] . The favorable effect on insulin sensitivity and glucose metabolism could be explained by the reduction of abdominal fat and visceral fat [254]. However, other study indicated that even without weight loss, mild level of exercise (walk training) improved glucose metabolism among middle-aged sedentary men [255]. Moreover, the method of diet control also plays an important role in glycemic control, given a similar weight loss. A one-year randomized clinical trial showed that low-carbohydrate diet was associated with more favorable HA1c levels, compared to a conventional diet (restricted

caloric intake by 500 calories per day with <30% of calories from fat) [256]. Several other studies have reported that bariatric surgery is effective for weight loss and improved insulin resistance and/or T2D [257]. Interestingly, the protective effects of bariatric surgery on insulin resistance and/or T2D differ across the types of surgical procedure [258]. Thus, it remains controversial whether weight loss per se or intervention benefits the glucose metabolism.

It is worth noting thatweight loss is not always associated with favorable health outcomes (phenomenon known as "obesity paradox"). Emerging evidence indicates that some subpopulation of obese population may not be benefit from weight loss, measured by improved health outcomes, for example adults with established CVD or T2D, weight cyclers, metabolically healthy obese adults, youth, older adults or "fit and fat" individuals [259].

2.5   CONCLUSION

In conclusion, obesity is a highly heterogeneous condition related to many cardiometabolic disorders. Many studies proved that the anatomical location of the fat is more important than the general fat accumulation in the development of cardiometabolic disorders. It is very important to identify the anatomical location of the fat, which is the strongest contributor to the risk of cardiometabolic disorders, determine the intendent impact on cardiometabolic disorders of each fat depot, and identify any potential race/ethnic, gender, and age differences. Previous studies have limitations such as small-sample size, cross-sectional design, and lack of information on incident cardiometabolic disorders. A well-designed longitudinal study on the general, regional, and ectopic fat distribution is warranted to identify the high-risk population, and the modifiable factors for development of cardiometabolic disorders.

3.0   METHODS

3.1   STUDY POPULATION

Between 1997 and 2003, men aged 40 and older were recruited for population-based prostate cancer screening for the first time on the island of Tobago, Trinidad & Tobago [260]. To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. Recruitment for the initial screening was accomplished by flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated and participation was representative of the island parishes. The recruited cohort was 97% African, 2% East Indian, <1% white, and <1% "other" as defined by participant-report of paternal and maternal grandparents' ethnicity [17].

3.2   ADIPOSTY MEASURES

3.2.1   Dual-energy X-ray absorptiometry (DXA)

DXA (QDR 4500 W, Hologic, Inc., Bedford, MA) scans were analyzed with QDR software version 8.26a for total, trunk, and lower body fat. The short-term precision measurements in 12 participants were all ≤1.16%.

3.2.2 Peripheral Quantitative Computed Tomography (pQCT)

pQCT scans were analyzed to measure ectopic skeletal muscle fat infiltration (myosteatosis) at the calf muscle. Studies that utilize CT scans for measures of myosteatosis examine either intermuscular fat (visible fat beneath the fascia lata) and/or muscle attenuation (lower skeletal muscle attenuation is indicative of greater intramuscular fat content) [261, 262]. In our study, both types of measures of myosteatosis were able to be obtained from the pQCT scans of the calf, which were performed using the Stratec XCT-2000 (QDR 4500 W, Hologic, Inc., Bedford, MA). At both study visits, 2.2 mm cross-sectional images of the calf skeletal muscle composition were obtained at 66% of the tibia length, proximal to the terminal end of the tibia, because this is the region with the largest circumference of the calf and has less variability between individuals [263]. All images were analyzed with STRATEC analysis software version 5.5D (Orthometrix, Inc., White Plains, NY) by a trained investigator unaware of the participants' disease status.

The mineral equivalent density of fat, muscle, and bone are different as 0, 80, 1200mg/cm^3, respectively, which can be distinguished by the pQCT scan. IM fat was determined by a shift of mineral equivalent density from 80 (muscle) to 0 (fat) mg/cm^3. Muscle attenuation was calculated as the ratio of muscle mass (mg) and muscle area (cm^2) [264]. Total muscle area (mm^2), total fat (mm^2), and subcutaneous fat (mm^2) were obtained in a similar method. The coefficients of variation (CV) for total, subcutaneous, and IM fat, muscle area, and muscle density respectively were 1.0%, 1.5%, 7.6%, 0.9%, and 1.1%, which were determined by repeat pQCT scanning in 15 individuals.

3.2.3   Anthropometric measures

Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg on a balance beam scale. Body mass index (BMI) was calculated as the ratio of weight and squared height (kg/m^2). Waist circumference (WC) was measured at the narrowest point of waist, or at umbilicus, if the narrowest point could not be identified.

3.3   CORRELATES

Standardized interviewer-administered questionnaires were performed to collect demographic information and correlate information. Former smoker was defined as men who smoked more than 100 cigarettes but did not currently smoke, and current smoker was defined as men who smoked more than 100 cigarettes and currently smoked. Alcohol consumption was defined as having 4 or more drinks per weeks in the past 12 months. As walking is the predominant form of physical activity in Tobago, whether or not a participant reported walking for exercise, to work, the store, or to church 4 or more times in the past 7 days was collected as a measure of physical activity. Sedentary lifestyle was assessed with hours of television watched with the cutoff point of "21 or more hours" per week considered sedentary. Self-reported overall health status compared to men of their own age was dichotomized as good/excellent versus fair/poor/very poor. Men were instructed to bring all prescription medications taken in the past 30 days to the clinic visit and were recorded by interviewers. The information from questionnaire was obtained on the same day as the DXA and pQCT scans.

3.4   CARDIOMETABOLIC DISEASES

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as (glucose $\times$ insulin) /22.5. Hypertension was defined as a systolic blood pressure $\geqslant$140 mmHg and/or diastolic blood pressure of $\geqslant$90 mmHg or currently taking antihypertensive medication. T2D was defined as fasting serum glucose $\geq$126mg/dl or currently taking anti-diabetic medication. Antihypertensive medication and anti-diabetic medication were defined with the standard of WHO ATC/DDD drug classification system [43]. Other comorbidities including prevalent CVD, renal disease, stroke, MI were self-reported yes/no using questionnaire.

3.5   MORTALITY STATUS

Death dates were obtained from death certificates and/or reports from a proxy (58% vs. 42% of study participants, respectively) between the start of the baseline visits in November 2003 and the end of mortality follow-up in May 2013.

3.6   SAMPLE SIZE

Aim 1: Power estimations were calculated for the association between adiposity measures and categorical correlates. SAS 9.4 software was used for the calculation based on two sample T-test for categorical correlates. The difference of the means between exposed group and unexposed group were 0.2, 0.4 and 2 using the known sample size of 1547 for DXA measures and 1480 for pQCT measures. The standard deviations were 0.8, 1, and 4. The difference of the means and the

standards deviations were selected based on the distribution of our adiposity measures. The group

weight was used as 1:4, which is approximately as sedentary lifestyle and type 2 diabetes presents.

Any group weight larger than 1:4 will have more power. Overall, we have >80% power to detect

the association between hypertension and total body fat, trunk fat and muscle attenuation (Table

1). However, we do not have enough power to detect the association between lifestyle factors, such

as physical activity and sedentary lifestyle with the adiposity measures.

For continuous variables, power estimates were calculated for Pearson correlation

estimates using coefficients of 0.1, 0.15, and 0.2. We have >80% power to detect correlations of

0.1 and greater (Table 2).

Aim 2: Power estimations were calculated for the association of adiposity measures with newly

developed hypertension. SAS 9.4 software was used for the calculation based on odd ratios of 1.2,

1.4 and 1.6 with the known sample size and a newly developed hypertension rate of 52.6%.

Overall, we have >80% power to detect the association between hypertension and adiposity

measures with an odd ratio of 1.6 (Table 4).

Aim 3: Power estimations were calculated for the association of adiposity measures with mortality

risk. SAS 9.4 software was used for the calculation based on odd ratios of 1.2 1.4 and 1.6 with the

sample size of 1652 and a mortality rate of 6.8%. Overall, we have >70% power to detect the

association between mortality risk and adiposity measures with an odd ratio of 1.6 (Table 5).

Table 1. Power Calculation for the Association between Adiposity Measures and Categorical Correlates

		Power Calculation	
Mean difference	Standard Deviation	DXA measures (N=1547)	pQCT measures (N=1480)
0.2	0.8	97.4%	97.0%
	1	87.7%	86.8%
	4	12.2%	12.0%
0.4	0.8	>99.9%	>99.9%
	1	>99.9%	>99.9%
	4	34.5%	33.7%
2	0.8	>99.9%	>99.9%
	1	>99.9%	>99.9%
	4	>99.9%	>99.9%

Table 2. Power Calculation for the Association between Adiposity Measures and Continuous Correlates

	Power Calculation	
Correlation Coefficient	DXA measures (N=1547)	pQCT measures (N=1480)
0.1	>97.5%	>97.1%
0.15	>99.9%	>99.9%
0.2	>99.9%	>99.9%

Table 3. Power Calculation for the Association between Adiposity Measures and Newly Developed Hypertension

Odd Ratios	Power Calculation	
	DXA measures (N=759)	pQCT measures (N=726)
1.2	23.9%	23.1%
1.4	62.9%	61.1%
1.6	88.9%	87.6%

Table 4. Power Calculation for the Association between Adiposity Measures and Mortality Risks

Odd Ratios	Power Calculation	
	DXA measures (N=1652)	pQCT measures (N=1652)
1.2	16.1%	16.1%
1.4	44.9%	44.9%
1.6	74.8%	74.8%

3.7 ANALYSES

Aim 1: Percent change in the pQCT and the DXA measures during follow-up was calculated as the difference between baseline and last follow-up measure divided by baseline measure and multiplied by 100%. The annualized rate of change in pQCT and DXA measures during follow-up was calculated as the percent of change in pQCT or DXA measures divided by duration (in years) between the two scans. Three types of annualized rates were calculated: the annualized rate of change in the DXA measures from visit 3 to visit 5, the annualized rate of change in the DXA measures from visit 1 to visit 5, and the annualized rate of change in the pQCT measures from visit 3 to visit 5. We categorized age at baseline by three groups: 40 to 54, 55 to 64 and 64 and above. For the description of age-associated rate of change, the annualized rates of change in the DXA measures from visit 1 to visit 5 and the annualized rate of change in the pQCT measures from visit 3 to visit 5 were used.

Age-adjusted contribution of each individual correlate to the annualized percent change in the pQCT measures and the DXA measures from visit 3 to visit 5 was evaluated using linear regression. The strength of correlation is presented with β coefficient and 95% confidence intervals. The multivariable analyses were performed using stepwise model selection method with age forced in. Only the correlates with a significant level lower than 0.05 were included in the final model. All analyses were performed using SAS statistical software version 9.3 (SAS Institute Inc., Cary, North Carolina).

Aim 2: Outliers were defined as any value outside the interval of $Quartile_3$ (75%ile) +3IQR (IQR=interquartile range) and $Quartile_1$ (25%ile) -3IQR and were deleted (11 were deleted for muscle density and 27 were deleted for IM fat) to increase the statistical power. All continuous variables were normally distributed. Distributions of continuous and categorical variables were

presented as mean ± standard deviation (SD) or frequency and analyzed with Student's T-test, Chi-square test or Fisher exact test, as necessary. Age-adjusted P-values were presented.

Age- and multivariable-adjusted hazard ratio (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazard models. The proportional hazards assumptions were confirmed by Schoenfeld residuals. The adjusted covariates were selected based on previous studies, biological plausibility and results from Table 1, including age, height, smoking status, alcohol intake, physical activity, TV viewing time, health status, and presence or absence of T2D, renal disease, stroke, cancer, MI and hypertension. Subcutaneous fat, IM fat and skeletal muscle density models were additionally adjusted for BMI and calf muscle area. Due to missing covariate data, the final N in multivariable models was 1495 for IM fat and 1375 for muscle density. Lastly, we performed fully adjusted, stratified analyses by age groups (< 65 and 65+).

Multicollinearity was checked with variation inflation factor (VIFs of included variables were less than 10). Sensitivity analyses with the outliers included confirmed the results (Appendix 1). Analyses were performed with SAS statistical software version 9.3 (SAS Institute Inc., Cary, North Carolina).

Aim 3: The absolute 6-year change in adiposity measures of interest, including calf muscle attenuation, calf IM fat, BMI, and waist circumference, were calculated for each individual as the difference between each measure from the follow-up visit and each measure at the baseline visit. We performed histogram and scatter plot analyses to inspect the distribution and potential outliers for continuous variables. Outliers were defined as any value outside the interval of $Q_3+3\times$IQR and $Q_1-3\times$IQR (IQR=interquartile range) and were deleted (19 for intermuscular fat and 2 for muscle attenuation) to increase the statistical power. The difference of continuous and categorical variables between individuals with newly developed hypertension and those with normal blood

pressure at follow-up are presented as mean ± standard deviation (SD) or frequency, and were analyzed with Student's T-test or Chi-square test, respectively.

Logistic regression was performed for association between the adiposity change measures and newly developed hypertension. Odds ratios are presented per SD change in adiposity measure over the 6.2 year follow-up period for BMI, WC, and calf IM fat and skeletal muscle attenuation (reflecting intramuscular fat). All fully adjusted models included age, marriage status, education level, smoking status, alcohol consumption, physical activity, sedentary lifestyle, HOMA-IR, and calf muscle area. Multicollinearity was tested and all variance inflation factors were less than 10. All analyses were performed using SAS statistical software version 9.3 (SAS Institute Inc., Cary, North Carolina).

