The Impacts of Adipose Tissue and the Gut Microbiome on Diabetes Risk among African-Caribbean Men

by

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Abstract

Type 2 diabetes (T2D) and its complications are increasing in prevalence and burden worldwide. Caribbean rates of T2D rival those of the United States, and mortality from T2D is exceptionally higher. The study of novel T2D risk factors, such as adipose tissue (AT) radiodensity, AT distribution, and the intestinal microbiome, yield greater insights into T2D pathophysiology and can inform targeted interventions. However, individuals of African ancestry, who have a higher burden of T2D compared to Caucasian counterparts, are underrepresented in this research. Thus, this dissertation fills the gaps by investigating associations of AT radiodensity, body composition, and the intestinal microbiome with T2D in a cohort of African Caribbean men from Tobago, Trinidad and Tobago.

The first dissertation paper investigates associations between AT radiodensity in the abdomen (visceral [VAT] and subcutaneous [SAT]) and thigh (intermuscular [IMAT]) with glucose, insulin, and insulin resistance. We demonstrate that lower radiodensity in any AT (indicating greater tissue lipid accumulation) was associated with higher insulin and insulin resistance, with independent contributions from thigh IMAT. The second dissertation paper models the associations of both AT and muscle from the abdomen and thigh with T2D. We report that abdominal SAT (but not VAT) was positively associated, and thigh muscle negatively associated, with higher odds of T2D. The third dissertation paper examines associations of the intestinal microbiome with sociodemographic, lifestyle, body composition, and T2D measures.

We identified sociodemographic factors as a main driver of microbial clustering, and several lifestyle and body composition measures as being differentially associated with taxonomic units, thus informing future prediction modeling of the microbiome with T2D.

These findings have significant public health implications. Our results somewhat differ from those reported in predominantly Caucasian cohorts, highlighting the importance of including racial/ethnic minorities in novel risk factor research. These papers also provide important methodological work, informing how body composition analyses are performed. Finally, this research produced the first nutritional and microbiome databases in Tobago, which can aid future T2D research. Taken together, information from this dissertation can be leveraged to inform future observational and interventional studies in T2D prevention, both in the Caribbean and worldwide.

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1.0 Introduction

1.1 Overall Background

1.1.1 Impact of obesity and T2D in the Caribbean

The United Nations cites an increase in obesity in Caribbean countries, with 58% of individuals in the Latin American/Caribbean region considered to be overweight and the highest rates of obesity (>30%) found in Caribbean countries (2). The rate of diabetes in many Caribbean countries is also increasing, with some rates surpassing those seen in the US (3). Several studies in the Caribbean have linked diabetes prevalence with increasing obesity (4-6), thus making control and prevention of obesity a main target for diabetes prevention in these countries.

Importantly, the rise in obesity and T2D is likely to lead to much greater increases in morbidity, mortality, and cost. Early 2000 projections estimated that age-adjusted cause-specific mortality for diabetes in Caribbean countries was 3-6 times higher than in the US, and that economic costs of diabetes were as high as $467 million (7). Studies have also indicated extremely high rates of amputations (8, 9), retinopathy (10, 11), and renal diseases related to diabetes (12, 13). While improved work is necessary for the access to care and treatment of diabetes in the Caribbean, it is imperative that action is also taken to reduce the increasing prevalence of diabetes.

1.1.2 Importance of fat distribution in development of T2D

While overall obesity remains a major risk factor for diabetes, the location of stored fat is also thought to play a role. Adipose tissue is found throughout the body, and the major depot for initial fat storage is thought to be subcutaneous fat (fat beneath the skin) (14). However, impairment of or inability of subcutaneous fat to store lipids results in fat storage in other depots (visceral, pericardial, hepatic, and intramuscular); such fat stores are called ectopic fat depots (14-16). Ectopic fat depots are often indicative of metabolic dysfunction in those areas, most notably with insulin resistance (16). Thus, fat distribution is considered to be a novel risk factor for cardiometabolic health and a likely contributor to T2D.

1.1.3 Importance of fat quality in development of T2D

Beyond the distribution of fat tissue, other characteristics of AT may reflect its functionality or pathogenicity. AT radiodensity, a CT-derived measure indicating the tissue's opacity to X-rays, was shown to reflect characteristics of AT, such as higher AT radiodensity being associated with beneficial aspects of adipose tissue such as smaller adipocyte cell size and lower lipid content (17, 18) and increased vascularity (19), as well as detrimental aspects such as potentially increased tissue fibrosis (20, 21). However, as an imaging measure it cannot distinguish between these different biological factors that may encompass the quality of fat tissue. Thus, AT radiodensity may serve as a surrogate marker of adipose tissue "quality". In observational studies, abdominal AT radiodensity has been associated with multiple cardiometabolic risk factors, even after accounting for AT volume (22-26). Lower abdominal VAT and SAT radiodensity was associated with higher HOMA-IR (24), increased odds of impaired fasting glucose (23, 24), and

increased glucose concentrations (22-24) in the Framingham cohort. Elevated glucose and prevalence of diabetes was also observed in individuals with lower vs. higher abdominal VAT, SAT, and IMAT radiodensity according to reports from the Multi Ethnic Study of Atherosclerosis (MESA) (25, 26). Thus, AT radiodensity may serve as a surrogate measure of AT quality and may be a novel biomarker of diabetes-related risk factors.

1.1.4 Dietary intake is associated with ectopic fat and T2D

It is well known that poor diet quality is associated with obesity and T2D. Poor diet quality is thought to be a major contributor to the obesity epidemic in the Caribbean (2). One way of measuring diet quality is through the use of dietary patterns, which take into account combinations of foods rather than specific foods or nutrients (27). While several dietary patterns exist (DASH diet, Alternative Healthy Eating Index, Mediterranean Diet Score, etc.), moderate evidence indicates that higher adherence to any healthy eating pattern is associated with reduced risk of obesity and T2D (27). A recent meta-analysis of prospective studies confirms that while differing in the food components that comprise the scores, dietary patterns are associated with diabetes incidence in apparently healthy populations (28). In addition to associations with obesity and T2D, a study in the Multi-Ethnic Study of Atherosclerosis (MESA) population also found associations between a Mediterranean-style dietary pattern and ectopic fat distribution (29). The authors of this study found that higher adherence to this dietary pattern was cross-sectionally associated with decreased visceral, pericardial, and liver fat, but not with subcutaneous fat. Preliminary data from the Tobago Health Study (30) also suggests associations between dietary patterns and various ectopic fat depot volumes. Dietary patterns, then, are not only associated with general obesity and risk of T2D but also with ectopic fat distribution.

1.1.5 Dietary intake is associated with the gut microbiome, which influences host metabolism

Diet quality not only impacts the persons eating foods, but also the function and composition of our resident microbial inhabitants. The gut microbiome consists of bacteria, viruses, fungi, and other microscopic organisms inhabiting our gastrointestinal tract. The human intestines are home to several trillion bacteria, outnumbering our own body cells (31). These intestinal bacteria survive in our gastrointestinal tract on undigested and unabsorbed foods which they are able to digest and utilize (32). It has even been suggested that the gut microbiome may function as an endocrine organ due to the ability to release hundreds of bacterial products into the circulatory system (33). The coexistence between host and microbiome, then, is thought to be mutually beneficial (32). Intestinal bacteria may modulate metabolism and disease through a variety of methods. For example, intestinal bacteria can break down food we cannot through fermentation, thus increasing energy harvest; additionally, fermentation products like short chain fatty acids have been implicated in energy metabolism (34). Microbes are also involved in the transformations of carcinogenic molecules and bile acids to the production of vitamins and amino acids (35).

Different dietary patterns are thought to help shape the composition and function of the gut microbiome. De Fillipo et al. demonstrated marked differences in bacterial diversity and the presence of specific bacterial taxa between children from Europe, who eat a predominantly Westernized-style diet (high animal proteins, fats, sugars, and starches, and low in fiber), and children in Africa, who eat a more agrarian diet (high in starches, fiber, and plant polysaccharides, and low in fat and animal protein) (36). A study comparing African American and African adults also found significant differences in microbiome makeup, microbial genes, and metabolites (37);

when diets were switched between individuals from these two populations for only two weeks, significant changes were observed in the bacteria present as well as differences in metabolites, intestinal mucosal inflammation and cell proliferation (38). A dietary intervention in Americans comparing a plant-based diet versus and animal-based diet for five days each also found differences in microbial composition and function, with between-person diversity significantly changing within one day of food reaching the distal colon (39). Thus, the influence of diet on the gut microbiome composition is rapid and contributes to changes in host metabolism.

1.1.6 The gut microbiome is associated with overall obesity and T2D

Differences in the gut microbiome at the phylum level and with respect to overall diversity are associated with obese phenotypes (40-42). This phenotype is likely due to a greater ability to harvest energy and is transmissible by fecal transplant in mice (43). Studies have also demonstrated associations between microbial profiles and function with T2D (44, 45). While findings have demonstrated associations between bacterial taxa, phyla, and diversity measures with obesity and T2D, these associations are inconsistent across studies (46, 47). Reasons for this inconsistency are likely due to methodological differences in sample processing and handling (46, 47), but may also be due to a lack of measuring confounders such as total caloric intake and diet (47). Additionally, these studies tend to look at BMI as a marker of obesity rather than separating lean and fat mass. Therefore, while specific relationships are inconsistent, the gut microbiome does seem to be consistently associated with overall obesity and T2D.

1.1.7 The gut microbiome is also associated with ectopic fat

While few in number, recent studies of humans indicate that the intestinal microbiome composition is associated with the development of fat in ectopic sites. An intervention study by Kadooka et al. demonstrated that probiotic administration could reduce visceral and subcutaneous fat depots as well as overall measures of adiposity (48). Beaumont et al. investigated associations between the gut microbiome and visceral fat in a large UK Twin cohort study (49). The authors found that specific taxa and decreased within-person diversity were associated with increased visceral adiposity. A study by Zhu et al. found increased levels of alcohol-producing bacteria in individuals with non-alcoholic steatohepatitis compared to obese and control individuals (50). Associations have also been found between gut microbial metabolites, namely lipopolysaccharide, with ectopic fat distribution. We previously published on the association of baseline serum lipopolysaccharide binding protein (a surrogate marker of lipopolysaccharide) with significant increases in trunk fat and borderline significant decreases muscle density (51). A large longitudinal study in Chinese individuals also found associations between baseline serum markers of bacterial lipopolysaccharide and increases in intrahepatic triglycerides (52). Taken together, these results suggest that the gut microbiome may be associated with various ectopic fat depots.