4.0 NATURAL HISTORY AND CORRELATES OF GENERAL, REGIONAL AND ECTOPIC SKELETAL MUSCLE FAT WITH AGING IN MEN OF AFRICAN ANCESTRY

4.1 ABSTRACT

Background: Longitudinal studies on the magnitude, pattern and determinants of body fat redistribution during the aging process are sparse, particularly in high-risk African ancestry populations. We evaluated the rate of change and determinants of general, regional and ectopic skeletal muscle fat with aging among African ancestry men.

Methods: Analyses were performed in the Tobago Health Study, a prospective longitudinal study of African ancestry men (age range 40 to 91 years). Calf intermuscular (IM) fat and skeletal muscle attenuation, a proxy for intramuscular fat, were measured using peripheral quantitative computed tomography (pQCT) in 1480 men. Total body, trunk and leg fat were measured with dual-energy X-ray absorptiometry (DXA) in 1547 men. Linear regression was used to identify the strength of the association between each determinant and the rate of change in adiposity measure.

Results: During 6.1 years of follow-up (range, 5.8-6.4 years) for pQCT measures, the annualized average rate of increase in IM fat was 12.3% and the annualized average rate of decrease in muscle attenuation was 0.5%. During 10.6 years of follow-up (range, 9.9-10.6 years) for DXA measures, the annualized average rates of increase in total and trunk fat were 2.5% and 0.49% respectively, and the annualized average rate of decrease in leg fat was 0.39%. Baseline prevalent hypertension (β coefficient (95% confidence intervals): -0.12 (-0.22, -0.02)) and type 2 diabetes (-0.18 (-0.31, -0.05)) were associated with greater decline in calf muscle attenuation, suggesting increases in

intramuscular fat. However, men with baseline hypertension had slower rates of increase in trunk fat (-0.16 (-0.27, -0.06)) and total body fat (-0.70 (-1.14, -0.25)), compared with men without hypertension. No lifestyle factors were associated with change in adiposity measures in fully adjusted models.

Conclusions: Our findings suggest that cardiometabolic diseases are predictors of fat redistribution during the aging process among African ancestry men. Further studies are warranted to collect more precise and objective measurements on lifestyle factors, to identify mechanisms linking cardiometabolic disease to fat distribution, and to replicate our results in other populations.

4.2   INTRODUCTION

Obesity is a worldwide health problem [1] and a well-established risk factor for cardiometabolic diseases [1, 2]. However, obesity is a highly heterogeneous condition given the phenomenon of "metabolically healthy obesity" [3] and "metabolically unhealthy normal weight" [4]. These phenomena suggest that rather than total body fat per se, the location of fat may be more important in driving metabolic disorders.

During the aging process, general body fat increases [33], and relocates from lower extremities to the trunk [39] and infiltrates non-adipose tissue and organs such as the heart, liver, and skeletal muscle [49]. Compared to Caucasians, African ancestry individuals are typically characterized by having lower total body fat percentage [7] and visceral fat [8], and similar trunk fat percentage [265], but paradoxically, having a greater accumulation of skeletal muscle fat [10], and greater risk of metabolic disorders [266, 267]. Despite the importance of the age-related

redistribution of body fat among this high-risk population, longitudinal studies focusing on state-of-the-art measures of body fat distribution in African ancestry populations are sparse.

Thus, our objective was to describe the longitudinal changes in general, regional and ectopic skeletal muscle fat, and to identify their correlates among middle-aged and elderly African ancestry men. We hypothesized that: 1) the rates of change in general body fat, trunk fat, leg fat, and ectopic skeletal muscle fat increase with advancing age; and that 2) excess alcohol consumption, current smoking, sedentary lifestyle, and medical histories are associated with greater changes in general body fat, trunk fat, leg fat, and ectopic skeletal muscle fat accumulation.

4.3   METHODS

Tobago Health Study

The first Tobago Health Study visit was conducted between 1997 and 2003 on the Caribbean island of Tobago with the original purpose of providing prostate cancer screening. To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. 3376 men (aged 40 years and above) were recruited regardless of their health status via flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. The samples was representative of the population with low admixture, including 97% African, 2% East Indian, <1% white, and <1% "other" participants. The study was approved by the Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health and Social Services and written informed consent was obtained from all participants before data collection.

Between 2000 and 2004, 2,589 participants were invited to receive a Dual-energy X-ray absorptiometry (DXA) scan to examine the general and regional fat (visit 1). During 2003 to 2013,

a total of 2204 (visit 3) and 1684 (visit 5) men completed another two follow-up assessments of DXA examinations, respectively (Figure 1). Between 2003 and 2007, 2,152 men were invited to participate in peripheral quantitative computed tomography (pQCT) scan of calf skeletal muscle composition. From 2010-2013, a total of 1618 men completed a follow-up assessment for pQCT (Figure 1). Both the baseline and follow-up visits followed the same procedures for questionnaire interviews, biospecimen collection, pQCT scans and DXA examinations [268]. Our analyses are limited to African ancestry men with questionnaire measurements and complete follow-up data. For the description of age-associated change in adiposity measures we used the full follow-up time span per measure type; thus, a total of 1480 men were included for pQCT measures (visit 3 to visit 5; Figure 1) and 1908 men were included for DXA measures (visit 1 to visit 5; Figure 2). Most correlates were collected in the Tobago Health Study at visit 3, so we used visit 3 as baseline for all correlate analyses. Therefore, there are a total of 1480 men for pQCT measures (Figure 1) and 1547 men for DXA for analyses (Figure 2).

Dual-energy X-ray absorptiometry (DXA)

The DXA (QDR 4500 W, Hologic, Inc., Bedford, MA) Scans were analyzed with QDR software version 8.26a for total, trunk and leg fat. The short-term precision measurements in 12 participants were all ≤1.16%.

Peripheral Quantitative Computed Tomography (pQCT)

Studies that utilize pQCT scans for measures of myosteatosis examine either intermuscular fat (IM fat, visible fat beneath the fascia lata) and/or muscle attenuation (lower skeletal muscle attenuation is indicative of greater intramuscular fat content) [261, 262]. In our study, both types of measures of myosteatosis were obtained by pQCT scans of the calf, which were performed

using the Stratec XCT-2000. At both study visits, 2.2 mm cross-sectional images of the calf skeletal muscle composition were obtained at 66% of the tibia length, proximal to the terminal end of the tibia, because this is the region with the largest circumference of the calf and has less variability between individuals [263]. All images were analyzed with STRATEC analysis software version 5.5D (Orthometrix, Inc., White Plains, NY) by a trained investigator unaware of the participants' disease status.

The mineral equivalent density of fat, muscle, and bone are different as 0, 80, 1200mg/cm^3, respectively, which can be distinguished by the pQCT scan. IM fat was determined by a shift of mineral equivalent density from 80 (muscle) to 0 (fat) mg/cm^3. Muscle attenuation was calculated as the ratio of muscle mass (mg) and muscle area (cm^2) [264]. Total muscle area (mm^2), total fat (mm^2), and subcutaneous fat (mm^2) were obtained in a similar method. The coefficients of variation (CV) for total, subcutaneous, and IM fat, muscle area, and muscle density respectively were 1.0%, 1.5%, 7.6%, 0.9%, and 1.1%, which were determined by repeat pQCT scanning in 15 individuals.

Anthropometric measures

Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg on a balance beam scale. Body mass index (BMI) was calculated as the ratio of weight and squared height (kg/m^2). Waist circumference (WC) was measured at the narrowest point of waist, or at umbilicus, if the narrowest point could not be identified.

Medical Conditions

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as (glucose $\times$ insulin) /22.5. Other comorbidities including prevalent hypertension, type 2 diabetes, cardiovascular diseases, renal disease, stroke, MI were self-reported yes/no using questionnaire.

Other measurements

Standardized interviewer-administered questionnaires were performed to collect demographic information, lifestyle factors and medication use. Former smoker was defined as men who smoked more than 100 cigarettes but did not currently smoke, and current smoker was defined as men who smoked more than 100 cigarettes and currently smoked. Alcohol consumption was defined as having 4 or more drinks per weeks in the past 12 months. As walking is the predominant form of physical activity in Tobago, whether or not a participant reported walking for exercise, to work, the store, or to church 4 or more times in the past 7 days was collected as a measure of physical activity. Sedentary lifestyle was assessed with hours of television watched with the cutoff point of "21 or more hours" per week considered sedentary. Self-reported overall health status compared to men of their own age was dichotomized as good/excellent versus fair/poor/very poor. Men were instructed to bring all prescription medications taken in the past 30 days to the clinic visit and were recorded by interviewers.

Statistical analysis

Percent change in the pQCT and the DXA measures during follow-up was calculated as the difference between baseline and last follow-up measure divided by baseline measure and multiplied by 100%. The annualized rate of change in pQCT and DXA measures during follow-up was calculated as the percent of change in pQCT or DXA measures divided by duration (in

years) between the two scans. Three types of annualized rates were calculated: the annualized rate of change in the DXA measures from visit 3 to visit 5, the annualized rate of change in the DXA measures from visit 1 to visit 5, and the annualized rate of change in the pQCT measures from visit 3 to visit 5. We categorized age at baseline by three groups: 40 to 54, 55 to 64 and 64 and above. For the description of age-associated rate of change, the annualized rates of change in the DXA measures from visit 1 to visit 5 and the annualized rate of change in the pQCT measures from visit 3 to visit 5 were used.

Age-adjusted contribution of each individual correlate to the annualized percent change in the pQCT measures and the DXA measures from visit 3 to visit 5 was evaluated using linear regression. The strength of correlation is presented with β coefficient and 95% confidence intervals. Multivariable analyses were performed using stepwise model selection method with age forced in. Smoking status was treated as dummy variable and were selected into or excluded from the models simultaneously. Only correlates with a significance level lower than 0.05 were included in the final model. All analyses were performed using SAS statistical software version 9.3 (SAS Institute Inc., Cary, North Carolina).

4.4   RESULTS

Annualized rate of change in pQCT measures across age groups

The mean follow-up time for pQCT measures was 6.1 years (range, 5.8-6.4 years). Averagely, IM fat in African ancestry men increased 12.3% per year, and muscle attenuation decreased 0.52% per year. The age-related patterns in the rate of change in muscle attenuation and IM fat were

examined by age group (Table 6). While the rate of increase in IM fat appeared to be similar across age groups, the rate of muscle attenuation loss increased with advancing age.

Annualized rate of change in DXA measures across age groups

The mean follow-up time for DXA measures was 10.6 years (range, 9.9-10.6 years). The average annualized rates of increase in total and trunk fat were 2.5% and 0.49% respectively, and the average annualized rate of decrease in leg fat was 0.39%. The age-related patterns in the rate of change in total, trunk, and leg fat percentage were examined by age group (Table 6). The rate of increase in total and trunk fat and the rate of decrease in leg fat across age groups showed as a linear relationship, such that older age at baseline was associated with lower rates of change in adiposity measures.

Age-adjusted correlates of the rate of change in pQCT measures

Table 7 presents the age adjusted contribution of each correlate to the rate of change in IM fat and muscle attenuation. The increase in IM fat was significantly greater in men with greater increases in weight, WC and BMI over the follow-up period. For example, every 1.2%/yr increase in body weight was associated with a 3.9%/yr increase in IM fat. Baseline lifestyle factors, cardiovascular diseases, myocardial infarction, renal diseases, stroke, cancer, grip strength, HOMA-IR and family history of diabetes were not associated with the rate of change in IM fat.

The rate of change in muscle attenuation was significantly lower in men with greater increases in weight, WC and BMI over the follow-up period (Table 7). For example, every 1.2%/yr increase in body weight was associated with a 0.07%/yr greater decrease in muscle attenuation. Additionally, men with obesity, type 2 diabetes or hypertension at baseline experienced significantly greater decreases in muscle attenuation, as well.

After using stepwise model selection, the rate of change in intermuscular fat was only associated with concurrent WC increase (β coefficient (95% confidence intervals, CI): 3.22 (1.29, 5.16); Table 8). However, greater age (-0.09 (-0.15, -0.04)), baseline hypertension (-0.12 (-0.22, -0.02)), baseline type 2 diabetes (-0.18 (-0.31, -0.05)) and concurrent WC increase (-0.09 (-0.16, -0.03)) were significantly associated with the rate of change in muscle attenuation. None of the other correlates were associated with the rate of change in pQCT measures.

Age-adjusted correlates of the rate of change in DXA measures

Table 9 presents the age-adjusted contribution of each correlate to the rate of change in total, trunk and leg fat percent. Men with high school and above education, obesity, hypertension, greater grip strength, and insulin resistance at baseline had significantly lower rates of total fat percent change. Also, men who had greater concurrent increases in body weight, WC or BMI had greater change in total body fat percent, as well.

Baseline hypertension and insulin resistance were associated with lower changes in trunk fat percent. In contrast, living with a spouse, or having a greater concurrent increase in body weight, WC or BMI was associated with greater changes in trunk fat percent.

The only factor associated with change in leg fat percent was that men with greater concurrent increases in body weight, WC or BMI had lower rates of leg fat percent change. This suggests that individuals who were gaining overall weight were preferentially adding it to the trunk, not the legs.

After using the stepwise model selection method (Table 10), total body fat percentage had a slower increase rate among the elderly at baseline (-0.29 (-0.53, -0.04)), and men with baseline hypertension (-0.70 (-1.14, -0.25)). Whereas, total body fat percentage had a greater increased rate among men with concurrent increases in WC (1.61 (1.40, 1.82)) and baseline former smoker (1.16

72

(0.45, 1.87)),. Trunk fat percentage had a slower increase rate among the elderly at baseline (-0.08 (-0.14, -0.02)) and men with baseline hypertension (-0.16 (-0.27, -0.06)). Whereas, trunk fat percentage had a greater increase rate among men living with a spouse at baseline (0.12 (0.01, 0.24)), baseline former smoker (0.27 (0.10, 0.44)), and men with concurrent increases in WC (0.17 (0.12, 0.22)). Leg fat percentage had a slower decrease in the elderly at baseline (0.29 (0.23, 0.35)) and had a greater decrease in the men with concurrent increase in WC (-0.11 (-0.17, -0.06)). No other correlates significantly contributed to the rate of change in DXA measures.

4.5   DISCUSSION

We report that with aging among African Ancestry men, fat appears to redistribute from the legs to the trunk, and accumulates ectopically within the skeletal muscle. With advancing age, the increase in total and trunk fat and the decrease in leg fat became slower. The decrease in muscle attenuation became greater. The rate of increase in inter-muscular fat was similar across age groups. In addition, we found that baseline T2D and hypertension are related to the rate of change in muscle attenuation, and total and trunk fat; however, lifestyle factors, such as alcohol consumption, smoking status, physical activity, and sedentary lifestyle, had no significant impact on the aging-associated redistribution of body fat or the accumulation of ectopic skeletal muscle fat in this cohort. Our findings support the hypothesis that cardiometabolic disorders are associated with the redistribution of body fat with aging.

Previous longitudinal studies similarly reported that the increase in general body fat with aging is independent of body weight change [34, 39]. The Health, Aging and Body Composition (Health ABC) Study reported that in black men, the approximate annualized rate of increase in

total body and trunk fat were 1% and 0.7%, respectively, with one- and two- years follow-up [37].

In our study, we showed similar annualized rates of increase in total body fat and trunk fat at 2.5%

and 0.5%, respectively, in our African ancestry men. Compared to our study, the participants of

the Health ABC were recruited to be older (aged 70-79 at the baseline), healthier, and very high-

functioning, and may have started to experience the deceleration in total body fat change that we

see in our older men. Moreover, our study was performed over 10-years of follow-up, which might

provide a more stable estimation of the rate of change in total and trunk fat. Thus, the results from

our study reflect the natural change of total body fat in middle-aged and elderly African ancestry

men recruited without regard to their health status.

Our study also found that the accumulation of skeletal intramuscular became greater, but

the accumulation of intermuscular fat was similar over age. Similarly, the Health ABC study

reported a similarly steady rate of intermuscular fat change with aging during 5-years of follow-

up [49]. They also reported a decrease in muscle attenuation, indicating an increase in

intramuscular fat, with aging in a cross-sectional study design [269].

We further examined the correlates that might contribute to the redistribution during aging,

including lifestyle factors and medical conditions. Previous studies suggested that physical activity

and sedentary lifestyle might modify the redistribution of general, regional and ectopic skeletal

muscle fat with aging. Moderate to vigorous physical activity was previously associated with a

favorable body fat distribution characterized by less total and trunk fats [270, 271]. Another

experimental study suggested that physical activity could prevent IM fat accumulation in the

elderly [89]. It was suggested that the protective effect of physical activity on IM fat accumulation

might depend on the intensity, suggesting that it must be vigorous enough to cause a detectable

change in IM fat. Indeed, a moderate physical activity intervention showed no association with IM

fat change in experimental [80] or observational [90] studies. In our study, physical activity was not associated with change in general, trunk or ectopic skeletal muscle fat. The discrepancy might be due to the method for the assessment of physical activity, because we used only self-reported information and did not collect information on intensity, duration, or type.

Sedentary lifestyle, mainly measured by television watching, is known to be associated with elevated risk of obesity, independent of physical activity [272]. However, the impact of sedentary lifestyle on regional body fat has been understudied and is inconsistent. Some previous studies suggested that the association between ectopic fat and sedentary lifestyle might be modified by the intensity of any physical activity that does take place [67, 93]. A previous longitudinal study indicated that increased television watching time is associated with an increase in WC, after accounting for physical activity and other covariates [91]. However, similar to our findings, a fourth, cross-sectional study found no association between intermuscular fat and sedentary lifestyle after adjusting for physical activity and BMI [273].

Baseline hypertension was inversely associated with the rate of increase in total and trunk fat, but was positively associated with the rate of decrease in muscle attenuation, indicating that men with hypertension tend to accumulate fat into skeletal muscle instead of total body or trunk. We have recently reported that in this African ancestry population, an increase in intermuscular fat is predictive of future T2D development [11], whereas a decrease in muscle attenuation, indicating an increase in intramuscular fat, is predictive of future hypertension development (Section 5.0; Zhao et al, manuscript under review). In the current study, men with insulin resistance, T2D or hypertension at baseline had greater decreases in muscle attenuation, indicating that muscle attenuation could also be a consequence, not only a predictor, of insulin resistance. Mechanistically, insulin resistance may be associated with mitochondrial dysfunction, which could

lead to decreases in both mitochondrial oxidative capacity and mitochondrial ATP synthesis, and may lead to elevated intramyocellular triacylglycerol [177]. To our knowledge, these are the first longitudinal studies to identify the bi-directional association between cardiometabolic disorders and ectopic skeletal muscle fat.

Our study has several limitations. First, our study is observational and thus causal relationship between the correlates and change of body composition could not be established. Second, the information of lifestyle factors and medical history were collected via self-reported questionnaires, which may not be completely accurate. Moreover, other facets of physical activity and sedentary lifestyle, such as intensity, type and duration should also be collected. Thus, our findings may suffer from information bias. Third, our findings may not be generalizable to women, other race/ethnic populations, or other geographic regions since we only focused on African ancestry men living in the Caribbean region. Fourth, we only included subjects who were healthy enough to receive pQCT and DXA measures, which might have introduced a "healthy participant" bias.

However, our study is the first to focus on the correlates and longitudinal changes in body fat distribution including ectopic skeletal muscle fat across a wide age range in a population at high-risk for cardiometabolic disease. With this longitudinal design, we are able to establish the temporality between baseline correlates and the change of total, regional and ectopic skeletal muscle fat.

In conclusion, this study shows that having a cardiometabolic disease at baseline is related to the longitudinal changes of general, regional, and ectopic skeletal muscle fat. In particular, our findings suggest that the temporal association between fat redistribution and cardiometabolic diseases may be bi-directional. We also show that the lifestyle factors measured in our study do

not affect the natural aging-associated redistribution of fat. These findings should be replicated in other race/ethnic groups and among women. Future studies are also warranted to collect more precise, detailed and objective measurements of lifestyle factors, to test if the association between cardiometabolic diseases and body fat distribution is independent of inflammation, and to identify the possible biological mechanisms underlying this relationship.

Table 5. Annualized Rate of Change in Pqct Measures and DXA Measures by Age Groups (Age at Visit 1 for DXA and Age at visit 3 for pQCT)

variables	average change	Men aged 40-54 years	Men aged 55-65 years	Men aged 65+ years years	P-value for trend
Intermuscular fat (%)	12.33	13.71	11.52	10.47	0.1739
Muscle attenuation (%)	-0.52	-0.43	-0.48	-0.77	<0.001
Total body fat (%)	2.54	2.76	2.22	2.07	0.0017
Trunk fat (%)	0.49	0.58	0.40	0.22	<.0001
Leg fat (%)	-0.32	-0.46	-0.19	0.05*	<.0001

*Rate of change is not significantly different from zero

Table 6. Baseline Correlates of the Rate of Change in IMAT and Muscle Attenuation among Men of African Ancestry during 6-Year Follow-Up

Variables	Mean ± SD or prevalence (%)	Unit	Age-adjusted rate of change in p-QCT measures	
			IMAT (%)	Muscle Attenuation (%)
Demographic characteristics				
Age	56.9±9.1	10	-1.30 (-3.40, 0.80)	**-0.10 (-0.20, -0.10)**
Education level	11.0%	1	5.55 (-0.62, 11.72)	-0.06 (-0.23, 0.10)
Marital status	71.1%	1	1.79 (-2.47, 6.05)	0.01 (-0.09, 0.12)
Lifestyle factors				
Current smoker	21.5%	1	0.14 (-4.64, 4.92)	-0.08 (-0.20, 0.04)
Former smoker	10.5%	1	-2.78 (-9.13, 3.56)	-0.02 (-0.18, 0.14)
TV watching	17.5%	1	-3.80 (-8.89, 1.29)	-0.00 (-0.13, 0.13)
Physical activity	50.4%	1	0.20 (-3.63, 4.04)	0.02 (-0.07, 0.12)
Alcohol consumption	10.6%	1	-2.21 (-8.44, 4.02)	0.11 (-0.05, 0.26)
Medical history				
Obesity	24.1%	1	-1.54 (-6.12, 3.03)	**-0.21 (-0.33, -0.10)**
Type 2 Diabetes	16.7%	1	-1.36 (-6.61, 3.90)	**-0.18 (-0.31, -0.05)**
Renal disease	0.2%	1	8.07 (-33.94, 50.07)	-0.72 (-1.64, 0.19)
Cardiovascular disease	3.4%	1	2.39 (-8.07, 12.86)	0.07 (-0.20, 0.35)
Stroke	1.4%	1	9.84 (-6.13, 25.80)	0.07 (-0.35, 0.50)
Cancer	6.5%	1	-1.11 (-9.04, 6.83)	0.03 (0.22, -0.17)
Myocardial infarction	0.68%	1	-10.15 (33.18, 12.88)	0.30 (-0.35, 0.95)
Hypertension	48.5%	1	-0.93 (-4.93, 3.08)	**-0.12 (-0.22, -0.02)**
Anthropometric measures				
Weight increase (%)	0.005±1.2	1.2	**3.90 (1.97, 5.83)**	**-0.07 (-0.12, -0.02)**
Waist circumference increase (%)	0.85±1.9	1.9	**3.22 (1.29, 5.16)**	**-0.08 (-0.15, -0.02)**
BMI increase (%)	0.01±1.2	1.2	**3.76 (1.84, 5.69)**	**-0.07 (-0.12, -0.03)**
Grip strength at visit 3	44.4±9.5	9.5	0.30 (-1.94, 2.52)	0.04 (-0.02, 0.09)
Other				
Family history of Type 2 Diabetes	38.0%	1	4.01 (-0.23, 8.25)	-0.05 (0.06, -0.15)
HOMA-IR	3.2±2.2	2.2	-1.74 (-3.68, 0.20)	**-0.06 (-0.11, -0.02)**

P<0.05 is shown in bold

Table 7. Multivariable Correlates of the Rate of Change in IMAT and Muscle Attenuation among Men of African Ancestry during 6-Year Follow-Up

Variables[†]	Unit	Rate of change in pQCT measures per unit	
		IMAT (%)	Muscle Attenuation (%)
Age (year)*	10	-1.36 (-3.50, 0.78)	**-0.09 (-0.15, -0.04)**
Prevalent of Type 2 Diabetes	1	-	**-0.18 (-0.31, -0.05)**
Prevalent of Hypertension	1	-	**-0.12 (-0.22, -0.02)**
Waist circumference increase (%)	1.9	**3.22 (1.29, 5.16)**	**-0.09 (-0.16, -0.03)**
Model R^2		0.01	0.04

P<0.05 is shown in bold

*Age was forced into the model

[†] Age, Education level, marital status, alcohol consumption, smoking status, physical activity, sedentary lifestyle, Type 2 Diabetes, stroke, cancer, myocardial infarction, hypertension, visit 3 grip strength, annualized waist circumference change (%) were entered into the model

Table 8. Baseline Correlates of the Rate of Change in Total, Trunk and Leg fat among Men of African Ancestry during 6-year Follow-Up

variables	Mean ± SD or prevalence (%)	Unit	Age-adjusted rate of change in DXA measures		
			Total body fat (%)	Trunk fat (%)	Leg fat (%)
Demographic characteristics					
Age	56.8±9.2	10	**-0.43 (-0.67, -0.18)**	**-0.11 (-0.17, -0.06)**	**0.29 (0.23, 0.35)**
Education level	11.1%	1	-0.72(-1.45,0.00)	-0.15 (-0.31, 0.01)	-0.12 (-0.30, 0.07)
Marital status	71.3%	1	-0.18(-0.68,0.33)	**0.12 (0.01, 0.23)**	-0.02 (-0.15, 0.11)
Lifestyle factors					
Current smoker	21.0%	1	0.28(-0.28,0.84)	-0.03 (-0.16, 0.10)	0.00 (-0.14, 0.14)
Former smoker	10.2%	1	**1.06 (0.31,1.81)**	**0.26 (0.09, 0.42)**	0.02 (-0.17, 0.21)
TV watching	17.3%	1	-0.16 (-0.77,0.45)	-0.10 (-0.24, 0.03)	-0.08 (-0.23, 0.07)
Physical activity	50.4%	1	0.40 (-0.06,0.85)	0.08 (-0.03, 0.18)	0.01 (-0.10, 0.13)
Alcohol consumption	10.3%	1	-0.18 (-0.92,0.57)	-0.01 (-0.17, 0.16)	0.02 (-0.17, 0.21)
Medical history					
Obesity	25.3%	1	**-1.13 (-1.66,-0.61)**	-0.10 (-0.22,0.02)	-0.07 (-0.21, 0.06)
Type 2 Diabetes	16.8%	1	-0.49 (-1.11,0.12)	0.03 (-0.11,0.17)	-0.09 (-0.24, 0.07)
Renal disease	0.2%	1	-1.58 (-6.69,3.53)	-0.37 (-1.51,0.77)	0.03 (-1.26, 1.31)
Cardiovascular disease	3.4%	1	0.39 (-0.88,1.65)	-0.09 (-0.38,0.19)	-0.12 (-0.43, 0.20)
Stroke	1.4%	1	1.70 (-0.24,3.64)	-0.27 (-0.70,0.17)	0.02 (-0.47, 0.51)
Cancer	6.7%	1	0.29 (-0.66,1.24)	-0.07 (-0.28,0.15)	0.13 (-0.11, 0.37)
Myocardial infarction	0.66%	1	-2.19 (-4.99,0.61)	-0.32 (-0.94,0.31)	-0.31 (-1.01, 0.40)
Hypertension	48.8%	1	**-0.89 (-1.36,-0.42)**	**-0.19 (-0.29,-0.08)**	-0.04 (-0.16, 0.09)
Anthropometric measures					
Weight increase (%)	0.0088±1.2	1.2	**2.68(2.51,2.86)**	**0.30 (0.25,0.35)**	**-0.21 (-0.26, -0.15)**
Waist circumference increase (%)	0.86±1.9	1.9	**1.64(1.42,1.85)**	**0.17 (0.12,0.22)**	**-0.12 (-0.17, -0.06)**
BMI increase (%)	0.013±1.2	1.2	**2.58 (2.40,2.76)**	**0.30 (0.25,0.35)**	**-0.21 (-0.26, -0.16)**
Grip strength at visit 3	44.5±9.6	9.6	**-0.33 (-0.59,-0.07)**	0.01 (-0.05,0.07)	-0.02 (-0.08, 0.05)
Other					
Family history of Type 2 Diabetes	38.0%	1	-0.29 (-0.78, 0.20)	-0.01 (-0.12, 0.10)	-0.12 (-0.24, 0.01)
HOMA-IR	3.2±2.3	2.3	**-0.66 (-0.88, -0.44)**	**-0.09 (-0.14, -0.03)**	-0.06 (-0.12, 0.00)