1.1.8 Knowledge gaps

1.1.8.1 Is adipose tissue quality associated with T2D risk factors in African Ancestry men?

African ancestry individuals are at a greater risk of T2D compared to Caucasian individuals at similar measures of general adiposity (53-55). There are also racial/ethnic differences in adipose tissue distribution, with African Ancestry individuals having less VAT (56-59) and greater IMAT

(60-62) compared to Caucasian counterparts. Results from MESA suggest that there may also be racial/ethnic differences in AT radiodensity (25). Given the associations of AT radiodensity with insulin and glucose, it is important to understand the contributions of AT radiodensity to T2D risk in both understudied ethnic populations (such as higher-risk African Ancestry individuals) and in understudied AT depots (such as thigh IMAT).

1.1.8.2 How does upper and lower body composition impact T2D risk?

Studies investigating upper and lower body composition have generally identified upper-body AT as being positively associated with T2D, while lower body AT may be protective (63). CT scans allow for good discrimination between AT and muscle, and studies utilizing images from abdominal and thigh CT scans (64-67) generally support the findings that upper body adiposity is harmful, while lower body SAT may be beneficial. However, these previous studies have generally low representation of African Ancestry individuals, who have greater risk of T2D and different body compositions compared to Caucasian counterparts. Additionally, models within those studies varied greatly in the tissues included in final models. By excluding tissues, it is likely that estimates obtained were biased. Further, tissues were entered into the models in a way that ignored the compositional nature of the data, whereby an increase in one tissue (holding body size constant) can only occur at the expense of some other tissue(s). This may further bias estimates and obscure true associations.

1.1.8.3 Is the intestinal microbiome associated with T2D risk factors in African Ancestry men?

The microbiome, which is associated with T2D (44, 45), can be impacted by geography (68), race/ethnicity (69, 70), and dietary intake (39), among many other factors. Studies looking at

the association of the microbiome with T2D risk have not yet been performed in the Caribbean region, which has countries like Tobago with high rates of T2D (71). Further, Tobago has a predominantly African Ancestry population (72) and lifestyle habits that may differ from other studied populations, such as dietary intake differences (**Table 1.2** compares Tobago and US dietary intakes). Given the impacts of race/ethnicity, geography, and lifestyle on the microbiome, it is unclear if associations between the microbiome and glucose and insulin levels are similar in Caribbean populations like Tobago as in other studied geographic and racial/ethnic groups. Additionally, studies investigating associations of the microbiome with T2D risk in African Ancestry individuals are lacking, with only one study specifically focusing on this racial/ethnic group (73).

1.1.9 Overall Background Summary

T2D is an increasing health risk in the Caribbean region. While causes of T2D are multifactorial, a more thorough understanding of the risk factors associated with T2D development is needed in order to better implement interventions. Traditional risk factors such as body composition, and more novel risk factors such as AT quality and the intestinal microbiome, show some promise in understanding the etiology of insulin resistance and T2D, but further research is needed to determine their importance in high-risk and understudied populations such as African Ancestry individuals.

1.2 Type 2 Diabetes: Biology and Risk Factors

1.2.1.1 Glucose, Insulin, and Insulin Resistance

Glucose is used as a source of energy in human cells, where it is metabolized to generate adenosine triphosphate (ATP). Glucose is obtained from the diet, absorbed in the intestines, and transported in the blood to other organs for use. Excess glucose can be stored in the liver and muscles by biochemically linking them together into a large polysaccharide called glycogen through a process called *glycogenesis* (74). When glucose levels are low, this glycogen can be easily broken down into monomeric glucose through a process called *glycogenolysis*; this glucose is released by the liver back into the blood stream (74). Alternatively, glucose can also be generated de novo in the liver from substances such as lactic acid in a process called *gluconeogenesis* (74).

Because of the importance of glucose in cellular energy production, regulation of blood glucose levels is needed, with a proper balance of glycogenesis, glycogenolysis, and gluconeogenesis. A typical non-fasting blood glucose level tends to fluctuate between 70-110 mg/dL (74). An improper balance can lead to a very low blood sugar (hypoglycemia) or a very high blood sugar (hyperglycemia). Chronic hyperglycemia can have long-term complications that damage blood vessels, kidneys, nerves, vision, and bones and joints, as well as increasing the risks of oral infections (75). In more acute and severe cases, it can also lead to life-threatening ketoacidosis or hyperglycemic hyperosmolar dehydration (75). Hyperglycemia can be brought on by chronic diseases (ex. type 1 or type 2 diabetes), excess eating and/or a lack of exercise, stress, or the “dawn phenomenon” where early-morning hormonal changes may impact blood sugar levels (76). In all of these cases, a unifying underlying abnormality occurs with the hormone insulin, with individuals having either too low of insulin levels or a resistance to the effects of insulin (76).

While several hormones contribute to glucose metabolism, the predominant two are glucagon and insulin. Glucagon is a protein secreted by pancreatic α-cells when blood glucose levels are low, and its main actions are to promote hepatic glucose production through stimulating glycogenolysis (77). Insulin, on the other hand, is secreted by pancreatic β-cells when blood glucose levels are high, and it acts to decrease in blood glucose levels (77). Blood glucose reductions are accomplished by three mechanisms: one, the stimulation of insulin-sensitive cells (such as adipocytes, hepatocytes, and myocytes) to increase glucose uptake; two, the promotion of glycogenesis; and three, inhibition of glucagon secretion from pancreatic α-cells, resulting in a halt of gluconeogenesis and glycogenolysis (77).

It is important to note that both glucagon and insulin are involved in metabolism beyond glucose control. Glucagon is also thought to play a role in regulating plasma lipids, promoting lipolysis and ketone-body production, and increasing energy expenditure and adipose tissue thermogenesis (78). Insulin inhibits adipose tissue lipolysis and the release of fatty acids, plays a role in lipoprotein metabolism and clearance, and prevents protein breakdown (79). Additionally, insulin is involved in cellular growth, proliferation, and migration, and can inhibit cellular apoptosis (80). Thus, circulating levels of these hormones play complicated roles in cellular and systemic metabolism.

Insulin resistance is the inability of previously insulin-sensitive cells or tissues to respond to the action of insulin, resulting in a compensatory increase in insulin secretion (81). Insulin resistance can occur in a multitude of ways, including genetic mutations, lipotoxicity from increased circulating free fatty acids and lipid storage in non-adipose tissues, inflammation, glycation of proteins due to hyperglycemia, mitochondrial dysfunction and endoplasmic reticulum stress, and hyperinsulinemia (82). These processes typically result in modifications that decrease

binding of insulin to insulin receptors or interrupt signaling cascades downstream of the insulin receptor (82). Given the myriad ways in which insulin resistance can occur, it has come to be associated with multiple pathological and chronic conditions, such as T2D, metabolic syndrome, polycystic ovarian syndrome (PCOS), non-alcoholic fatty liver disease (NAFLD), cancer, and more (83).

Despite associations with multiple diseases, insulin resistance in the right context may be beneficial. It has been hypothesized that insulin resistance was actually an evolutionary adaptation that allows for glucose mobilization in times of energy demand, such as fasting, stress, infection, and pregnancy (84). These times of energy demand are temporary; however, in a modern environment of high calories, chronic stress, and chronic low-grade inflammation, insulin resistance would become chronic and maladaptive (84).

1.2.1.2 Type 2 Diabetes Definitions

Diabetes mellitus is a chronic condition in which blood glucose levels are elevated, with the underlying mechanism being either a defect in insulin secretion or in insulin signaling (85). There are various forms of diabetes, including type 1 diabetes (an inability to produce insulin, generally diagnosed in younger individuals), T2D (an inability to effectively use insulin due to high insulin resistance, sometimes accompanied by lower insulin production), gestational diabetes (a potentially temporary diabetes occurring during pregnancy), and rare genetic forms of diabetes (85). As T2D is the most common form of diabetes mellitus (comprising 90-95% of all diabetes cases (86)) and the focus of this dissertation, this section will focus exclusively on T2D.

In 2014, it was estimated that 422 million adults had some form of diabetes, with the greatest increase since 1980 occurring in low- and middle-income countries (86). Diabetes is a leading cause of global mortality, with 2012 statistics estimating 1.5 million deaths as a direct

result of diabetes and 2.2 million indirectly associated with diabetes (87). It is also a huge economic burden, with estimates of global healthcare costs around $850 billion (3). T2D is treatable, but not curable; thus, prevention of T2D and its complications is of the utmost importance. Poor management of diabetes can lead to a variety of complications affecting multiple organ systems, including increased risk of cardiovascular diseases like stroke and coronary artery disease, kidney disease, hypertension, neuropathy, increased risk of lower limb amputation, retinopathy, periodontitis, and pregnancy complications (88). Diabetes may also increase the risk of dementia by as much as two-fold (89).

Symptoms of diabetes include increased thirst, urination, and hunger; fatigue; blurred vision; unexplained weight loss; numbness or tingling in the hands or feet; and sores that do not heal (90). Individuals with T2D may be asymptomatic or may not recognize symptoms due to the slow and progressive nature of the disease (90).

T2D is diagnosed by first diagnosing diabetes mellitus, and second using clinical and family history factors to identify the T2D subtype of diabetes mellitus. The World Health Organization (WHO) currently recommends four methods of diabetes diagnosis (86), which are also recognized by the American Diabetes Association (ADA) (91). Each diagnostic test consists of a measure of plasma glucose or glycated hemoglobin (HbA1c) under some specific condition. The four diagnostic tests recommended by both organizations include:

1) A fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL)
2) A plasma glucose ≥ 11.1 mmol/L (200 mg/dL) following a 2-hour OGTT
3) An HbA1c ≥ 6.5% (48 mmol/mol)
4) A random blood glucose of ≥ 11.1 mmol/L (200 mg/dL) if an individual has signs and symptoms of diabetes

For diagnostic tests 1-3 (occurring in asymptomatic individuals), a second positive test is usually administered to confirm the diagnosis, with the suggestion of repeating the same test. If different tests are used, then diabetes is diagnosed if both tests meet their diagnostic thresholds; if results are discordant, than the test which exceeded the diagnostic threshold should be repeated (86, 92). It is important to note that discordant results between tests are not uncommon, as they are likely measuring different underlying pathophysiological mechanisms which lead to the elevated glucose levels (93, 94).