P<0.05 is shown in bold

Table 9. Multivariable Correlates of the Rate of Change in Total, Trunk and Leg Fat Percentage among Men of African Ancestry during 6-year Follow-Up

Variables[†]	Unit	Rate of change in DXA measures per unit		
		Total body fat (%)	Trunk fat (%)	Leg fat (%)
Age (year)*	10	-0.29 (-0.53, -0.04)	-0.08 (-0.14, -0.03)	0.29 (0.23, 0.35)
Marriage status	1	-	0.12 (0.01, 0.24)	-
Current smoker	1	0.35 (-0.17, 0.88)	-0.03 (-0.15, 0.10)	-
Former smoker		1.16 (0.45, 1.87)	0.27 (0.10, 0.44)	-
Prevalent of Hypertension	1	-0.70 (-1.14, -0.25)	-0.16 (-0.27, -0.06)	-
Waist circumference change (%)	1.9	1.60 (1.39, 1.82)	0.17 (0.12, 0.22)	-0.11 (-0.17, -0.06)
Model R^2		0.14	0.05	0.06

P<0.05 is shown in bold

*Age was forced into the model

[†] Age, Education level, marital status, alcohol consumption, smoking status, physical activity, sedentary lifestyle, Type 2 Diabetes, stroke, cancer, myocardial infarction, hypertension, visit 3 grip strength, annualized waist circumference change (%) were entered into the model

```
┌─────────────────────┐         ┌─────────────────────┐
│  Original Tobago     │         │  Participated in pQCT│
│  Health Study for    │────────▶│  measurements at     │
│  prostate cancer     │         │  visit 3             │
│  screening           │         │  (n=2,512)           │
│  (n=3,376)           │         └─────────────────────┘
└─────────────────────┘                    │
                                           │         ┌─────────────────────────┐
                                           ├────────▶│ Participants who were    │
                                           │         │ lost of follow-up        │
                                           │         │ (n=894)                  │
                                           ▼         └─────────────────────────┘
                               ┌─────────────────────┐
                               │ Participated in pQCT │
                               │ measurements at      │
                               │ visit 5              │
                               │ (n=1618)             │
                               └─────────────────────┘
```

Participants who were lost of
follow-up
(n=894)

Participants with 1 of the following
were excluded
-no African ancestry
-age less than 40 at baseline
-missing value on pQCT
measurements
(n=138)

Total number of eligible
participants
(n=1480)
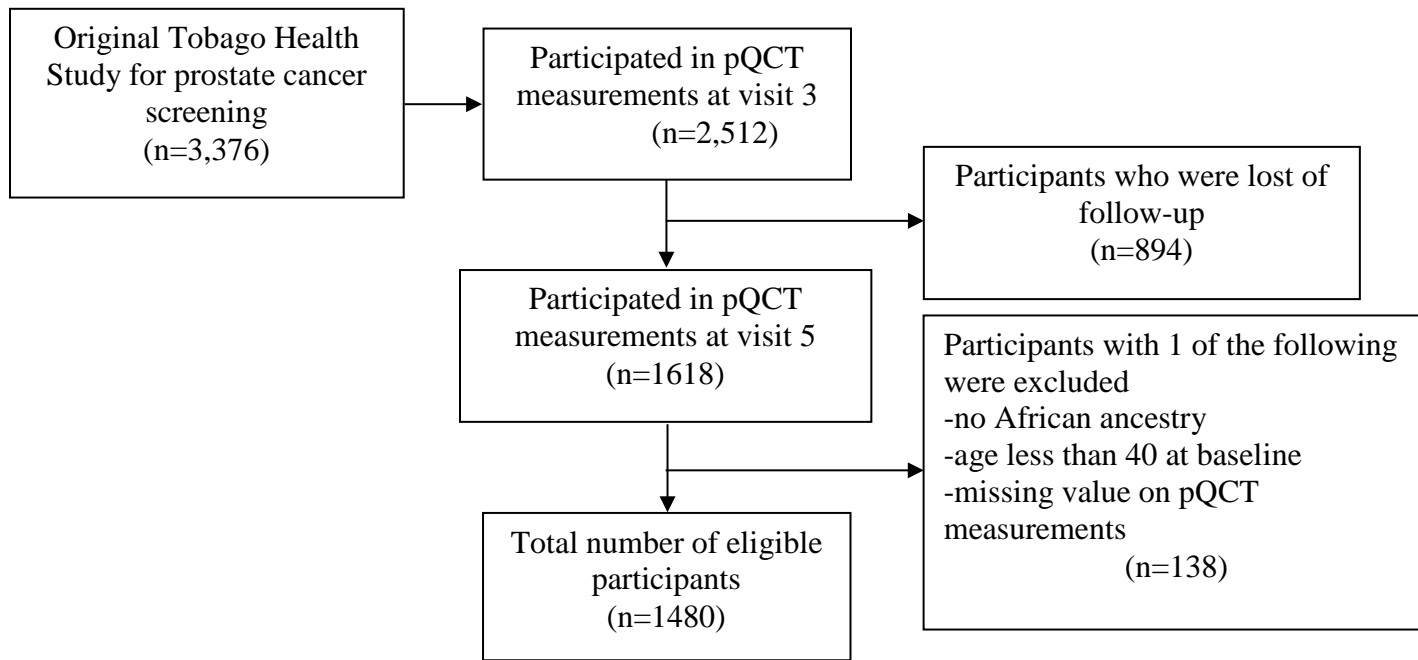
Figure 1. Flow chart for the pQCT measures
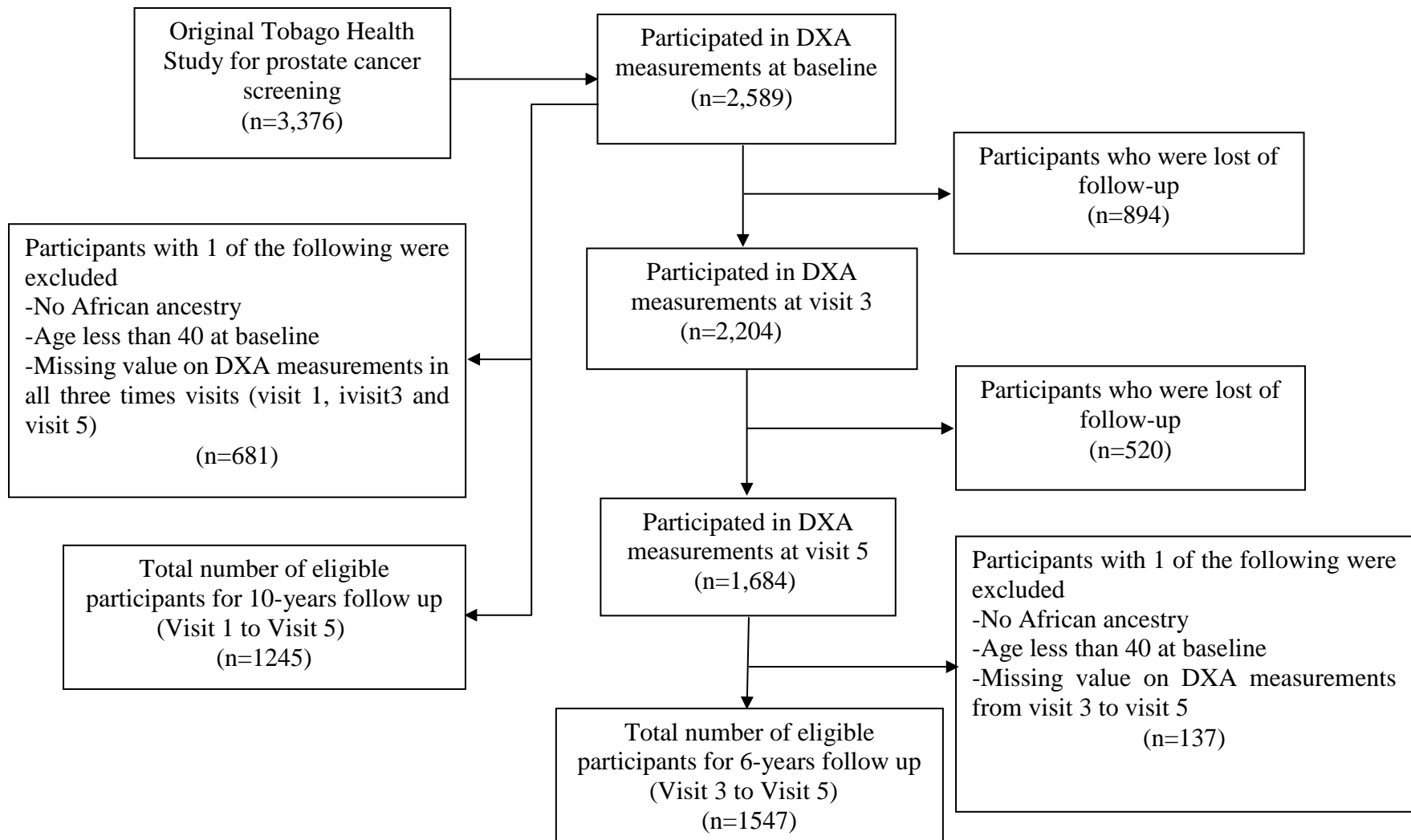
Figure 2. Flow chart for the DXA measures

5.0 GREATER SKELETAL MUSCLE FAT INFILTRATION IS ASSOCIATED WITH HIGHER ALL-CAUSE MORTALITY AMONG MEN OF AFRICAN ANCESTRY

5.1 ABSTRACT

Background: Fat infiltration within and around skeletal muscle (i.e. myosteatosis) increases with aging, is greater in African versus European ancestry men, and is associated with poor health. Myosteatosis studies of mortality are lacking, particularly among African ancestry populations.

Methods: In the Tobago Health study, a prospective longitudinal study, we evaluated the association of all-cause mortality with quantitative computed tomography (pQCT) measured lower leg myosteatosis (intermuscular fat (IM fat) and muscle density) in 1652 African Ancestry men using Cox proportional hazards models. Date of death was abstracted from death certificates and/or proxy.

Results: 112 deaths occurred during follow-up (mean 5.9 years). In all men (age range 40 to 91 years), higher all-cause mortality was associated with greater IM fat (HR (95% CI) per SD: 1.29 (1.06-1.57)) and lower muscle density (HR (95% CI) per SD lower: 1.37 (1.08-1.75)) in fully adjusted models. Similar mortality hazard rates were seen in the subset of elderly men (aged ≥65 years) with greater IM fat (1.40 (1.11-1.78) or lower muscle density (1.66 (1.24-2.21)) in fully adjusted models.

Conclusions: Our study identified a novel, independent association between myosteatosis and all-cause mortality in African ancestry men. Further studies are needed to establish if this association is independent of other ectopic fat depots and to identify possible biological mechanisms underlying this relationship.

5.2   INTRODUCTION

Ectopic fat infiltration within and around skeletal muscle (myosteatosis) is associated with metabolic disorders and poor musculoskeletal health [241, 242, 263, 274-276]. In particular, myosteatosis may play an important role in the development of insulin resistance and type 2 diabetes (T2D) [241, 242, 276-280], decreased muscle strength [281, 282], and mobility loss [274, 283]. Versus Caucasians, African Ancestry individuals have greater myosteatosis [10], shorter lifespan expectancy, and higher mortality from diabetes and heart disease [165]. Despite the emerging clinical importance of myosteatosis, studies examining the relationship of this important fat depot and mortality are sparse, particularly among high-risk African ancestry individuals. Therefore, we assessed myosteatosis and mortality in a large population-based cohort of African Ancestry men aged 40 years and above, regardless of their health status. We hypothesized that greater myosteatosis would be associated with elevated risk of all-cause mortality independent of age, lifestyle, comorbidities and BMI.

5.3 METHODS

Tobago Health Study

Between 1997 and 2003, 3376 men aged 40 and above were recruited for population-based prostate cancer screening on the Caribbean island of Tobago [260]. To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. Recruitment for the initial screening was accomplished by flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. The population representative sample included 97% African, 2% East Indian, <1% white, and <1% "other" [17] .

Between 2003 and 2007, men from the prostate cancer study were invited via phone for peripheral quantitative computed tomography (pQCT) scan of calf skeletal muscle composition, which serves as the baseline for the analysis. Death dates were obtained from death certificates and/or reports from a proxy (58% vs. 42% of study participants, respectively) between November 2003 and May 2013. Of the 2029 with some adiposity measurements at baseline, 377 men lost to follow-up with no information on date-of-censor were excluded. Therefore, our analysis is limited to men with baseline pQCT, known vital status at follow-up and African Ancestry (N=1652). Written informed consent was obtained from all participants. This study has been approved by the Institutional Review Boards of the Tobago Division of Health and Social Services and the University of Pittsburgh.

Myosteatosis Measures

Myosteatosis measures included intermuscular fat (IM fat, visible fat beneath the fascia lata) and muscle density (fat between muscle fibers and fat within the muscle cell) from pQCT scan of the calf performed with a Stratec XCT-2000 scanner (Orthometrix, Inc.; White Plains,

NY). Lower skeletal muscle density from pQCT is indicative of greater intra-muscular fat content [262]. A site at 66% of the calf length, proximal to the terminal end of the tibia was scanned, since it has the largest circumference and the lowest variability among individuals [263]. All images were analyzed with STRATEC analysis software version 5.5D (Orthometrix, Inc., White Plains, NY) and performed by a trained investigator unaware of the participant's disease status.

With the pQCT scan, fat, muscle, and bone can be distinguished by different mineral equivalent density of 0, 80, 1200mg/cm^3, respectively. IM fat can be detected as a shift of mineral equivalent density from 80 (muscle) to 0 (fat) mg/cm^3. Muscle density was determined as the ratio of muscle mass (mg) and muscle area (cm^2) [264]. Similarly, total muscle area (mm^2), total fat (mm^2), and subcutaneous fat (mm^2) were obtained. The coefficients of variation (CV) determined by repeat pQCT scanning in 15 individuals were 1.0%, 1.5%, 7.6%, 0.9%, and 1.1% for total, subcutaneous, and IM fat, muscle area, and muscle density respectively.

General adiposity measures

Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm. Weight was measured on a balance beam scale to the nearest 0.1 kg. Body mass index (BMI) was calculated from measured height and weight (kg/m^2). Waist circumference was measured at the narrowest point of waist, or at umbilicus, if the narrowest point could not be identified.

Medical Conditions

T2D was defined as fasting serum glucose ≥126mg/dl or currently taking anti-diabetic medication. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg or currently taking antihypertensive medication. Other comorbidities including "ever having" cancer, CVD, renal disease, stroke, MI were self-reported yes/no using questionnaire.

Other measures

Covariate information was collected with standardized interviewer-administered questionnaires. Participants were instructed to bring in all prescription medications taken in the past 30 days to their clinic visit and interviewers recorded the medications. Smoking status was categorized as nonsmoker (men who smoked less than 100 cigarettes total in their lifetime), former smoker (men who smoked more than 100 cigarettes but did not currently smoke) or current smoker and treated as nominal variables. Walking is the predominant form of physical activity in Tobago, so physical activity was assessed by whether or not participants walked for exercise, to work, the store or church 4 or more times in the past 7 days. Hours of television watching was assessed, with the cutoff point of "21 or more hours" per week as showing some versus no sedentary behavior. Self-reported health status was categorized as good/excellent versus fair/poor/very poor. All baseline information provided by pQCT measures or questionnaires was obtained at the same day.

Statistical analysis

Outliers were defined as any value outside the interval of $Quartile_3$ (75%ile) +3IQR (IQR=interquartile range) and $Quartile_1$ (25%ile) -3IQR and were deleted (11 were deleted for muscle density and 27 were deleted for IM fat) to increase the statistical power. All continuous variables were normally distributed. Distributions of continuous and categorical variables were presented as mean ± standard deviation (SD) or frequency and analyzed with Student's T-test, Chi-square test or Fisher exact test, as necessary. Age-adjusted P-values were presented.

Age- and multivariable-adjusted hazard ratio (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazard models. The proportional hazards assumptions were confirmed by Schoenfeld residuals. The adjusted covariates were selected based on previous studies, biological plausibility and results from Table 1, including age, height, smoking status,

alcohol intake, physical activity, TV viewing time, health status, and presence or absence of T2D, renal disease, stroke, cancer, MI and hypertension. Subcutaneous fat, IM fat and skeletal muscle density models were additionally adjusted for BMI and calf muscle area. Due to missing covariate data, the final N in multivariable models was 1495 for IM fat and 1375 for muscle density. Lastly, we performed fully adjusted, stratified analyses by age groups (< 65 and 65+).

Multicollinearity was checked with variation inflation factor (VIFs of included variables were less than 10). Sensitivity analyses with the outliers included confirmed the results (Appendix 1). Analyses were performed with SAS statistical software version 9.3 (SAS Institute Inc., Cary, North Carolina).

5.4 RESULTS

Baseline characteristics

Of the 2029 men with baseline data, 377 were lost to follow-up. Of the 1652 men with known mortality status and baseline pQCT, 112 participants died during a mean follow-up of 5.9 years (median (IQR) 6.0 years (5.5-6.4 years); Table 11). At baseline, the mean age of the men was 57.7 years (Table 1). The majority reported excellent or good health (93.2%). Deceased men were older and with a poorer health status and a lower BMI, had higher prevalence of T2D, renal disease, cardiovascular disease, stroke, cancer and hypertension, and higher anti-diabetic drug use. Additionally, deceased men had greater IM fat and lower muscle density after adjustment for age (both $p<0.05$).

Myosteatosis and All-Cause Mortality

IM fat (per SD greater) and muscle density (per SD lower) were associated with 21% (95%CI: 2%-44%) and 36% (95%CI: 11%-67%) higher hazard of mortality, respectively, independent of age (Table 12). These results slightly attenuated after additionally adjusted for comorbidities and lifestyle factors (HR (95%CI) per SD greater IM fat: 1.20 (0.99-1.45) and per SD lower muscle density: 1.30 (1.03-1.63)), and in models with further adjustment for BMI and muscle area ((HR (95%CI) per SD greater IM fat: 1.29 (1.06-1.57) and per SD lower muscle density: 1.37 (1.08-1.75)). No other measure of adiposity was associated with mortality, regardless of the degree of adjustment.

Myosteatosis and all-cause mortality by age groups

Among the 1242 middle-aged men (aged 40-64 years), 35 deaths occurred. Neither IM fat nor muscle density was associated with all-cause mortality among middle-aged men in any model (Table 13). Among 410 elderly men (aged 65+ years), 77 deaths occurred. In these men, both greater IM fat and lower muscle density were associated with greater all-cause mortality in both age-adjusted and fully adjusted models (p<0.05 for all).

5.5   DISCUSSION

We report an association between both intermuscular fat and muscle density and increased hazards of all-cause mortality among African ancestry men, even after adjustment for important lifestyle and medical covariates, and general adiposity.

91

Despite the growing appreciation of a potential role for myosteatosis in metabolic health and aging independent from overall adiposity [241, 242, 276-280], few studies have examined the association between myosteatosis and mortality. Existing studies have focused exclusively on older Caucasians [150, 284] or highly selected individuals [285]. Similarly to our findings, the Walking and Leg Circulation Study II (WALCS II) reported a higher mortality risk associated with lower calf muscle density among 434 patients with lower extremity peripheral arterial disease (PAD) [285]. The Osteoporotic Fractures in Men Study (MrOS) further extended the finding to community-dwelling older Caucasian men (N=1063, mean age=77) and found that muscle density, but not intermuscular fat was associated with an increased risk of mortality [150]. The AGES-Reykjavik study [286] in Icelandic individuals aged 66 to 96 years found that greater thigh inter-muscular fat was independently associated with elevated mortality risk in men, but not in women, suggesting a potential gender difference in the adverse impact of intermuscular fat. Our study extends these findings to middle-aged and elderly African ancestry men, a high-risk population segment inadequately studied thus far. Contrary to our findings, the "Invecchiare in Chianti" (InCHIANTI) study [284], a prospective population-based study among Italians (N=934, mean age=74.5) found no association between muscle density and all-cause mortality. The discrepancy may due to differences in the studied populations as ours is younger, yet, less healthy as determined by comorbidity prevalence.

Ectopic fat increases with the aging process. Our results showed an adverse impact of myosteatosis on mortality risk in a combined sample of middle-aged and elderly African ancestry man. In the fully adjusted model of men aged 65 years and older, the mortality risk was 40% (95%CI: 11%-78%) and 66% (95%CI: 24%-121%) greater per one SD greater IM fat and one SD lower muscle density, respectively. This indicates that maintaining muscle density and prevention

of muscle fat infiltration may be crucial for successful aging in elderly men. However, we were unable to detect a significant association in middle-aged men alone. The adverse impact of greater myosteatosis on mortality risk may be due to an acceleration of muscle density loss and ectopic fat infiltration around age 65.

Insulin resistance is hypothesized to be a culprit linking myosteatosis and mortality. The accumulation of inter-muscular fat may impair nutritive blood flow to muscles, and lead to impaired insulin action and insulin diffusion capacity [241]. Additionally, the accumulation of intra-muscular fat may impair the glucose metabolism via the insulin receptor substrate 1/phosphatidylinositol 3-kinase and growth-factor-regulated protein kinase B pathways [239], and eventually lead to impaired insulin signaling and insulin resistance [238]. Myosteatosis could also increase local inflammation in the muscle fibers [248]. This increased inflammation could lead to increases in oxidative stress, resulting in a decrease in insulin-stimulated tyrosine phosphorylation [240] and a decrease in the activity of downstream signaling molecules; thus, resulting in insulin resistance [240]. However, in our study, the significant association between myosteatosis and all-cause mortality risk persists even after adjusting for T2D or HOMA-IR (results not shown), which suggests that there are also other mechanisms underlying these associations.

Subcutaneous fat is generally thought to be protective for health outcomes because it stores excess fat and prevents it from overflowing into other deleterious ectopic depots [278]. Several studies have linked thigh subcutaneous fat to a favorable glucose or lipid profile, independent of abdominal fat [206]. Although, we have previously linked lower calf subcutaneous fat to a higher prevalence of T2D [278], we found no association between subcutaneous fat and all-cause mortality.

No other measures of adiposity were associated with mortality, despite the fact that some previous studies revealed a relation of BMI or waist circumference with mortality risk [287]. However, it has been hypothesized that this association may be attenuated with increasing age due to the associated redistribution of body fat, or shrinkage of height [288]. Thus, it is possible that the wide age range of our population (40 to 91 years) may have weakened the associations between BMI or waist circumference and mortality.

However, the inclusion of men with a wide age range is also a major strength of our study as we were able to test for an age effect. Other strengths of our study include the population-based design, which allows us to study the effects of myosteatosis within the normal process of aging. And, most importantly, ours is the first mortality study of myosteatosis in African ancestry men. Given similar total body fat across ethnicities, the greater myosteatosis observed in African ancestry men might explain the relatively higher risk of mortality, especially from T2D and heart disease [165]. However, further analyses in comparable, ethnically diverse cohorts are needed to definitively address this hypothesis.

Our study has some limitations. First, our study is observational, and causality cannot be determined. Second, 377 men were lost to follow-up. Compared to individuals with complete follow-up information, the censored individuals were older and less healthy (Appendix 2) and, therefore, likely had a greater risk of mortality. Even with a possibly underestimated mortality rate, we were still able to identify a significant association of myosteatosis and mortality. Third, some bias may have been introduced from missing covariate data in multivariable models. Fourth, the bias caused by reverse causation, a phenomenon that obesity-related disease may lead to both greater myosteatosis and elevated risk of mortality, is unavoidable. In order to minimize this bias, our analyses were adjusted for several major comorbidities related to obesity at baseline. Fifth,

including only the men who were well enough to have pQCT measures may have resulted in a "healthy participant" bias and could limit the generalizability of our findings. Sixth, fat located in other ectopic storage depots, such as the liver, pancreas and heart, should be taken into consideration. Lastly, other untested potential mechanisms such as inflammation or mitochondrial function may be residual confounders.

In conclusion, our study identified a previously unreported, independent association of both intra- and inter- muscular fat with mortality in middle-aged and elderly African ancestry men. Further, it highlights the potential importance of maintaining muscle density in the elderly. These results illustrate that myosteatosis may be a novel independent marker of aging with a potential impact on healthy aging and expected lifespan. Future studies are needed to replicate our findings in other populations of African ancestry, to establish if the association is independent of other ectopic fat depots, and to identify the precise biological mechanisms underlying this relationship.