Additionally, not reaching the threshold for diabetes diagnosis does not indicate a low risk for diabetes. Prediabetes is a state of elevated plasma glucose that is not high enough to be diagnosed as diabetes (92). These can be measured using tests 1-3 listed above with lower criteria; however, there is a lack of consistency in these cutoffs (3, 92). Prediabetes also goes by different names depending on the method used: if based on fasting plasma glucose, it is referred to as impaired fasting glucose (IFG); if based on an OGTT, it is referred to as impaired glucose tolerance (IGT). The ADA also has suggested cut-offs for HbA1c values and prediabetes diagnosis (92). **Table 1.1** summarizes all of the diagnostic methods and test cut-offs for diabetes and prediabetes. While these cut-offs for diabetes and prediabetes diagnoses are useful in the clinical and research setting, it is also important to note that regardless of the test method used, risk of plasma glucose is continuous and increases from below the range of prediabetes diagnosis to beyond the limit for diabetes diagnosis (92).

Table 1.1 Diagnostic Criteria for Prediabetes/Diabetes

Diagnosis	Fasting Plasma Glucose (FPG)	Oral Glucose Tolerance Test (OGTT)	Glycated Hemoglobin (HbA1c)	Random Blood Glucose
Prediabetes (WHO/IDF)	6.1–6.9 mmol/L (110–125 mg/dL) AND OGTT < 7.8 mmol/L (140 mg/dL) [IFG]	7.8–11.0 mmol/L (140–199 mg/dL) AND FPG < 7.0 mmol/L (126 mg/dL) [IGT]	--	--
Prediabetes (ADA)	5.6 – 6.9 mmol/L (100–125 mg/dL) [IFG]	7.8–11.0 mmol/L (140–199 mg/dL) [IGT]	5.7%–6.4% (39–47 mmol/mol	--
Diabetes	≥ 7.0 mmol/L (126 mg/dL)	≥ 11.1 mmol/L (200 mg/dL)	≥ 6.5% (48 mmol/mol)	≥ 11.1 mmol/L (200 mg/dL) with signs/symptoms

Once diabetes is confirmed, it must be differentiated into one of the many subtypes that exists, based on factors such as age at diagnosis, family history, and any other clinical characteristics or presentations (86). T2D typically occurs after puberty, and factors that suggest T2D over other forms include being overweight or obese, having strong family history of T2D, the presence of acanthosis nigrans, having elevated or normal C-peptide, no detectable pancreatic autoantibodies or history of autoimmune disease, and a lack of rapid weight loss (86).

1.2.1.3 Risk Factors for Type 2 Diabetes

Some risk factors, such as increasing age, having a family history of diabetes, and being in specific racial/ethnic groups (such as being of African ancestry) are non-modifiable factors which contribute to diabetes risk (95). While non-modifiable risk factors have importance in diabetes

risk, modifiable risk factors provide the greatest potential for reducing diabetes risk as they can be intervened upon.

Obesity is a well-known risk factor for diabetes, with over half of US individuals with diabetes being obese and over 85% being overweight or obese (96). Studies have demonstrated that individuals who gain weight are at a higher risk for developing incident diabetes (97), and that intentional weight loss can improve risk (98). Mechanisms linking obesity to diabetes are multifactorial, including inflammation, insulin resistance, and increased circulation of fatty acids (99). Further contributions of obesity to diabetes risk may come from the distribution and quality of adipose tissue in the body, discussed later in this document.

Dietary intake is a contributor to both obesity and T2D risk. The overall caloric content of an individual's diet may increase risk if an individual's caloric intake exceeds their caloric output, resulting in a net weight gain. However, the quality of a diet also plays a role. This may include differences in the quality or source of specific nutrients, such as dietary fats and fibers (100); the consumption of particular food groups, such as increased risks seen with higher consumption of red and processed meats (101, 102) and sugar-sweetened beverages (102); or with overall combinations of food groups, in which greater adherence to healthy dietary patterns were associated with reduced T2D risk (27, 28).

As presented in a joint position statement by the American College of Sports Medicine and the American Diabetes Association (103), multiple observational and interventional studies have provided extensive evidence that increased physical activity (aerobic or resistance exercise) can help with prevention of and maintenance of T2D through reductions in blood glucose levels and improvements in systemic insulin sensitivity. A review including analyses for sedentary behavior and T2D risk identified a few studies that demonstrated associations between increased sedentary

time and increased risk of T2D, though these associations were not significant after adjustment for BMI (104).

Other lifestyle factors such as smoking and alcohol intake can also influence the risk of T2D. Cigarette smoking impacts multiple cardiovascular disease risk factors. In an intervention study comparing the effects of smoking cigarettes in healthy smokers and healthy never-smokers, cigarette smoking was found to lead to impaired glucose tolerance and increases in blood pressure and serum cholesterol in both groups, while leading to decreased insulin sensitivity and increased LDL-cholesterol and triglycerides in the healthy smoker group (105). A meta-analysis of observational studies found that active smoking increased the risk of incident T2D, with a pooled Relative Risk of 1.44 (95% CI: 1.31-1.58); the study results were also consistent with a dose-response relationship between smoking and risk for T2D, as well as an increased (but reduced) risk for previous smokers (106). The association of alcohol consumption with T2D risk, however, is less consistent. Meta-analyses have demonstrated a U-shaped association between amount of alcohol consumption and T2D risk, with individuals who have low or moderate alcohol consumption exhibiting a decreased risk and those with high consumption having an increased risk (107-109); however, these protective effects may be more pronounced in women (108) or completely limited to women and non-Asian populations (107), or may be affected by the type of alcohol consumed (109). Furthermore, trajectory analyses indicate that differences in risk seen between participants with T2D may be affected by earlier-life heavy drinking (110) and that differences in risk between those who do or don't develop T2D are reduced when controlling for confounders (111).

Being of a lower socio-economic position can impact both risk of T2D as well as management of disease in those with T2D. A meta-analysis of socio-economic position and T2D

incidence found that socio-economic status, measured as education level, occupation, or income, was inversely associated with T2D incidence regardless of country-level income (112). How socio-economic factors contribute to T2D prevalence and maintenance is complex, as one's socio-economic position can impact personal-level factors (household income, education, occupational status) as well as factors beyond the individual (neighborhood, school, and work environments), all of which can shape an individual's choices, their resulting health behaviors, and their access to and utilization of healthcare services (113, 114).

Other cardiometabolic diseases may place an individual at risk for T2D. Hypertension and T2D are risk factors for each other, likely due to a shared pathophysiology of a variety of factors including obesity, inflammation, oxidative stress, inappropriate activation of the renin-angiotensin-aldosterone system (RAAS), and impaired insulin-mediated vasodilation (115). Associations of elevated blood pressure or blood pressure category and incident diabetes have been documented in multiple studies of differing ethnic backgrounds (116-121), as well as a potential increased risk with use of beta-blockers (118, 119). However, one study looking at incidence in African Americans compared to Caucasians found that although hypertension increased risk more than two-fold for developing diabetes in African Americans compared to being normotensive, adjustments for BMI, fasting glucose, and lipids greatly attenuated these results (121), suggesting that risk for diabetes may be more strongly associated with other cardiometabolic risk factors rather than blood pressure in this population.

Similar to hypertension, dyslipidemia (having high triglycerides, high low-density lipoprotein (LDL)-cholesterol, and low high-density lipoprotein (HDL)-cholesterol) is also common in individuals with T2D and serves as a risk factor for T2D (122, 123). Studies using nuclear magnetic resonance to measure lipoprotein particles found associations between

lipoprotein size and concentrations with incidence of T2D, with increased triglycerides, larger very low-density lipoproteins (VLDLs), and smaller HDL particles being associated with increased incidence of T2D across most studies (124-127). As reviewed by von Eckardstein and Sibler (122), lipoprotein particles may differentially affect pancreatic β-cell function, proliferation, and survival. Additionally, changes in lipids due to therapy with statins may increase risk of T2D development (123).

1.3 Impact of T2D in Tobago, Trinidad and Tobago

1.3.1 Trinidad and Tobago History and Current State

1.3.1.1 History

A brief history of the Republic of Trinidad and Tobago, as told by the country's first Prime Minister Dr. Eric Williams, is summarized in this paragraph (72). The islands of Trinidad and Tobago, located in the southern Caribbean, were separately colonized in the early 1500's by Spain for the purpose of establishing sugar plantations. As with most Caribbean islands, while enslavement of Amerindians first took place, African slaves were later brought to Trinidad and Tobago as substitutes for Amerindian slaves given the racist belief that Africans were more accustomed to hard labor. With the rise and fall of colonial empires, both Tobago and Trinidad underwent ownership by different powers, with Tobago being occupied by multiple colonial powers while Trinidad remained more consistently under Spanish, French, or British rule. After the abolition of slavery, Britain sought to avoid paying freed Black workers a fair price and instead brought in indentured servants from India as competition, thus changing the ethnic makeup of the

Trinidadian population greatly. However, Tobagonians remained (and still remain) a predominantly Black island. The islands were later merged under British rule, but became independent in 1962.

1.3.1.2 Current State

Statistics presented in this section are for the entire Republic of Trinidad and Tobago unless explicitly stated otherwise. Island-specific reporting tends to be uncommon in governmental reports; this may be due to a strong push for unity and nationality as a rebuke of the racist policies and actions from previous colonial rule. Indeed, as part of his Independence Day speech, Prime Minister Williams stated "Division of the races was the policy of colonialism. Integration of the races must be the policy of Independence" (72). Additionally, while there are reports of health studies performed in "Trinidad and Tobago", a majority of these have occurred primarily in the island of Trinidad with little inclusion of individuals from Tobago. Many socioeconomic and cultural differences likely exist between the sister islands, as will be highlighted below; thus, national statistics and study results should be taken with a grain of salt when thinking specifically of the island of Tobago.

1.3.1.2.1 **Government**

The governmental system of Trinidad and Tobago is a parliamentary republic, currently headed by President Paula-Mae Weekes and Prime Minister Keith Rowley (128). Tobago has its own local authority, the Tobago House of Assembly, which has ten divisions comprising its Legislative and Executive arms (129). One such division is the division of Health, Wellness, and Family Development, currently under the direction of Councillor Tracy Davidson-Celestine (130). The Tobago House of Assembly and the Division of Health, Wellness, and Family Development have provided support and in-kind services to population research programs such as the Tobago Health Study (see section **1.3.4**).

1.3.1.2.2 **Economy**

Trinidad and Tobago is considered to be a high-income country, with a 2017 GDP of almost $22.1 billion (131). The main driver of the economy is the energy sector, with oil and natural gas accounting for a majority of the country's exports and about 40% of its GDP (128). The US is a major trading partner for Trinidad and Tobago (128); however, regional instability with neighboring country Venezuela, a currency crisis in Argentina, uncertainties surrounding Brexit, and a combination of US trade disputes, sanctions, and trade renegotiations have led to some future economic uncertainties (132).

Though the energy sector is the main driver of the nation's economy, Trinidad is the primary beneficiary of this sector (133). Instead, the predominant economy of Tobago is tourism, an industry that has fallen by 75% in the past decade due to competition with other Caribbean islands (133). The 2017 unemployment rates for Trinidad and Tobago were at 4.8% with projected increases in unemployment in 2018 (132); however, if similar to 2015 trends, this may disproportionately affect the younger population (aged 15-19) and females (134). While there are no current estimates of poverty in Trinidad and Tobago, it was estimated that 18.9% of the population in 2009 were living in poverty, with reductions of about 2% per year by 2015 (135).

1.3.1.2.3 **Demographics**

Trinidad and Tobago has a population around 1.35 million individuals, with about 91.4% of individuals living in rural areas (136). Trinidad and Tobago has undergone an epidemiological transition since the 1960's, with non-communicable diseases now the primary source of health problems (136). Along with this transition, there has been a demographic transition as well, creating a shift in population structure. There has been notable greater growth in older age groups, with almost 45% of the population being between the ages of 25-54, 13.31% between 55-64, and 11.1% being 65 or older (128).

Trinidad and Tobago has an ethnically diverse population, with over a third of the population being of East Indian descent, another third being of African descent, about 20% being mixed race, and the remaining listed as other/unspecified (128); though as previously mentioned, Tobago is predominantly African descent.

1.3.1.2.4 **Healthcare**

Healthcare in Trinidad and Tobago consists of government-funded free health services as well as services from private and non-governmental organizations (136). The Ministry of Health is responsible for financial and regulatory oversite as well as policy and legislation; beyond the Ministry of Health, there are five Regional Health Authorities, one of which is located in Tobago, which help deliver health care through hospitals, district health facilities, and health centers (137). Health expenditures in 2016 in Trinidad and Tobago was 6.51% of the country's GDP; as a comparison, the number was around 17% of GDP for the United States that same year (138).

1.3.2 Health Profile and Type 2 Diabetes Burden

As a country having undergone an epidemiologic transition, the burden of health issues in Trinidad and Tobago can mainly be attributed to chronic non-communicable diseases (NCDs). Between 2010-2015, NCDs accounted for half of all hospital admissions and remain in the top leading causes of death for older adults aged 45-60 (137). Importantly for this dissertation, diabetes mellitus ranked as the second leading cause of death overall and in males (113.32 and 114.26 deaths per 100,000 live births, respectively) and the first leading cause of death among females (112.4 deaths per 100,000 live births) (137).

In 2016, the overall diabetes prevalence was estimated at 12.5%, with a higher prevalence in females compared to males (14.1% to 10.9%, respectively) (71). Despite this high prevalence, no diabetes registry currently exists (71). This lack of a registry has implications: a 2010 white paper highlights some difficulties in providing accurate morbidity/mortality statistics of diabetes

in Trinidad and Tobago, as data collected by the country's Ministry of Health may be undercounting diabetes cases (data not collected from all public hospitals and none from private hospitals, as well as a failure to separate first-time admissions and repeat admissions), and data from the International Diabetes Federation (IDF) on diabetes in many Caribbean countries are estimates derived using Jamaican data and adjusted for country age distributions (139). Still, while true prevalence estimates are not accurately known, all estimates indicate that Trinidad and Tobago have some of the highest diabetes rates globally.

In addition to high prevalence rates of diabetes, the burden of diabetes complications in Trinidad and Tobago is high. Individuals with diabetes had 2-4 times the rates of heart disease and stroke; diabetes was the leading cause of blindness and contributed to increased dialysis needs; over 60% of individuals with diabetes had mild-to-sever neuropathy, with many individuals requiring lower-limb amputations and suffering resulting depression; a 74% prevalence of erectile dysfunction in men with diabetes for longer than 15 years; and increased risk of periodontal disease (139). These high rates of morbidity indicate poor disease management among individuals with diabetes.

Importantly, the management of diabetes involves factors at both the health care provider and patient levels. A survey of health care providers from Trinidad and Tobago identified multiple barriers in providing adequate diabetes care including a lack of resources for the care of persons with diabetes, inadequate screening and evaluating time, and low education of cardiovascular complications risks (140). One study from the Trinidad and Tobago Health Sciences Initiative's (TTHSI) Diabetes Outreach Program also found that patients lacked proper understanding of diabetes management and the effects of diabetes on the body, poor lifestyle factors such as high rates of sedentary behavior and low quality of diet, and financial barriers to management (141).

Similarly, a small study of diabetic individuals in a Trinidad health center found low participant knowledge of the causes diabetes and how to care for it, as well as low rates of regular exercise and low diet quality (142). An additional TTHSI report also found high rates of diabetes complications and low utilization of eye and foot exams (143). Thus, a multitude of barriers at many levels are impacting diabetes care and self-management.

Local and national initiatives have been implemented to address many of the issues surrounding diabetes reporting and management. The Eastern Regional Health Authority in Trinidad, covering about one third of the island, has had a diabetic registry initiated in 2007 and found some disparities by gender, ethnicity, and geographic location, though the registry data was considered to be of poorer quality (144). The Ministry of Health has also implemented several initiatives to target modifiable risk factors, such as the "Healthy Me" childhood obesity prevention camp, the "Fight the Fat" initiative, and the "Annual Wellness Campaign" (137). Additional government-funded programs exist to help residents of Trinidad and Tobago including the Chronic Disease Assistance Programme, food subsidy grants, government-provided housing, free medical equipment, and home improvements (137), all of which can impact multiple socioeconomic barriers to diabetes management and care. Finally, international collaborations for diabetes-related research also have occurred, including the Tobago Health Study (current) and the aforementioned TTHSI Diabetes Outreach Program (2007-2014).

1.3.3 Non-Modifiable and Modifiable Risk Factors for Diabetes in Trinidad and Tobago

Given the high rates of diabetes in Trinidad and Tobago, it is important to understand how various factors contribute to excess risk. Importantly, many risk factors are interrelated. Some risk factors, such as increasing age, having a family history of diabetes, and being in specific

racial/ethnic groups (such as being of African ancestry) are non-modifiable factors which contribute to diabetes risk (95). These risk factors are important to diabetes risk in Tobago; indeed, small studies from Trinidad indicated that increasing age and family history of diabetes were significantly associated with greater prevalence of diabetes (145, 146), and our Tobago Health Study has previously reported diabetes family history estimates of about 48.3% (147). African ancestry individuals also have a greater risk for T2D, independent of overall obesity (53-55), and given that Tobago is predominantly African ancestry, this is likely to be an important contributor to diabetes risk as well.

While non-modifiable risk factors have importance in diabetes risk, modifiable risk factors provide potential for reducing diabetes risk as they can be intervened upon, providing the greatest impact on reducing diabetes burden in Tobago. In this section, I will make some reference to the Tobago Health Study (further discussed in section **1.3.4**) in order to give more Tobago-centric data when possible; however, these statistics will be limited to men aged 40 and older, who we estimate at our most recent visit to have an overall prevalence of T2D at 23.6%. This estimate is slightly higher than the self-reported national prevalence of 18.1% for men aged 45-64 (148), but when restricted to a similar age range (50-64) is somewhat similar (Tobago: 21.1%). This data will also rely heavily on the 2011 Trinidad and Tobago Chronic Non-Communicable Disease Risk Factor Survey (Pan American STEPS) (148), which I will refer to as the STEPS survey.

1.3.3.1 Obesity

Rates of overweight and obesity in Trinidad and Tobago are high and increasing, with the nation considered to have one of the highest obesity rates in the Caribbean at ~30% (149). The STEPS survey showed gender disparities in overweight/obesity, with rates being much higher in women compared to men (59.0% vs 52.3%, respectively) (148). Age disparities were also noted,

with obesity increasing after age 24 and then decreasing again in older age groups (148). In men aged 45-64, about 23.3% of men were considered to be obese (148); this is much less than that reported in the Tobago Health Study, where men aged 50-64 had an obesity prevalence of 31.5%. Waist circumference was also measured in the STEPS survey and showed men with an overall larger waist size compared to women (mean 104.9 cm vs. 89.3 cm, respectively); this gender disparity persisted in all age groups (148). Men from the Tobago Health Study tended to have slightly smaller waist circumference sizes, with the average size in men aged 50-64 being 99.0 cm.

1.3.3.2 Diet and Nutrition

The different population makeups and histories of Trinidad and Tobago have resulted in a rich and varied food culture. African dishes such as callaloo and accra are still present (72); there is use of native plants once eaten by Amerindians such as maize, sweet potato, and custard apple (72); and there is a noticeable Indian-influence of foods such as roti and curries (150). Additionally, the differences in economy and urbanization that exist between the islands may contribute to differences in purchasing and eating patterns.

In addition to ethnic influences, other factors may be contributing to current dietary intake in Trinidad and Tobago. A focus on the oil and energy sectors came at the expense of agricultural policies, which may contribute to a high reliance on imported foods (151). These foreign imported foods, according to anthropologists, may also be associated with feelings of wealth and status, a connection which may find historical roots in social hierarchies between slaves who worked in the fields compared to those who worked in houses (and had access to foreign foods) (151). Conversely, more modern social identities seem to equate individuality with fast food, with some Trinidadian youth saying that Kentucky Fried Chicken “to some extent it is what makes us Trinidadian” (151). Other indications that age may be a factor in healthy food intake comes from

the STEPS report which indicated a slight increase in mean servings of daily fruit and vegetable intake across increasing age groups, though the overall average was still low (overall fruit mean: 1.0 , 95% CI: 0.9-1.0; overall vegetable mean: 1.3, 95% CI: 1.2-1.4) (148).

Dietary intake likely differs between individuals in Trinidad and Tobago with the United States, as there are large overall differences in economy, culture, climate, and history. **Table 1.2** below shows a rough comparison of food intakes between men aged 40 and older from the Tobago Health Study and men from the National Health and Nutrition Examination Survey (NHANES) 2013-2014 (152). It is important to note that due to differences in collection methodologies (Tobago used a monthly 146-item semi-quantitative Food Frequency Questionnaire validated for the Trinidad and Tobago population (153), while NHANES used 24-hour dietary recall interviews), it is not possible to perform a direct comparison of intakes. Still, these preliminary results suggest that men from Tobago report consuming larger daily intakes of fruits, vegetables, whole grains, and seafood, while reporting lesser intakes of refined grains, red and processed meats, dairy, and alcohol, compared to their American counterparts. The high intakes of fruits and vegetables is in contrast to that reported in the STEPS survey; this may be in part due to differences in how diet was ascertained (Tobago Health Study: 146-item questionnaire of foods consumed in the past month vs STEPS: survey questions on amount and frequency of fruits or vegetables consumed in a typical week). However, it is also possible that there are island-specific differences in food consumption which was not reported on through the STEPS survey.