Table 10. The Distributions of Baseline Demographic Characteristics, Lifestyle Factors and Comorbidity by Vital Status in 1652 Tobago Men

	All (n=1652)	Survivors (n=1540)	Deceased (n=112)	*P*-value*	Age- adjusted *P*-value
Socio-demographic					
Age	57.7±9.6	56.9±9.1	68.9±9.4	<0.001	-
Education level, n (%)					
High school and above	181(11.1)	172(11.3)	9(8.0)	0.287	0.737
Marriage status, n (%)					
Married or live with a spouse	1167(71.1)	1089(71.1)	78(70.9)	0.969	0.993
Lifestyle factors					
Smoking, n (%)					
Former smoker	364(22.0)	334(21.7)	30(26.8)	0.137	0.020
Current smoker	176(10.7)	160(10.4)	16(14.3)		
Alcohol consumption, n (%)					
>3 drinks per week	172(10.4)	163(10.6)	9(8.0)	0.393	0.899
Physical activity, n (%)					
>3 times per week	822(49.8)	766(49.7)	56(50.0)	0.958	0.759
TV viewing, n (%)					
>21 hours per week	290(17.6)	272(17.7)	18(16.1)	0.654	0.864
Health status, n (%)					
Excellent/good	1530(93.2)	1444(94.3)	86(78.2)	<0.001	<0.001

Table 10 Continued

Comorbidities

Prevalence of Type 2 Diabetes, n (%)	305(18.9)	258(17.1)	47(42.3)	<0.001	<0.001
Renal disease, n (%)	5(0.3)	3(0.2)	2(1.8)	0.039	0.059
Cardiovascular disease, n (%)	62(3.8)	52(3.4)	10(8.9)	0.007	0.096
Stroke, n (%)	26(1.6)	21(1.4)	5(4.5)	0.027	0.169
Cancer, n (%)	129(7.8)	100(6.5)	29(25.9)	<0.001	0.002
Myocardial infarction, n (%)	13(0.8)	11(0.7)	2(1.8)	0.218	0.204
Hypertension, n (%)	823(49.8)	746(48.4)	77(68.8)	<0.001	0.506
Medication use					
Antihypertensive drugs, n (%)	342(60.5)	301(61.1)	41(56.9)	0.505	0.148
Lipid lowering drugs, n (%)	71(4.5)	69(4.6)	2(2.8)	0.769	0.413
Anti-diabetic drugs, n (%)	189(11.7)	156(10.4)	33(29.7)	<0.001	0.004
Body composition characteristic					
BMI (kg/m^2)	27.4 ± 4.4	27.5 ± 4.4	26.3 ± 4.6	0.005	0.148
Waist Circumference (cm)	93.1 ± 11.1	93.1 ± 11.1	92.9 ± 11.3	0.853	0.664
Calf Total Fat (mm^2)	1794.8 ± 765.7	1792.8 ± 762.0	1823.2 ± 817.5	0.689	0.718
Calf Subcutaneous Fat (mm^2)	1367.0 ± 669.1	1375.1 ± 670.8	1254.3 ± 636.6	0.069	0.625
Calf Intermuscular Fat (mm^2)	249.7 ± 17.3	241.9 ± 210.5	361.8 ± 276.6	<0.001	0.014
Calf Skeletal Muscle Density (mg/cm^3)	73.5 ± 3.9	73.7 ± 3.7	70.4 ± 5.2	<0.001	<0.001
Calf Muscle Area (mm^2)	7556.4 ± 1295.5	7614.2 ± 1279.8	6757.2 ± 1251.5	<0.001	0.006
Total body fat (%)	20.7 ± 5.8	20.6 ± 5.8	21.8 ± 6.3	0.0348	0.9675
Trunk fat (%)	48.7 ± 5.3	48.7 ± 5.3	47.7 ± 6.1	0.0533	0.0243
Leg fat (%)	33.0 ± 4.6	33.0 ± 4.5	33.8 ± 5.2	0.0664	0.0280

Values are unadjusted mean ± SD, unless indicated otherwise
*P-values for comparisons between the survivors and deceased from two-sample T-tests or Chi-square tests or Fisher's Exact Test
Age-adjusted P-values were obtained from logistic regression for categorical variables and linear regression for continuous variables

Table 11. Hazard Ratios (95% Confidence Interval) for All-Cause Mortality per SD Greater Baseline Adiposity or Muscle Measure

	Age adjusted	Multivariable[1]	Multivariable[1] + BMI and muscle area
BMI (kg/m^2)	0.88 (0.71-1.10)	0.88 (0.70-1.11)	-
Waist Circumference (cm)	1.01 (0.82-1.25)	1.01 (0.80-1.28)	-
Calf Total Fat (mm^2)	1.01 (0.84-1.21)	1.04 (0.85-1.26)	-
Calf Subcutaneous Fat (mm^2)	0.94 (0.76-1.16)	0.94 (0.76-1.16)	1.04 (0.78-1.39)
Calf Intermuscular Fat (mm^2)	1.21 (1.02-1.44) [†]	1.20 (0.99-1.45)	1.29 (1.06-1.57) [†]
Calf Skeletal Muscle Density (mg/cm^3)*	1.36 (1.11-1.67)[†]	1.30 (1.03-1.63) [†]	1.37 (1.08-1.75) [†]
Calf Muscle Area (mm^2)	0.77 (0.61-0.98) [†]	0.85 (0.65-1.09)	-
Total body fat (%)	1.06 (0.86-1.29)	1.02 (0.82-1.26)	-
Trunk fat (%)	0.93 (0.76-1.13)	0.96 (0.77-1.19)	-
Leg fat (%)	1.06 (0.86-1.30)	1.05 (0.84-1.31)	-

[1]adjusted for age, height, smoking status, alcohol consumption, physical activity, TV viewing time, health status, cancer, T2D, renal disease, stroke, myocardial infarction, hypertension
*per SD lower
[†]P-value< 0.05 [‡]P-value < 0.01
Hazard ratios and P-values were obtained with Cox proportional hazard models.
Fully adjusted multivariable model N's for BMI, waist circumference, calf total fat, calf subcutaneous fat, and calf muscle area were 88 deaths and 1435 survivors. N for calf intermuscular fat was 82 deaths and 1413 survivors. N for calf muscle density was 72 deaths and 1303 survivors.

Table 12. Age Stratified Adjusted Hazard Ratios (95% Confidence Interval) of All-Cause Mortality Risk by Baseline Myosteatosis Measures*

Model	Age group			
	Under 65 years (N died/survived: 35/1207)		65 years and above (N died/survived: 77/333)	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Intermuscular Fat (mm^2)				
Age adjusted	1.08 (0.75-1.55)	0.675	1.26 (1.03-1.53)	0.025
Multivariable[1] adjusted	1.16 (0.74-1.82)	0.527	1.40 (1.11-1.78)	0.005
Muscle density (mg/cm^3)				
Age adjusted	0.97 (0.61-1.53)	0.880	1.50 (1.19-1.90)	<0.001
Multivariable[1] adjusted	0.87 (0.51-1.48)	0.601	1.66 (1.24-2.21)	0.007

[1]adjusted for age, height, smoking status, alcohol consumption, physical activity, TV viewing time, health status, cancer, T2D, renal disease, stroke, myocardial infarction, hypertension, BMI and calf muscle cross-sectional area
*per 1 SD increase in IM fat and 1 SD decrease in muscle density
Hazard ratios and *P*-values were obtained with Cox proportional hazard models.

6.0   INTRAMUSCULAR FAT IS ASSOCIATED WITH NEWLY DEVELOPED HYPERTENSION IN AFRICAN ANCESTRY MEN

6.1   ABSTRACT

Background: Previous studies have shown that increased ectopic fat infiltration in the skeletal muscle (i.e. myosteatosis) is associated with insulin resistance and diabetes, but studies examining the association between myosteatosis and other cardiometabolic diseases are lacking, particularly in high-risk African ancestry populations. We sought to determine if change in myosteatosis predicts subsequent development of hypertension in African ancestry men.

Methods: In the Tobago Health Study, a prospective longitudinal study of African ancestry men (age range 40 to 91 years), calf intermuscular fat and skeletal muscle attenuation, a proxy for intramuscular fat, were measured with computed tomography. Blood pressure was assessed at two time points. Hypertension was defined as a systolic blood pressure over 140 mmHg, or a diastolic blood pressure over 90 mmHg, or receiving anti-hypertensive medications. Logistic regression was performed with the adjustment for age, demographic characteristics, lifestyle factors, HOMA-IR, baseline body mass index (BMI) and 6-year change in BMI, baseline waist circumference (WC) and 6-year change in WC, and 6-year change in muscle area.

Results: Among 726 normotensive men at baseline, 309 men (43%) developed hypertension during mean 6.2 years follow-up. There were no significant associations between increases in BMI or WC and newly developed hypertension after adding calf intermuscular fat or skeletal muscle attenuation to the models. A decrease in skeletal muscle attenuation, indicative of an increase in intramuscular fat, was robustly associated with elevated odds of newly developed hypertension

after adjustment for the baseline and the change in BMI (OR (95% CI) per SD: 1.32 (1.06,1.64))

or WC measurements (OR (95% CI) per SD: 1.35 (1.08, 1.68)). In contrast, an increase in calf

intermuscular fat was not associated with newly developed hypertension in any model.

Conclusions: Our novel findings show that increased intramuscular fat is associated with newly

developed hypertension among African ancestry men, independent of general and central adiposity

and insulin resistance. Further studies are needed to establish if this observed association is

independent of inflammation, visceral or other ectopic fat depots, to identify possible biological

mechanisms underlying this relationship, and to confirm our findings in other populations.

6.2   INTRODUCTION

Storage of fat around and within non-adipose tissue organs (known as ectopic fat) is now

recognized as a risk factor for type 2 diabetes (T2D) and cardiovascular disease (CVD),

independent of general adiposity [289-296]. Compared to Caucasians, African ancestry individuals

have greater burden of T2D [266] and hypertension [297], and greater ectopic fat infiltration in the

skeletal muscle (i.e. myosteatosis) [47, 298]. However, they also have less general [299] and

visceral fat [6]. This suggests that myosteatosis may be a key fat depot contributing to the increased

risk of cardiometabolic diseases in African ancestry populations. Despite the fact that myosteatosis

has been associated with insulin resistance [246] and T2D [244] the studies on the association

between myosteatosis and hypertension are sparse, particularly in this high-risk population group.

Therefore, our objective was to prospectively evaluate the relationship between myosteatosis and

newly developed hypertension among middle-aged and elderly African ancestry men, while

accounting for potential confounding factors, including total and central adiposity, and insulin

resistance. We hypothesized that greater myosteatosis would be associated with the development of hypertension, and that the association will be independent of general and central adiposity, and insulin resistance.

6.3   METHODS

Tobago Health Study

The first Tobago Health Study visit was conducted between 1997 and 2003 on the Caribbean island of Tobago with the original purpose of providing prostate cancer screening. To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. 3089 men (aged 40 years and above) were recruited regardless of their health status via flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. The samples was representative of the population with low admixture, including 97% African, 2% East Indian, <1% white, and <1% "other" participants.

Between 2003 and 2007, 2,152 men from the original cohort were invited via phone to participate in peripheral quantitative computed tomography (pQCT) scan of calf skeletal muscle composition, which served as the baseline clinic visit for these analyses. From 2010-2013, we invited these men to return for repeat pQCT scans of calf skeletal muscle composition. A total of 1618 men completed the follow-up assessment (80% of survivors). Both the baseline and follow-up visits followed the same procedures for questionnaire interviews, biospecimen collection, and pQCT scans [268]. Hypertension was defined as a systolic blood pressure over 140 mmHg, or a diastolic blood pressure over 90 mmHg, or currently taking anti-hypertensive medications. Men

102

with hypertension or a history of CVD at baseline were excluded. A total of 726 initially normotensive men were included in the final analyses.

The Institutional Review Boards of the University of Pittsburgh and the Tobago Division of Health and Social Services approved this study and all participants provided written informed consent before data collection.

Peripheral Quantitative Computed Tomography (pQCT) Quantification of Myosteatosis

Studies that utilize pQCT scans for measures of myosteatosis examine either intermuscular fat (visible fat beneath the fascia lata) and/or muscle attenuation (lower skeletal muscle attenuation is indicative of greater intramuscular fat content) [261, 262]. In our study, both types of measures of myosteatosis were obtained by pQCT scans of the calf, which were performed using the Stratec XCT-2000. At both study visits, 2.2 mm cross-sectional images of the calf skeletal muscle composition were obtained at 66% of the tibia length, proximal to the terminal end of the tibia, because this is the region with the largest circumference of the calf and has less variability between individuals [263].All images were analyzed with STRATEC analysis software version 5.5D (Orthometrix, Inc., White Plains, NY) by a trained investigator unaware of the participants' disease status.

The mineral equivalent density of fat, muscle, and bone differ as 0, 80, 1200mg/cm^3, respectively, which can be distinguished by the pQCT scan. IM fat was determined by a shift of mineral equivalent density from 80 (muscle) to 0 (fat) mg/cm^3. Muscle attenuation was calculated as the ratio of muscle mass (mg) and muscle area (cm^2) [264]. Total muscle area (mm^2), total fat (mm^2), and subcutaneous fat (mm^2) were obtained in a similar method. The coefficients of variation (CV) for total, subcutaneous, and IM fat, muscle area, and muscle density respectively

were 1.0%, 1.5%, 7.6%, 0.9%, and 1.1%, which were determined by repeat pQCT scanning in 15 individuals.

General adiposity measures

Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg on a balance beam scale. Body mass index (BMI) was calculated as the ratio of weight and squared height (kg/m^2). Waist circumference (WC) was measured at the narrowest point of waist, or at umbilicus, if the narrowest point could not be identified.

Medical Conditions

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as (glucose × insulin) /22.5. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg or currently taking antihypertensive medication. Antihypertensive medication was defined with the standard of WHO ATC/DDD drug classification system [43]. Other comorbidities including prevalent CVD, renal disease, stroke, MI were self-reported yes/no using questionnaire.

Other measurements

Standardized interviewer-administered questionnaires were used to collect demographic information and other factors known to be related to hypertension. Former smoker was defined as men who had smoked more than 100 cigarettes but did not currently smoke, and current smoker was defined as men who smoked more than 100 cigarettes and currently smoked. Alcohol consumption was defined as having 4 or more drinks per weeks in the past 12 months. As walking is the predominant form of physical activity in Tobago, whether or not a participant reported walking for exercise, to work, the store, or to church 4 or more times in the past 7 days was

collected as a measure of physical activity. Sedentary lifestyle was assessed with hours of television watched with the cutoff point of "21 or more hours" per week considered sedentary. Self-reported overall health status compared to men of their own age was dichotomized as good/excellent versus fair/poor/very poor. Men were instructed to bring all prescription medications taken in the past 30 days to the clinic visit and were recorded by interviewers. The information from questionnaire and anthropometric measurements were obtained on the same day as the pQCT scan.

Statistical analysis

The absolute 6-year change in adiposity measures of interest, including calf muscle attenuation, calf IM fat, BMI, and waist circumference, were calculated for each individual as the difference between each measure from the follow-up visit and each measure at the baseline visit. We performed histogram and scatter plot analyses to inspect the distribution and potential outliers for continuous variables. Outliers were defined as any value outside the interval of $Q_3+3\times IQR$ and $Q_1-3\times IQR$ (IQR=interquartile range) and were deleted (19 for intermuscular fat and 2 for muscle attenuation) to increase the statistical power. The difference of continuous and categorical variables between individuals with newly developed hypertension and those with normal blood pressure at follow-up are presented as mean ± standard deviation (SD) or frequency, and were analyzed with Student's T-test or Chi-square test, respectively.

Logistic regression was performed for association between the adiposity change measures and newly developed hypertension. Odds ratios are presented per SD change in adiposity measure over the 6.2 year follow-up period for BMI, WC, and calf IM fat and skeletal muscle attenuation (reflecting intramuscular fat). All fully adjusted models included age, marriage status, education level, smoking status, alcohol consumption, physical activity, sedentary lifestyle, HOMA-IR, and

calf muscle area. Multicollinearity was tested and all variance inflation factors were less than 10. All analyses were performed using SAS statistical software version 9.3 (SAS Institute Inc., Cary, North Carolina).

6.4 RESULTS

Baseline characteristics

Of the 726 men without hypertension or CVD at the baseline, 309 developed hypertension over the follow-up time (median (IQR) 6.2 years (5.9-6.4 years); Table 14). Men who developed hypertension were older, and more likely to be sedentary and insulin resistant, at baseline (all p<0.05). Moreover, men who developed hypertension tended to have greater baseline BMI, calf total fat and calf IM fat, and lower baseline calf muscle attenuation (all *p*<0.05).

The association of 6-year absolute change in overall, central, and myosteatosis measures with newly developed hypertension

There was no significant association between absolute change in overall adiposity, as measured by BMI, and odds of newly developed hypertension when adjusting for baseline BMI, age, demographic characteristics, lifestyle factors, and HOMA-IR (OR (95%CI): 1.00 (0.84, 1.18); Table 15). In a model containing both baseline and change in BMI with muscle attenuation, a 1 SD (2.87 mg/cm^3) decrease in muscle attenuation, signifying increased intramuscular fat, was associated with increased odds of newly developed hypertension (OR (95%CI): 1.30 (1.05, 1.61), Table 15), while change in overall adiposity remained an insignificant predictor (P=0.5789).

However, there was a significant association between absolute change in central adiposity, as measured by WC, and odds of newly developed hypertension when adjusting for of baseline WC, age, demographic characteristics, lifestyle factors, and HOMA-IR (OR per SD change in WC (95%CI): 1.19 (1.00, 1.42); Table 15). In a model containing both change in WC and change in muscle attenuation, a 1 SD (2.87 mg/cm^3) decrease in muscle attenuation, signifying increased intramuscular fat, was associated with increased odds of newly developed hypertension (OR (95%CI): 1.31 (1.06, 1.63); Figure 3) and the association with change in central adiposity was attenuated (P=0.1174; Table 2). Figure 3 demonstrates the relationship between 6-year decrease in muscle attenuation and odds of newly developed hypertension by quartile (linear P=0.0297). The quartile with the greatest reduction in muscle attenuation was significantly associated with elevated risk of developing hypertension (OR (95%CI): 1.96 (1.09-3.51)).

There were no significant associations between 6-year absolute increase in calf IM fat and odds of newly developed hypertension in any model (Table 15).

6.5   DISCUSSION

We report a novel association between decreased skeletal muscle attenuation, a surrogate measure of greater intramuscular fat, and increased odds of newly developed hypertension after 6-years of follow-up in African ancestry men. These results were independent of and stronger predictors than general or central adiposity measures measured by BMI and WC, respectively. Therefore, our findings support the hypothesis that intramuscular fat may be a more important risk factor for hypertension than general or central adiposity.

To our knowledge, this is the first longitudinal study on the impact of intramuscular fat and hypertension. The few existing cross-sectional studies linking hypertension to myosteatosis have been inconclusive. The Health, Aging and Body Composition (Health ABC) Study first revealed that thigh intermuscular fat was associated with prevalent hypertension in elderly African ancestry men and women but not elderly Caucasians, and was especially evident among elderly men (age 70 or above). However, in the same study, there was no significant association with thigh intramuscular fat measured by muscle attenuation and prevalent hypertension [250]. In a report by the Framingham Study on 2949 middle-aged participants, there was a significant association between paraspinous intramuscular fat (measured by muscle attenuation) and prevalent hypertension, though the association was lost after further adjustment for visceral fat or BMI [251]. The discrepancy in results between the previous studies and ours might signify that there are muscle-specific associations [246], that the previous results were specific to cross-sectional data, and/or that the duration of hypertension should be taken into consideration as it may alter the subsequent accumulation of skeletal muscle fat.

The mechanisms that link intramuscular fat and increased risk of newly developed hypertension are not yet understood. Insulin resistance seems to be a direct regulator of the complex crosstalk of myosteatosis and hypertension [300], although other indirect mechanisms, such as inflammation [300, 301] or oxidative stress [300, 302], may also be involved. Pathophysiology has shown that hypertension per se is a status of insulin resistance as several studies have described an interaction between insulin signaling and the renin-angiotensin systems (RAAS) [300, 303]. Angiotensin II inhibits insulin signaling and insulin induced nitric oxide production, and leads to elevated insulin level [301]. In addition, inhibition of the RAAS improves insulin sensitivity and slows the progression of T2D [301]. Furthermore, insulin resistance induced

hyperinsulinemia leads to hyperfiltration, causes structural changes in the kidney (glomerular hypertrophy and focal segmental glomerulosclerosis) and substantially lowers glomerular filtration rate and increases arterial pressure [300]. However, in our study, an association between muscle attenuation, a measure of intramuscular fat, and hypertension persisted even after adjusting for insulin resistance, suggesting that this fat depot might be an independent risk factor for hypertension. In addition to insulin resistance, ectopic fat becomes infiltrated with macrophages and secretes pro-inflammatory cytokines, such as IL-6 and TNF-α, which can then trigger the activation of the RAAS [304]. Finally, the accumulation of lipid in muscle cells increases tricarboxylic acid cycle activity and generates excess lipid intermediates [305], resulting in oxidative stress, which may be associated with activation of the RAAS [306].

In our study, intermuscular fat was not associated with newly developed hypertension in any models (results not shown), despite the fact that we have previously reported an association between intermuscular fat and incident risk of T2D in this population [307]. This indicates that inter- and intra- muscular fat might have different impact on the development of cardiometabolic complications. The underlying mechanisms are unknown. The different locations of intermuscular fat (visible fat beneath the fascia lata) and intramuscular fat (fat infiltration within muscle fibers and muscle cells) might be one explanation to this paradox since they may trigger the metabolic changes via different pathways. The accumulation of intermuscular fat may have a negative impact on insulin action and insulin diffusion capacity via reducing nutritive blood flow to muscles [241], whereas the accumulation of intramuscular fat may induce insulin resistance via impaired insulin signaling [238]. Indeed, the insulin receptor substrate 1/phosphatidylinositol 3-kinase and growth-factor-regulated protein kinase B pathways are known to interact with the activation of RAAS [239].

Our study is the first to focus on myosteatosis and hypertension and provides a novel perspective in understanding the pathophysiology of hypertension and the heterogeneity of obesity. A major strength of our study is the use of longitudinal data to assess newly developed hypertension instead of prevalent hypertension, which minimized the prevalence-incidence bias. The longitudinal study design helps to establish the temporality between decreased muscle attenuation (reflecting greater intramuscular fat) and the development of hypertension. Moreover, we included a wide age range, which enabled us to detect longitudinal changes of myosteatosis throughout the aging process.

Our study also has some limitations. Although we used a prospective study design, our study was not designed to definitively establish a causal relationship between myosteatosis and newly developed hypertension. Second, we focused on African ancestry men living in the Caribbean region, which limits the generalizability to women and other race/ethnic populations and other geographic regions. Third, we only included subjects who were healthy enough to receive CT measures, which might have introduced a "healthy participant" bias. Fourth, our study could not exclude the impact of other ectopic fat, such as visceral, liver, heart, perivascular and kidney fat on our findings. Fifth, intramuscular fat could be further divided into extra- (lipid storage in interstitial adipose tissue) and intra- myocellular fat (lipid droplets storage in muscle cells), which may have different biological impact on cardiometabolic disorders, but cannot be distinguished with CT imaging.

In summary, this study reports a previously unidentified adverse impact of intramuscular fat on the development of hypertension, independent of general or central adiposity among high-risk African ancestry men. We also show that greater intramuscular fat may be more important than greater general and central adiposity in driving metabolic disorders among African ancestry

110

men. Our findings may provide an explanation and suggest a new intervention target for the cardiometabolic "resilience" in African ancestry population, who are at high risk for cardiometabolic disease despite having less whole body and visceral fat. Intramuscular fat might be a novel and modifiable risk factor that is important for the prevention of cardiometabolic disorders and for healthy aging. Our results should be replicated in other racial and ethnic groups and among women. Future studies are also needed to delineate whether the accumulation of intramuscular fat is a marker or the cause of the development of hypertension, to test for potential mediation effects of insulin resistance and the RAAS system, to test if the association between intramuscular fat and hypertension is independent of inflammation, and to identify the possible biological mechanisms underlying this relationship.

Table 13. Baseline Characteristics by Newly Developed Hypertension Status

	Follow-up Hypertension status			
	Hypertensive (N=309)	**Non-hypertensive (N=417)**	**Crude OR[a] (95%CI)**	**P-value***
Age (year)	56.3 (8.1)	52.9 (7.3)	1.058 (1.037, 1.079)	**<0.001**
Education level, n (%)				
High school and above	38 (12.5)	46 (11.3)	1.123 (0.711, 1.775)	0.619
Marital status, n (%)				
Married or live with a spouse	231 (75.2)	289 (69.6)	1.325 (0.950, 1.849)	0.0971
Current smoker, n (%)	61 (19.7)	72 (17.3)	1.140 (0.776, 1.675)	0.394
Former smoker, n (%)	37 (12.0)	61 (14.6)	0.816 (0.523, 1.275)	0.3008
Alcohol consumption, n (%)				
>3 drinks per week	27 (8.7)	43 (10.3)	0.831 (0.501, 1.377)	0.4711
Physical activity, n (%)				
>3 times per week	151 (48.9)	213 (51.1)	0.915 (0.682, 1.229)	0.5556
Sedentary lifestyle, n (%)				
>21 hours per week	61 (19.8)	59 (14.2)	1.494 (1.009, 2.214)	**0.0443**
HOMA-IR	3.2 (1.9)	2.5 (1.7)	1.262 (1.149, 1.386)	**<0.001**
BMI (kg/m^2)	27.2 (4.4)	25.9 (4.1)	1.363 (1.169, 1.589)	**<0.001**
Waist circumference (cm)	92.0 (10.6)	88.0 (10.3)	1.493 (1.275, 1.748)	**<0.001**
Calf subcutaneous fat (mm^2)	1347.9 (620.6)	1294.8 (670.5)	1.085 (0.937, 1.257)	0.2765
Calf intermuscular fat (mm^2)	247.0 (286.2)	196.3 (196.2)	1.249 (1.063, 1.467)	**0.0069**
Calf muscle attenuation (mg/cm^3)	77.0 (3.1)	77.7 (3.3)	0.788 (0.666, 0.933)	**0.0044**
Total body fat (kg)[†]	3.0 (3.9)	3.0 (3.2)	0.988 (0.855, 1.141)	0.8688
Trunk fat (%)[†]	1.3 (2.6)	1.7 (2.8)	0.865 (0.748, 1.000)	0.0496
Leg fat (%)[†]	-0.8 (2.3)	-0.9 (2.4)	1.075 (0.930, 1.241)	0.3279

*P-values for comparisons between the hypertensive and non-hypertensive subjects from two-sample T-tests or Chi-square tests or Fisher's Exact Test

[a]per SD change for continuous variables except for age (per year)

[†]the total number for DXA measurements was 759, of these

Table 14. Multivariable Adjusted Odds Ratios for Newly Developed Hypertension per SD Difference in Absolute Baseline and Longitudinal Myosteatosis Measures

	OR (95% CI)	P value
Multivariable Effects Adjusted for Overall Adiposity (BMI)*		
Model with BMI only		
6-year increase in BMI	1.00 (0.84, 1.18)	0.9666
Baseline BMI	**1.27 (1.06, 1.53)**	**0.0110**
Model with BMI and Calf Muscle Attenuation		
6-year decrease in Muscle attenuation	**1.30 (1.05, 1.61)**	**0.0165**
6-year increase in BMI	1.06 (0.86, 1.31)	0.5789
Baseline Muscle attenuation	0.79 (0.61, 1.00)	0.0505
Baseline BMI	1.13 (0.88,1.44)	0.3367
Model with BMI and Calf IM Fat		
6-year increase in IM Fat	1.05 (0.87, 1.25)	0.6296
6-year increase in BMI	0.97 (0.81, 1.16)	0.7237
Baseline IM Fat	1.00 (0.78, 1.28)	0.9917
Baseline BMI	1.22 (0.99, 1.51)	0.0622
Multivariable Effects Adjusted for Central Adiposity (WC)*		
Model with WC only		
6-year increase in WC	**1.19 (1.00, 1.42)**	**0.0493**
Baseline WC	**1.33 (1.11, 1.60)**	**0.0025**
Model with WC and Calf Muscle Attenuation		
6-year decrease in Muscle attenuation	**1.31 (1.06, 1.63)**	**0.0136**
6-year increase in WC	1.18 (0.96, 1.44)	0.1174
Baseline Muscle attenuation	0.81 (0.64, 1.04)	0.0974
Baseline WC	1.15 (0.90, 1.46)	0.2656
Model with WC and Calf IM Fat		
6-year increase in IM Fat	1.02 (0.85, 1.22)	0.8482
6-year increase in WC	1.16 (0.97, 1.39)	0.0962
Baseline IM Fat	0.98 (0.77, 1.25)	0.8833
Baseline WC	**1.30 (1.06, 1.60)**	**0.0132**

*All models are additionally adjusted for: age, education, marriage status, alcohol consumption, physical activity, current smoker, former smoker, sedentary lifestyle, baseline HOMA-IR, and 6-year change in calf muscle area.