Table 1.2 Comparison of Tobago and NHANES Men, 40+, using Food Patterns Equivalent Database (FPED)

Food Groups

Food Group	Tobago N=799	Non-Hispanic White N=692	Non-Hispanic Black N=336	Non-Hispanic Asian N=156	Hispanic N=347
Citrus, Melons, and Berries (cup eq)	0.83 (0.04)	0.20 (0.02)	0.17 (0.04)	0.25 (0.06)	0.20 (0.03)
Other Fruit (cup eq)	2.09 (0.09)	0.45 (0.05)	0.44 (0.04)	0.94 (0.14)	0.57 (0.05)
Fruit Juice (cup eq)	0.11 (0.01)	0.23 (0.03)	0.35 (0.04)	0.25 (0.06)	0.31 (0.05)
Total Fruit (cup eq)	3.04 (0.12)	0.88 (0.07)	0.96 (0.06)	1.45 (0.21)	1.07 (0.06)
Dark Green Vegetables (cup eq)	0.76 (0.03)	0.12 (0.02)	0.14 (0.03)	0.27 (0.05)	0.11 (0.03)
Tomatoes (cup eq)	0.19 (0.01)	0.34 (0.02)	0.27 (0.05)	0.24 (0.03)	0.38 (0.03)
Other Red/Orange Vegetables (cup eq)	0.71 (0.02)	0.10 (0.01)	0.12 (0.02)	0.19 (0.04)	0.09 (0.02)
Total Red/Orange Vegetables (cup eq)	0.91 (0.02)	0.44 (0.02)	0.38 (0.05)	0.42 (0.05)	0.47 (0.02)
Potatoes (cup eq)	0.17 (0.01)	0.46 (0.03)	0.44 (0.05)	0.32 (0.05)	0.31 (0.03)
Other Starchy Vegetables (cup eq)	1.06 (0.03)	0.08 (0.01)	0.13 (0.03)	0.12 (0.03)	0.13 (0.02)
Total Starchy Vegetables (cup eq)	1.24 (0.03)	0.53 (0.03)	0.56 (0.06)	0.44 (0.05)	0.44 (0.03)
Other Vegetables (cup eq)	1.16 (0.03)	0.57 (0.02)	0.36 (0.03)	0.86 (0.11)	0.65 (0.07)
Total Vegetables (cup eq)	4.06 (0.07)	1.67 (0.06)	1.45 (0.10)	1.99 (0.16)	1.67 (0.10)
Legumes as Vegetables (cup eq)	0.14 (0.01)	0.10 (0.01)	0.18 (0.05)	0.24 (0.06)	0.29 (0.04)
Legumes as Protein (oz eq)	0.58 (0.03)	0.40 (0.04)	0.71 (0.20)	0.96 (0.23)	1.17 (0.14)
Whole Grains (oz eq)	2.19 (0.07)	1.10 (0.08)	0.94 (0.07)	1.89 (0.19)	0.75 (0.11)
Refined Grains (oz eq)	2.87 (0.09)	5.83 (0.22)	5.54 (0.46)	5.97 (0.47)	7.29 (0.18)
Total Grains (oz eq)	5.06 (0.11)	6.93 (0.21)	6.48 (0.46)	7.86 (0.46)	8.04 (0.19)

Table 1.2 Continued

Meat (oz eq)	0.37 (0.02)	1.76 (0.11)	1.58 (0.18)	1.83 (0.30)	2.17 (0.27)
Cured Meat (oz eq)	0.35 (0.02)	1.33 (0.13)	1.30 (0.11)	0.50 (0.11)	0.75 (0.11)
Organ Meats (oz eq)	0.03 (0.00)	0.05 (0.03)	0.08 (0.04)	##	0.08 (0.03)
Poultry (oz eq)	1.29 (0.04)	1.58 (0.10)	2.24 (0.19)	1.64 (0.32)	2.03 (0.28)
Seafood High N-3 (oz eq)	1.30 (0.04)	0.26 (0.09)	0.23 (0.08)	0.53 (0.18)	0.20 (0.10)
Seafood Low N-3 (oz eq)	1.36 (0.04)	0.51 (0.14)	1.13 (0.20)	0.82 (0.16)	0.61 (0.11)
Total Meat, Poultry, and Seafood (oz eq)	4.69 (0.10)	5.48 (0.22)	6.56 (0.38)	5.32 (0.51)	5.84 (0.29)
Eggs (oz eq)	0.34 (0.01)	0.59 (0.04)	0.79 (0.10)	0.47 (0.09)	0.91 (0.09)
Soybean Products (oz eq)	0.04 (0.00)	0.05 (0.01)	0.04 (0.02)	0.23 (0.07)	0.03 (0.01)
Nuts and Seeds (oz eq)	0.12 (0.01)	1.10 (0.16)	0.83 (0.15)	0.72 (0.13)	0.54 (0.11)
Total Protein (oz eq)	5.20 (0.10)	7.22 (0.30)	8.22 (0.48)	6.74 (0.59)	7.32 (0.31)
Fluid Milk (cup eq)	0.56 (0.02)	0.88 (0.04)	0.50 (0.04)	0.58 (0.08)	0.61 (0.05)
Yogurt (cup eq)	0.02 (0.00)	0.06 (0.01)	0.02 (0.01)	0.08 (0.03)	0.03 (0.00)
Cheese (cup eq)	0.35 (0.01)	0.83 (0.05)	0.65 (0.12)	0.35 (0.05)	0.86 (0.11)
Total Dairy (cup eq)	0.97 (0.03)	1.81 (0.05)	1.19 (0.15)	1.03 (0.11)	1.56 (0.15)
Oils (g)	19.05 (0.32)	30.05 (1.04)	30.16 (2.52)	25.12 (1.67)	25.31 (1.64)
Solid Fats (g)	25.37 (0.49)	40.34 (0.90)	40.84 (3.49)	23.33 (2.07)	38.06 (1.74)
Added Sugars (tsp eq)	6.49 (0.15)	17.85 (1.12)	20.39 (1.52)	8.90 (0.75)	16.38 (0.90)
Alcoholic Drinks (# drinks)	0.39 (0.04)	1.16 (0.13)	1.04 (0.23)	0.41 (0.09)	0.80 (0.07)

A comparison of calculated food group equivalents from the Tobago Health Study and NHANES. Men from the Tobago Health Study excluded individuals who were missing 10% or more food items on a questionnaire or who reported energy intakes < 600 kcal/day or > 5,000 kcal/day.

Importantly for diabetes risk in the Caribbean, these nutritional and dietary patterns are not unique to Trinidad and Tobago. Many of the Caribbean islands, while having different developmental histories, share similarities in colonial pasts, in being huge importers of food, and in having increasing food patterns of poorer nutritional quality (154). Thus, nutrition and dietary intake are major risk factors for diabetes risk in the Caribbean.

1.3.3.3 Physical Activity and Sedentary Time

In Trinidad and Tobago, physical activity tends to be seasonal (136). The vigorousness of activity levels differ between men and women, with twice as many men having reported high level physical activity compared to women, and significantly more women participating in low-to-moderate physical activity compared to men, in the STEPS survey (148). This difference in activity and its seasonality was attributed to men participating in more laborious occupational work (148). In addition to seasonal or occupational contributions, other facilitators and barriers of physical activity may play a role. A survey of Trinidadians identified a lack of time for physical activity, low motivation to be physically active, and a lack of affordable or accessible places to be physically active as major barriers to being more active (155). Increasing population age is another factor, with the STEPS survey indicating that prevalence of low physical activity level generally increased with age while high physical activity level decreased with age (148).

Physical activity definitions are found in **Appendix A Table 1**. In the STEPS survey, 47.2% of men aged 45-64 had low levels of physical activity while 32.2% had high levels of physical activity. These men also reported ~3 hours of sedentary activity per day (148). Our men also have low objectively-measured physical activity and low subjectively measures of walking with high rates of sedentary behavior. Men from our most recent visit (restricted to those aged 50-64) reported roughly 2.4 hours per week of watching television (our marker of sedentary behavior),

and self-reported high vigorous activity was 2.2 hours/day and light physical activity was 9.0 hours/day. Using objectively-measured physical activity in a subset of men from this most recent visit, men on average spent 27% of waking hours in light physical activity, 4.9% in moderate physical activity, and 0.6% in vigorous or very vigorous physical activity, with over 67% of the time spent in sedentary activity. These survey reports and objectively-measured physical activity reports indicate both low levels of physical activity and high levels of sedentary behavior, both likely to contribute to an increased risk for T2D.

1.3.3.4 Smoking and Alcohol Use

National smoking rates are high in Trinidad and Tobago, with an estimated prevalence of 21.1% and a higher prevalence in men (33.5%) compared to women (9.4%) (148). Current smoking status generally increased with age in men but decreased with age in women (148). Most individuals who smoke use manufactured cigarettes and smoked on average 11.5 cigarettes per day (136). National alcohol consumption is also high, with an overall reported prevalence of drinking in the past 30 days at 40.4% and a higher prevalence in men (50.6%) compared to women (30.9%) (148). Daily consumption was reported to be much lower, with only 3.3% of men and 0.2% of women reporting daily consumption over the past year (148). While the number of drinking occasions increased with increasing age group in men, the number of drinks per drinking occasion decreased; both drinking occasions and number of drinks decreased with age in women (148). Binge drinking rates were reported as high (~34% in males and ~17% in females), and almost 60% of men and women reported rarely or never drinking with meals (148).

These reported national statistics for smoking differ compared to those reported in our Tobago Health Study, with our men reporting lower smoking prevalence. Using data from the Tobago Health Study at a similar time frame to the STEPS survey (Tobago: 2010-2014 vs. STEPS:

2011), our men aged 45-64 reported a current smoking prevalence of 11.3% and a former smoker prevalence of 20.5%. This is much lower than that reported for men 45-64 in the STEPS survey, who had a current smoking prevalence of 35.9% (former smoking not reported) (148). Results also differed for alcohol consumption, where men aged 45-64 from our study report higher rates of any drinking but a lower number of drinks consumed per week compared to national averages. In Tobago, 67.5% of men reported having had any alcohol consumption in the past year, with 28.9% of drinkers having had at least 1 drink per week, 19.4% of drinkers having had 4+ drinks per week, and 4.7% of drinkers having had 15+ drinks per week. This is in contrast to men in the STEPS survey, which indicated a frequency of 61.2% ever drinkers in the past year but, among current drinkers (drank in last 30 days), about 9.2% had 20+ drinks in the past week (148). These results may reflect cultural differences of tobacco and alcohol use in Tobago as compared to overall national statistics; however, if there are cultural differences in perceptions of smoking or alcohol intake, this may also lead to an underreporting of these risk factors in our study. It may also be impacted by differences in how questions were asked between interviews, especially concerning alcohol intake. Still, these results suggest an overall favorable profile for smoking and alcohol intake in our Tobago population, which may beneficially impact T2D risk.