BMI: body mass index; IM: intermuscular: WC: waist circumference

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┌─────────────────────────┐       ┌─────────────────────────┐
│   Original Tobago Health │       │  Participated in pQCT    │
│  Study for prostate cancer│──────▶│  measurements at baseline│
│        screening          │       │       (n=2,512)          │
│        (n=3,089)          │       └───────────┬─────────────┘
└─────────────────────────┘                    │         ┌─────────────────────────┐
                                               │────────▶│ Participants who were lost of│
                                               │         │        follow-up          │
                                               │         │         (n=894)           │
                                               ▼         └─────────────────────────┘
                                   ┌─────────────────────────┐
                                   │  Participated in pQCT    │
                                   │ measurements at follow-up│       ┌──────────────────────────────┐
                                   │       (n=1618)           │       │   Participants with 1 of the   │
                                   └───────────┬─────────────┘        │   following were excluded      │
                                               │──────────────────────▶│ -with cardio-vascular diseases │
                                               │                       │ -with baseline hypertension    │
                                               ▼                       │ -no African ancestry           │
                                   ┌─────────────────────────┐         │         (n=892)                │
                                   │ Total number of eligible │         └──────────────────────────────┘
                                   │      participants        │
                                   │        (n=726)           │
                                   └─────────────────────────┘
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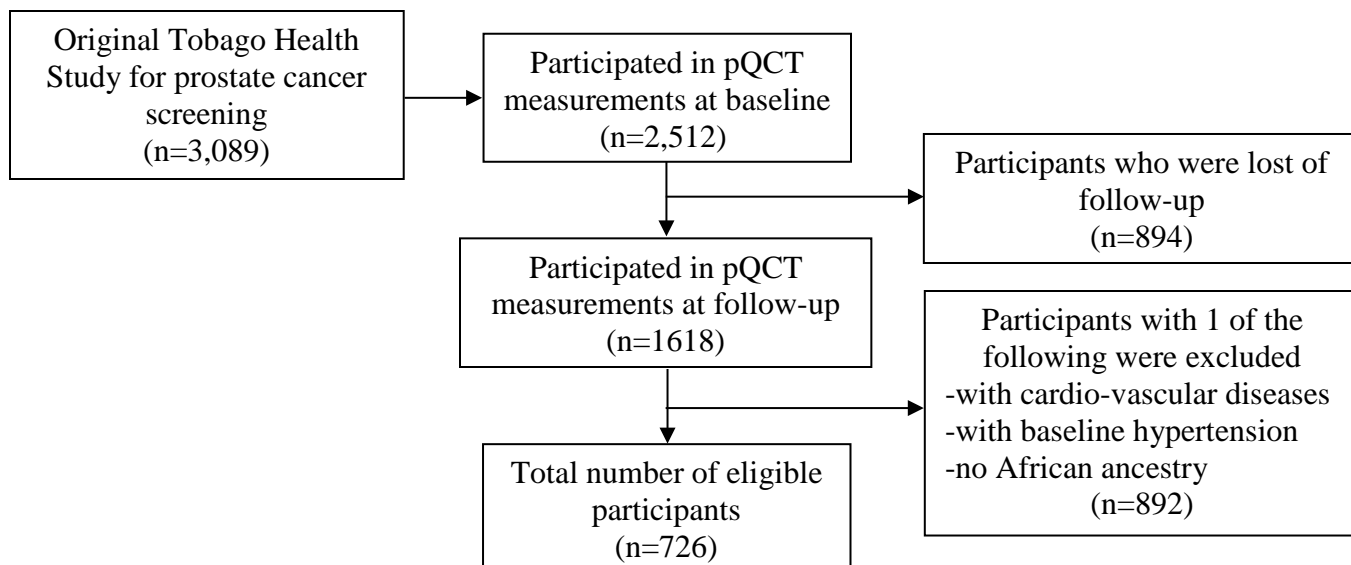
Figure 3. Flow chart of Tobago Health Study
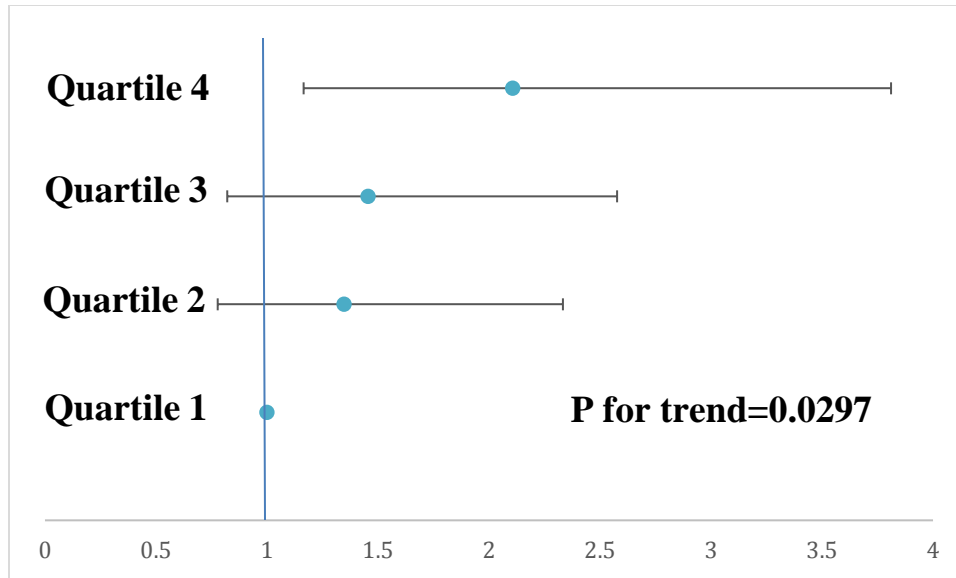
Figure 4. Risk of newly developed hypertension increase with 6-year absolute decrease of muscle attenuation

All models are additionally adjusted for: age, education, marriage status, alcohol consumption, physical activity, current smoker, former smoker, sedentary lifestyle, baseline HOMA-IR, and 6-year change in muscle area, baseline WC and 6-year increase of WC.

The models with the adjustment of baseline BMI and 6-year increase of BMI showed a similar association between newly developed hypertension and 6-year absolute increase of muscle attenuation (Figure not shown).

7.0   SYNTHESIS

7.1   OVERVIEW OF RESEARCH FINDING

This dissertation used the Tobago Health Study cohort to study the epidemiology of general, regional and ectopic skeletal muscle fat. Specifically, we assessed the magnitude, pattern and determinants of the natural changes in these adiposity measures, the impact of these adiposity measures on the development of cardiometabolic diseases, and the association with elevated mortality risks. Our research identified that the increase in total body fat and trunk fat, and the decrease in leg fat became slower in the elderly . Additionally, the increase in intramuscular fat (reflecting by the decrease in muscle attenuation) became greater in the elderly. We also revealed a bi-directional association between cardiometabolic disease (hypertension and diabetes) with ectopic skeletal muscle fat, which means that cardiometabolic diseases are both predictor and consequence of the accumulation of ectopic skeletal muscle fat. In addition, we revealed that ectopic skeletal muscle fat was significantly associated with elevated risk of mortality, independent of general body fat, which added the knowledge to the different risk of cardiometabolic diseases across races/ethnicities.

7.2   THE LIMITATIONS AND STRENGTHS

Our study has some limitations. First, our study is observational. Thus, we are unable to establish causality between general, regional, and ectopic skeletal muscle fat with correlates, newly

developed hypertension, or mortality. Moreover, our findings in African ancestry men in the Caribbean may not be generalizable to women, other race/ethnic populations, or other geographic regions.

Our study may suffer from minor selection bias. As a longitudinal study, we have censored individuals during the follow-up period. Compared to individuals with complete follow-up information, the censored individuals were older and less healthy and, therefore, likely had greater risk of developing hypertension and mortality, and greater change in adiposity measures. Even with a possibly underestimated hypertension and mortality rate, we were still able to identify a significant association of ectopic skeletal muscle fat with hypertension and mortality. These indicate the robustness of our findings. However, we are unable to detect a significant impact of lifestyle factors on the change in adiposity measurements, which may due to the missing value in adiposity measurements caused by censored data. Another potential selection bias may be due to the "healthy participant" effect, since we only included men who were well enough to have pQCT and DXA measurements.

Our findings may also suffer from information bias. The information of the correlates, such as lifestyle factors and medical history were collected via self-reported questionnaires, which might be inaccurate. Other facets of physical activity and sedentary lifestyle, such as intensity, type and duration were not taken into consideration. Moreover, missing values of the correlates may induce bias in multivariable models.

Our study has several unmeasured confounders, for instance, fat located in other ectopic storage depots, such as the liver, pancreas and heart, inflammation factors, and mitochondrial function, which may impact the associations we observed. In addition, the association between adiposity measurements and mortality risks may be biased by reverse causation, which means that

obesity-related disease may lead to both greater adiposity and elevated risk of mortality. In order to minimize this bias, our analyses were adjusted for several major comorbidities related to obesity at baseline. Lastly, intramuscular fat could not be further divided into extra- (lipid storage in interstitial adipose tissue) and intra- myocellular fat (lipid droplets storage in muscle cells), which may have different biological impact on cardiometabolic disorders, but cannot be distinguished with our CT imaging.

However, our study also has several strengths including the longitudinal study design, which allows us to establish temporality between correlates and the redistribution of adiposity measures, as well as, temporality between the adiposity measures and the development of cardiometabolic diseases or death. We are the first longitudinal study to reveal a novel association between ectopic skeletal muscle fat and hypertension. By using newly developed hypertension instead of prevalent hypertension, we are able to minimize the prevalence-incidence bias. Thus, our findings provide a novel perspective in understanding the pathophysiology of hypertension and the heterogeneity of obesity. We are also the first longitudinal study to describe the magnitude, pattern and determinants of age-associated redistribution of adiposity. Lastly, the population-based study design that included men across a wide age range enabled us to detect associations within the normal aging process.

7.3   PUBLIC HEALTH SIGNIFICANCE

Our findings have significant public health importance. We identified that men with insulin resistance, T2D and hypertension at baseline had a greater decrease in muscle attenuation, indicating that intra-muscular fat may be either a consequence or a marker of insulin resistance.

These findings may provide a basis for a new diabetes prevention and/or treatment target. In addition, although our study found no association between lifestyle factors, including alcohol consumption, smoking status, physical activity, and sedentary lifestyle, with a negative redistribution of body fat or the accumulation of ectopic skeletal muscle fat, it is possible that self-reported information on lifestyle factors, especially physical activity and sedentary lifestyle, may be inadequate to draw a powerful conclusion.

Our findings also support the hypothesis that intramuscular, but not intermuscular, fat may be a more important risk factor for hypertension than general or central adiposity. This indicates that inter- and intra- muscular fat might have different impact on the development of cardiometabolic complications. The different locations of intermuscular fat (visible fat beneath the fascia lata) and intramuscular fat (fat infiltration within muscle fibers and muscle cells) may be one explanation to this paradox, since they may trigger the metabolic changes via different pathways. Thus, our findings illustrate the potential importance of separately studying intra- and inter- muscular fat in determining risk of cardiometabolic diseases. Furthermore, we provide an explanation and suggest a new intervention target for the cardiometabolic "resilience" in African ancestry population, who are at high risk for cardiometabolic disease despite having less general body and central fat. Therefore, intramuscular fat may be a novel and important modifiable factor in preventing cardiometabolic disorders and in promoting healthy aging.

Our study also identified a previously unreported, independent association of both intra- and inter- muscular fat with mortality in middle-aged and elderly African ancestry men. These results illustrate that skeletal muscle fat may be a novel independent marker of aging with a potential impact on healthy aging and expected lifespan. Moreover, this association persisted even after taking into consideration T2D or insulin resistance. Our study suggests that mechanisms other

than insulin resistance may also play an important role in driving premature mortality. No other adiposity measurements were associated with mortality, which implies that during the aging process, ectopic skeletal muscle fat may be a more adverse fat depot compared to other locations. In conclusion, our study indicates that maintaining high muscle density and prevention fat infiltration in the muscle may be crucial for successful aging in elderly African ancestry men.

7.4   FUTURE DIRECTIONS

Previous studies on the epidemiology of general, regional and ectopic skeletal muscle fat in African population are sparse. There are likely differences between the Caribbean and American cultures, and future studies of should also include other male populations of African ancestry, as well as, women.

In addition, future studies are needed to collect more precise, detailed and objective measurements on lifestyle factors. Specifically, information on physical activity and sedentary lifestyle should include intensity, duration, style and other dimensions.

Other ectopic fat depots, including heart, liver, kidney should be also taken into consideration in future studies, since these ectopic fat depots may have local impact on the function of related organs and subsequently drive cardiometabolic disease. Although our findings indicate that maintaining high muscle density and preventing infiltration of fat to the muscle would be beneficial for healthy aging, it is important to rule out the impact of other ectopic fat on this association. Furthermore, with all major ectopic fat depots simultaneously studied, the biological function of the adiposity in different locations could be illustrated. Additionally, we could better

understand whether the redistribution of body fat is simply a marker of aging or a cause of future diseases.

Mechanisms linking these adiposity measures and adverse health outcomes are another direction for future studies. Previous studies suggested that insulin resistance might be a direct link, whereas, inflammation status, oxidative stress, mitochondrial dysfunction, et cetera might serve as indirect links. Our findings show that the significant association of ectopic skeletal muscle fat with hypertension and mortality persists even after adjusting for insulin resistance, suggesting other mechanisms underlying these associations still remain to be identified.

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