1.3.3.5 Socio-economic Factors

Despite the availability of free education (137), the level of education attainment is relatively low, with almost 30% of the population having only completed a primary level education and less than 15% with education beyond high-school level (156). This lack of educational attainment is more stark in age groups 50 and older, where over half the population in each age group did not have a stated educational qualification (156). As mentioned previously (section **1.3.1.2.2**), national poverty rates in 2009 were estimated at around 18.9%, and the primary driver

of the Tobagonian economy (tourism) has taken a hit in recent decades. These factors of lower education and high poverty rates are likely to impact an individual's income, occupational status and opportunities, and resulting health behaviors. Indeed, as previously discussed, cost was reported as a barrier to both increased physical activity (through access to fitness facilities) and to healthy eating (through purchasing of healthy foods) (155). Markers of low socio-economic status were inversely associated with diabetes morbidity in a sample of diabetic Trinidadian patients (157), suggesting that social determinants of may also play a role in diabetes prevalence and management in Trinidad and Tobago.

1.3.3.6 Hypertension and Dyslipidemia

In Trinidad and Tobago, around 26.3% of individuals are estimated to have hypertension (SBP ≥ 140 and/or DBP ≥ 90 mmHg or on antihypertensive medication), with a majority of them being men (29.8% in men vs. 23.1% in women) (148). Among men, rates were higher in older age groups, with a prevalence of about 52.4% in men aged 45-64 (148). Men from our study have a slightly higher rate, with men from our most recent visit aged 50-64 having a prevalence of 55.3%. However, these Tobago Study rates and the national rates are based off of older criteria; given the new criteria (SBP ≥ 130 and/or DBP ≥ 80 mmHg or on antihypertensive medication) (158), the prevalence in our study is closer to 71.9%.

Rates of high cholesterol were similar, with an overall prevalence of 23.5% of individuals having a total cholesterol ≥ 240 mg/dl or being on a lipid-lowering medication, with a higher prevalence in men compared to women (28.3% vs. 18.9%, respectively) (148). Having a low HDL-cholesterol (< 40 mg/dl in men or < 50 mg/dl in women) was more prevalent in women (34.7%) than in men (9.9%); similarly, having triglycerides ≥ 150 mg/dl was also higher in women than in men (51.4% vs. 47.9%, respectively) (148). These results were not stratified by race, an important

confounder as African ancestry individuals tend to have better lipid profiles compared to other ethnic groups (159). We currently do not have lipids measured at our most recent Tobago Study visit. However, a study comparing a subset of our Tobago men with Caucasian and African American men in the Cardiovascular Health Study (160) found more favorable lipoprotein profiles in African ancestry men compared to Caucasians and lower levels of various lipoproteins (triglycerides; small, medium, and large VLDLs; and small and medium LDLs) in Tobago men compared to both Caucasian and African American men. In total, these results suggest that hypertension, but potentially not dyslipidemia, may have a greater impact on diabetes risk in the Tobago population.

1.3.4 Our Study: The Tobago Health Study

1.3.4.1 Origins

The Tobago Health Study is a longitudinal study of men from the island of Tobago, Trinidad and Tobago, described in further detail in the next section (**1.3.4.2**). The study, which began recruitment in 1997 (161), was originally started as a population-based prostate cancer screening study. For this reason, the study population is restricted to only middle-aged and older males. However, men from the Tobago Health Study have returned for multiple follow-up visits, at which time various clinical and body compositional data collections were performed. While many of the clinical measures were collected using standardized procedures over the study visits, body compositional measures and anatomical locations assessed varied depending on the visit. For example, CT measures for ectopic fat assessment and microbiome data collection were only performed at one visit (between 2014-2018). Thus, while the study is a longitudinal study of

Tobago men, data for this dissertation (which will utilize CT and microbiome measures) can only be cross-sectionally analyzed.

1.3.4.2 Study Description

Figure 1.1 shows the flowchart of the study participants. Between 1997 and 2003, 3,170 previously unscreened men were recruited for a population-based prostate cancer screening study on the Caribbean island of Tobago, Trinidad and Tobago (161). To be eligible, men had to be aged 40 years or older, ambulatory, noninstitutionalized, and not terminally ill. Recruitment for the survey was accomplished by flyers, public service announcements, and posters; by informing health care workers at local hospitals and health centers; and by word of mouth. Approximately 60% of all age-eligible men on the island participated, and participation was similar across the island parishes. All men were invited to participate in a follow-up clinic examination between 2004 and 2007, and 2,031 men (70% of survivors) and 451 new participants completed the visit. A second follow-up examination was performed between 2010 and 2013, and a total of 1,611 men completed this follow-up assessment (82% of survivors). Between 2014 and 2018, a convenience sample of N=768 participants from the prior (2010-2013) visit had CT scans of the chest, abdomen, and mid-thigh for ectopic fat assessment performed, and a subset of men (N=262) participated in an ancillary study of fecal collection for microbiome analysis. Data from the 2014-2018 visit was used for this dissertation.

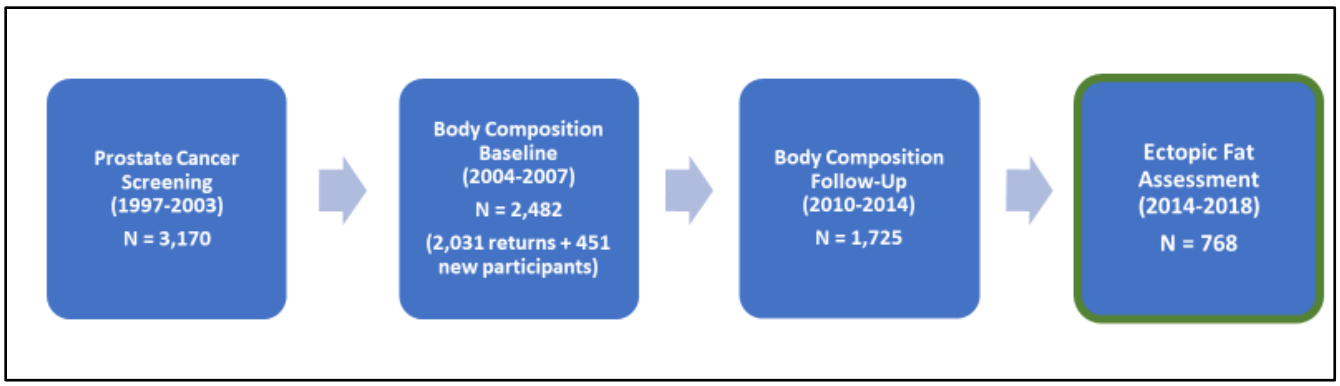

Figure 1.1 Flowchart of Tobago Study Visits

To be eligible for the 2014-2018 study, participants had to be aged 40 or older, ambulatory (able to walk without assistance of another person; assistive devices permitted), and willing and able to give informed consent. Participants could be withdrawn from the study if they were unable to answer or complete the study questionnaires due to cognitive impairment or dementia, or if participants were unable to complete most of the other clinical procedures. Potential participants were identified from men who participated in the previous 2010-2013 visit of the Tobago cohort study (n=1,611) and were contacted by those who are directly involved in the participants' care in the study to determine their eligibility and willingness to participate in the extended CT exam. Participants were re-contacted by Tobago Study staff via telephone call. Written informed consent was obtained before enrollment.

Beginning in June 2017, participants who had completed CT visits were re-contacted to participate in an ancillary microbiome study. IRB approval was obtained for this ancillary study. Participants were contacted by phone, with emphasis for recruitment placed on those who most recently completed a CT visit. Informed consent was obtained.

1.4 The Role of Adipose Tissue in Type 2 Diabetes Risk

1.4.1 Adipocytes and Adipose Tissue Biology

1.4.1.1 Adipose Tissue as Energy Storage

Adipose tissue is comprised of multiple cell types, including those of the stromal vascular fraction (consisting of a diverse population of cells such as pericytes, immune cells, endothelial cells, and vascular smooth muscle cells) and the adipocytes (162), though mature adipocytes are the major cell type found (163). Adipose tissue can be further broken down into two main tissue types: white adipose tissue (WAT) or brown adipose tissue (BAT). BAT is composed of mitochondria-rich adipocytes, is well-innervated and well-vascularized, and is responsible for converting chemical energy into heat through non-shivering thermogenesis; however, BAT tissue is a minority of the AT in adult humans and is found in localized areas in thoracic and supraclavicular regions (164). WAT on the other hand is the predominant AT in adult humans, is a major endocrine organ, and is involved in energy homeostasis (163).

WAT is a major site of triglyceride storage and fat mobilization by secretion of nonesterified fatty acids (165). As previously mentioned (section **1.2.1.1**), insulin can act to inhibit adipocyte lipolysis. This allows the adipocyte to continue to store lipids, which in mature adipocytes fuse to form a characteristic large lipid droplet (166). Additionally, the storage of lipids in adipose tissue also can prevent the circulation of free fatty acids, which can cause insulin resistance (section **1.2.1.1**). Thus, fatty acid storage and mobilization is linked to insulin action and resistance.

1.4.1.2 Adipose Tissue as an Endocrine/Paracrine Organ

In addition to fatty acids, AT is a major secretor of several proteins collectively referred to as adipokines. There are at least 50 different documented adipokines (167), allowing cross-talk between AT and several organ systems such as the brain, skeletal muscle, immune system, adrenal cortex, and cardiovascular systems (167, 168). I will not go into a review of different adipokines and their roles in such cross-talks; however, the main purpose of this section is to highlight the complex role AT plays in whole-body energy homeostasis through both positive and negative regulation of multiple tissues.

1.4.1.3 Adipose Tissue Growth: Hypertrophy vs. Hyperplasia

AT expands in response to overnutrition as a mechanism to store excess calories. This expansion is accomplished in predominantly two ways: cellular hypertrophy and cellular hyperplasia. Hypertrophy is thought to be the first mode of expansion, where the size of the adipocyte increases to accommodate an ever-growing lipid droplet (169). Upon reaching a critical size, additional cells are recruited for energy storage through adipocyte proliferation or the differentiation of preadipocytes in a process called hyperplasia (169).

As adipose tissue grows, remodeling of the tissue to accommodate the increased cellular size and number is needed. This requires changes in the tissue vasculature to meet nutrient and oxygen needs of the tissue, as well as changes in tissue extracellular matrix to accommodate the growing size. An inability to accommodate the tissue size can lead to hypoxia, cellular stress, and inflammation, all of which negatively impact the ability for the tissue depot to store lipids (169, 170). This can lead to adipocyte cell death and subsequent proliferation of new adipocytes, contributing to a net growth of AT (169); additionally, it may promote the storage of fat in non-traditional and ectopic sites such as the liver and muscle (16).

1.4.2 Adipose Tissue Distribution

Adipose tissue is found throughout the body from locations under the skin to within bone marrow. Given the wide variety of locations and changes in naming conventions, Shen et al propose a classification system that helps to separate AT depots by location and function (1). These classifications are visualized in **Appendix A Figure 1** and **Appendix A Figure 2**. Briefly, the most broad classifications of adipose tissue are the subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and non-VAT. SAT is the adipose tissue located beneath the skin, and can be further divided into superficial and deep SAT with respect to reference to the fascial plane (1). VAT is an umbrella term for the AT located within the thoracic, abdominal, and pelvic cavities. This tissue surrounds internal organs such as the heart, intestines, kidneys, and liver. Depending on location, they may have different local or systemic effects; for example, intraperitoneal adipose tissues within the VAT drain into the portal system, whereas retroperitoneal drain into the inferior vena cava, thus providing intraperitoneal adipose tissue a greater ability to impact multiple organ systems involved in metabolism in comparison to retroperitoneal tissues (1). Finally, non-VAT tissues predominantly make up those within and surrounding the musculature. These include intramuscular AT, which is located between muscle fascicles, intermuscular AT, which is located between muscles, and paraosseal AT, which is found between muscle and bone (1).

The reasons for differences in accumulation of AT in differing depots is not fully known, though several hypotheses exist. One hypothesis is the AT expandability hypothesis, which suggests that impaired expandability of AT leads to lipid storage in non-adipose tissues (171). SAT is the largest AT depot in the human body and therefore should contribute most to lipid storage. A modification of this hypothesis is that even preceding the inability of adipocytes to continue expansion, an impaired triglyceride turnover ability of adipocytes may also contribute to a

propensity to store fat in non-adipose tissues (172). Another hypothesis suggests that nutritional status in the womb may predispose an individual to store lipids in VAT as a mechanism to help fight intraabdominal infections (173). Beyond these hypotheses, other aspects are also noted to play a role. Gender is associated with distribution, with men being more likely to accrue fat centrally and women (prior to menopause) storing fat in a gluteal-femoral pattern (174). Racial/ethnic differences have also been identified in fat distribution patterns (56-62, 175), with some suggestions of underlying genetic associations (176); however, environmental interactions may have a predominant or modifying role in these associations as well (177-179).

The various AT depots have differing storage and lipolytic activities (172), as well as differing functional and genetic profiles (180). Combined with their proximities to insulin-sensitive tissues or major circulatory pathways, this may lead to greater risk of metabolic disturbances if those particular tissues become dysfunctional. For example, IMAT may have a great impact on skeletal muscle insulin sensitivity (181), whereas VAT depots may have more of an impact on hepatic insulin sensitivity (182).

1.4.3 Adiposopathy: Potential Connection between Adipose Tissue and Inflammation

"Adiposopathy" is a term describing pathological fat tissue function, wherein the fat tissue has an abnormal release of hormones, cytokines, and molecules resulting in non-fat tissue damage and disease development (183). Hallmarks of adiposopathy include enlarged hypertrophic adipocytes, increased visceral fat accumulation, and increased ectopic fat deposition (184), all of which are associated with the inflammatory and metabolically dysfunctional states of unhealthy obesity. Additional measures of quality include macrophage infiltration of AT (185, 186), angiogenesis (187), arteriolar dysfunction (188), and tissue hypoxia (189).

1.4.3.1 Measuring Body Composition with CT Scans

CT scans use X-rays to generate cross-sectional images that, if performed in series, can be additionally used to generate 3D images (190). Thus, CT scans can provide cross-sectional area measurements or 3D volumetric measurements of tissues. Images are divided into a grid of voxels, which each hold an X-ray attenuation value for that voxel location (given in Hounsfield units, HU) and are colored on a grey scale (191). Water has an attenuation of 0 HU, while air has an attenuation of -1000 HU; thus, more positive HU values indicate more-dense tissues. There is inconsistency in the field on exact locations to be used for measuring different AT depots and the HU thresholds used to define adipose tissue (ranging from -20 to -250 HU) (191). However, the HU may range considerably over AT voxels.

Organ and tissue boundaries are delineated manually or semi-automatically by trained technicians and software (191). This allows for precise measurements of AT area or volume, but can be laborious and influenced by technician skill (191). However, it also allows for quantification of AT in more difficult areas of imaging such as intermuscular deposition (192). CT also exposes individuals to radiation, which may place them at a small but increased risk for development of radiation-induced cancer (193).

1.4.3.2 Radiodensity: A Measure of Adiposopathy?

In addition to AT volume, a recently novel measure of average AT attenuation has been proposed. This average attenuation, also referred to as AT radiodensity, is the average HU value across the defined tissue. Human biopsy studies and studies in animal models indicate that higher AT radiodensity can reflect beneficial aspects of AT such as smaller adipocyte cell size and lower lipid content (17, 18) and increased vascularity (19). Thus, AT radiodensity may serve as a surrogate marker of AT “quality”.

CT-derived AT radiodensity is emerging as a marker of increased risk for cardiometabolic disease independent of tissue volume (22-26). Several reports using the Framingham cohort observed lower abdominal VAT and SAT radiodensity associated with worse cardiometabolic profiles, including higher HOMA-IR (24), increased odds of impaired fasting glucose (23, 24), and increased glucose concentrations (22-24). Studies from the Multi Ethnic Study of Atherosclerosis (MESA) have also reported that individuals with lower vs. higher abdominal VAT, SAT, and IMAT radiodensity had greater levels of glucose and diabetes (25, 26).

1.5 The Intestinal Microbiome: A Novel Risk Factor for Type 2 Diabetes

1.5.1 Defining the Intestinal Microbiome and its Importance to Health

Humans are host to a variety of microorganisms, covering all domains of life (bacteria, archaea, and eukaryotes) as well as viruses (194). Collectively, the genomes of these microbiota are noted as the "microbiome" (32). While studies have predominantly focused on the microbiome in the gastrointestinal tract, communities of resident microorganisms have also been documented in the oral cavity, urogenital tract, skin, airways, placenta, and eyes (68). These microbiota are not inert, and can influence host health through metabolic functions as well as be influenced by the host environment in a mutualistic relationship (32).

A large portion of microbiome research has focused on microbes in the gastrointestinal tract, and more specifically with bacteria. As this is also the focus of this dissertation, I will also focus predominantly on bacteria in the intestinal tract; the term *microbiome* will be used to refer to bacteria specifically in this case, as is done frequently in the literature. It is important to note,

however, that populations in other anatomical sites, as well as other types of microorganisms (archaea, eukaryotes, and viruses) also play integral roles in the relationships between the whole microbiome and human health.

The influence of gut bacteria and other microbes on host health is not specific to humans. The co-evolution of microbes and their hosts may exist across many species, with mammals showing stronger similarities among members of the same species compared to others, and similarities that tracked with diet and host phylogeny (195). Studies involving the absence of a microbiome, such as the use of gnotobiotic mice, as well as those that then repopulate the guts of gnotobiotic mice with specific bacterial communities, have demonstrated effects on the immune system, metabolism, reproduction, and behavior (196). Thus, these bacterial organisms have a storied and profound effect on how multiple systems in our bodies function, making them an important key in helping to understand human disease.

Large population-based microbiome projects in recent years have contributed significantly to our understanding of the gut microbiome and human health. These include the U.S.-based Human Microbiome Project (HMP1) (197) and the Integrative Human Microbiome Project (HMP2) (198), the European-based Metagenomics of the Human Intestinal Tract (MetaHIT) (199), and studies from China (44). Studies from other countries and across a range of urbanicity environments are also being conducted, providing a large amount of new information across geographies, race/ethnicity, and culture (200). It is the hope that data from the Tobago Health Study may add to this growing field, providing a new geographical and dietary insight into the connection between gut microbiota and human health.

1.5.2 Measuring the Intestinal Microbiome

There are two ways to look at the microbiome. The first is a descriptive analysis, which asks what bacteria are present in a sample. This is derived from a sample's bacterial DNA, which I will describe below. The second is a functional analysis, which asks what the bacteria are doing (or are capable of doing) in a sample. This can be estimated using predictions from DNA (201), but can be more precisely measured using transcriptomics, proteomics, metabolomics, and other –omics methodologies (202). Given that our project will be descriptive in nature, I will focus in this section on the collection, process, and analysis of samples for describing microbial taxonomies using 16S DNA sequences. However, the functional analyses are an incredibly important component as well for understanding host/microbiome interactions and health consequences.

1.5.2.1 Sample collection methods

Methods of intestinal microbiome sampling in large population-based studies typically involve the collection of fecal matter from an individual. Based on the previous sections of this document, fecal samples are unlikely to be representative of the entire intestinal microbiome, given differences in concentration along the gastrointestinal tract, intestinal lumen, and the constant shifts in bacterial populations. Multiple methods of collection exist, including collection of whole fecal samples or the use of swabs or cards (203). In addition to different methods of collection (as well as different manufacturer kits for collection), other choices must be made such as the use of stabilizers, storage temperature and anticipated number of freeze-thaw cycles, transportation method and timing from toilet to lab, and aliquoting of sample (if applicable) (204). These considerations are made based on the ease of sample collection for study participants and the type of analyses prepared for the samples, and may have variable impacts on results (205).

1.5.2.2 Process from sample to DNA

Once collected, the bacterial DNA must be separated from the rest of the stool using physical and chemical methods. Typical commercial kits use beaded tubes and shakers to mechanically homogenize and lyse bacterial cells, after which DNA is isolated using chemical extraction procedures (204). Differences in extraction kits and processes, as well as potential contamination from the kits themselves, can result in some between-kit differences (204); however, a comparison of extraction protocols used in the HMP and MetaHIT studies found that the largest variation was still attributable to inter-individual differences (206).

1.5.2.3 DNA Sequencing

Once DNA has been isolated, the investigator next chooses what portions and how much of the DNA to sequence. Ideally, one would want to obtain as much genetic information from a sample as possible to get as close to species identification as possible. Shotgun metagenomics can accomplish this by fragmenting DNA into small pieces, sequencing the pieces, and aligning overlapping pieces to reconstruct parts (or nearly all) of the genome of an organism (207). Using a metagenomics approach allows for the identification of bacteria (and any other microbes) as well as a description of many functional genes; however, such an approach is computationally and analytically complex due to the large volume of data, potential issues in overlapping of DNA sequences, inclusion of host DNA, and detection and handling of contamination (207).

An alternative approach is the use of amplicon sequencing, where a specific and identifying region of DNA is isolated and amplified through polymerase chain reaction (PCR) and then sequenced (194). In bacteria and archaea, a portion of the 16S ribosomal RNA subunit is typically amplified as this region contains multiple hypervariable regions that can differentiate between many bacteria (194). While much simpler to perform in comparison to shotgun metagenomics,

16S rRNA sequencing is less sensitive to differences in some bacterial taxa, and the same sample may yield different results depending on the hypervariable region sequenced and PCR primers used (194). For the Tobago study, we will be using 16S rRNA sequencing on our fecal samples.

1.5.2.4 Taxonomy, Analytical Pipelines, and the OTU

It is important to give a brief primer on taxonomy and its issues with respect to bacteria. Taxonomy is the system by which organisms are classified, encompassing the taxa (i.e. groups) of Domain, Kingdom, Phylum, Class, Order, Family, Genus, and Species. The rules for classifying bacteria at the species level have strict criterion involving the percentage of sequences shared as well as phenotypic discrimination; however, there is less uniformity in rules for classification at higher taxonomic ranks (208, 209). This is further complicated by the sheer number of detected and uncultured microorganisms being discovered with improving bioinformatics methods, which are outnumbering the classified cultured microbes in reference databases (208). The prior choice of DNA sequencing method can also impact resolution of your taxonomic assignment, as 16S rRNA is not necessarily sensitive to the species level (194). For this reason, groups of closely-related sequences are often clustered together into what is called an operational taxonomic unit (OTU) (210).

Following DNA sequencing, the data must be cleaned and placed into taxonomic bins. These processes are performed using dedicated bioinformatics tools such as QIIME (211) and mothur (212). The choice of pipeline used may have an impact on the classification of some genera, but results are generally comparable between programs (213). For 16S rRNA sequences, a next choice comes from methods of binning sequences together into OTUs based on their similarities (210). OTU construction is based on algorithms that either use a reference database to help assign OTUs or create them de novo, often with differing results (210). Sequences and OTUs are then

aligned to a reference database, allowing a taxonomic assignment (214). Importantly, the choice of reference database may also impact assigned taxonomy (214).

1.5.2.5 Structural Characteristics of a Microbiome

There are three main characteristics that can be ascertained for the microbiome: diversity, stability, and resilience. Diversity refers to structural or functional differences within a sample and/or between samples (215). Stability refers to the identification of distinct and longer-lasting community signatures, such as the identification of enterotypes (216). Resilience refers to the ability of the microbiome to return to a steady state following some perturbation, such as dietary change, antibiotics, and invasion by new bacterial species (215). Stability and resilience likely require multiple measures to ascertain. However, diversity measures can be obtained with a single measure; therefore, I will focus a bit more on the different diversity measures in this section.

Once taxonomy has been assigned, measures of structural differences between individual's microbiomes can be determined. As reviewed by Lozupone and Knight (217), there are three main considerations for looking at the diversity of microbiomes: 1) the choice of diversity within an individual community (α diversity) and diversity between individuals (β diversity), 2) looking for the presence or absence of a taxon (qualitative) versus both presence and abundance of the taxon (quantitative), and 3) treating the species or OTUs as being equally related to one another (species-based) or allow for different species/OTUs to not be equally related to one another (divergence-based).

Measures of α diversity try to answer the question "Which samples have the most diverse compositions?" (215). This can typically be split into two types of measures: richness, which is the total number of species in a sample, and evenness, which is the abundance of a particular species (217). Some indices combine both measures, such as the Shannon's index (217). Measures

of β diversity try to answer the question "How different are our samples from each other?" (218). These include phylogeny-based measures such as the weighted and unweighted UniFrac and non-phylogeny based such as Bray-Curtis (217, 218).

1.5.2.6 Comparing Intestinal Microbiomes

When thinking of comparing intestinal microbiomes in public health, we are often interested in identifying what microbiomes are "healthy" and which are not. Defining a healthy microbiome, though, is not simple. As reviewed by Bäckhed et al (219), microbiome health can be looked at from an ecological standpoint as community stability (measured as resistance to change under stress or as resilience in returning to a prior state post-stress), from a functional standpoint (as containing a core set of "healthy" genes and pathways), and from a taxonomic standpoint (as the presence or absence of particular taxa). Additional issues come with the actual comparison of population samples to a chosen reference "healthy" microbiome, as microbiomes may vary significantly by geographic location independently of host health and impact the use of that reference for building diseases models (220). Thus, in making a comparison of microbial communities, the choice of a reference microbiome and the definition of "healthy" are important factors to consider and can affect the generalizability of results.

Additionally, comparisons should only be made with references obtained using standardized protocols, as issues affecting comparability can arise at all steps of microbial collection and analysis. These include factors such as sample collection (when and at what sites samples are collected, sampling methods such as swabs versus stool collection, differences in manufacturer kit choice, choice to homogenize samples), sample storage (how samples are stored, length of storage, use of protectants), extraction kit choice and methods, choice of 16S hypervariable region and primer set, sequencing platforms used, pipelines used to process and

analyze data, quality control methods, and method of OTU picking, among others (214). Together, this can impact not only which microbiomes are considered to be "healthy" references, but can also impact the comparisons of your results to those found in other studies.

1.5.2.7 Visualization and Analysis

The richness and evenness of specific taxa can be visualized using bar graphs and phylogenetic trees. β diversity measures can be visualized using principal coordinates analysis (PCoA), which uses a scatterplot of components of the variability in the microbial communities to allow for a cluster-based analysis (218). Heat maps can show relationships between bacterial taxa and metadata, and dendrograms can be overlayed to show the relationships and clusters (218). Co-occurrence analyses and network analyses can help demonstrate relationships between bacteria at structural and functional levels (218).

Structural measures can also be included in statistical models. For example, diversity indices, principal coordinates, and abundance levels can all be used as model variables (218). However, modeling generally requires correction for multiple comparisons given the large number of OTUs in a sample, and transformations or zero-inflated models would be needed if OTUs are rare and only present in a few samples (218).

1.5.3 Factors Influencing the Intestinal Microbiome

The gastrointestinal tract is a continuity of our outer environment, as it is topographically connected to the outside of the body. The bacteria in the intestines have a constant cross-talk with both this external environment brought inward (through ingested materials) as well as with the internal environment (through interactions with host cells and products). It is therefore connected

to many parts of host physiology and the external environment, making the impacts of various factors on gut bacteria complex and numerous. I will focus here on a limited number of modifiable and non-modifiable factors, though countless others are likely to exist.

1.5.3.1 The Microbiome at Birth

The human intestinal microbiome is impacted by factors from the prenatal stage onward. It is not clear if there are bacteria which can inhabit the fetus during development, as low biomass and contamination preclude definitive evidence (221); however, the maternal vaginal microbiome (222, 223) and potentially the intestinal microbiome (224) also experience changes during pregnancy. These changes can help support the health of the mother and fetus by helping to prevent infection and increase energy harvest (221). Additionally, these are the first bacterial species to colonize the newborn in vaginal deliveries (225). Other factors associated with the first major introduction to the microbiome are mode of delivery (cesarean section babies are colonized mostly be skin microbes), breast feeding (which confers additional microbes and prebiotics), and the introduction to solid foods (which leads to increased diversity and increases in adult-associated microbes) (225).

1.5.3.2 Genes versus Environment

The microbiome is predominantly determined by environmental factors, with limited contributions from genetics. A study of twin pairs in the United Kingdom found low heritability, with less than 10% of taxa being heritable and the highest heritability for a taxon was 0.42, an estimate they state is lower than for systolic blood pressure, anxiety, and serum Vitamin D from the same population (226). Still, some taxa were very heritable, such as bacteria from the family Christensenellaceae who had a heritability of 42% (226). A study in an Israeli population with

differing genetic ancestries found that similarities were strongest among individuals who shared households, regardless of genetic relation, and that up to 20% of the variance in between-person bacterial diversity could be determined by looking at environmental factors (227). That there is low genetic influence on most of the microbiome suggests that changes in many lifestyle factors (such as those associated with T2D) are likely to have a greater impact on microbial composition; however, the higher heritability of some microbial taxa also highlights the importance of host genetic makeup.

1.5.3.3 Age and Sex

Both age and sex are non-modifiable risk factors which may be associated with intestinal bacterial populations. A study of US, Malawian, and Amerindian populations found that bacterial diversity increased with age and, together with geography and cultural traditions, explained most of the variation in their data (228). Changes in bacterial populations are also noted between infants and adults and between adults and the elderly, with infants and elders sharing a similarly low *Firmicutes*/*Bacteroidetes* ratio compared to adults (229). These changes were also similarly reflected in a large study of US, UK, Columbian, and Chinese individuals, where a positive association between age and within-individual diversity was observed in non-Chinese individuals, but the association plateaued in middle age (230). This may be due to an increase in factors associated with poor health, or an increasing "biological age" (231). It is possible that these age-related differences are due to differences in lifestyle factors over time, as discussed in sections below.

The microbiome may also differ by sex. Gender was found to be a significant covariate of the microbiome in two European cohorts (232). In one of these cohorts (the LifeLines-DEEP Netherlands cohort), females had a more diverse microbiome both taxonomically and functionally;

however, the contribution of sex to the microbial variation was small (0.5%) and mainly driven by differential medication use (233). Still, studies in mouse models (234) and human clinical populations (235) suggest that sex-specific interactions may impact immune-related diseases, providing a role for the importance of sex in some microbiome/health related contexts.

1.5.3.4 Geographic Location

The microbiome varies by geographical location, with variation in both global gut microbial signatures as well as at the strain level (68). It is unclear how much of geography may be due to other factors, such as dietary and other lifestyle differences between compared areas (200). Additionally, comparisons across populations can be affected by a variety of factors (section **1.5.2.5**), further complicating the understanding of geographical impact on the microbiome. Still, a study in China utilizing standard procedures found that most microbial variation was due to the location within one of 14 districts in the province they studied, with effects being noticeable even when comparing communities within the same neighborhood (220). Thus, exploration of the microbiome in different geographic settings may shed light on important similarities and differences with respect to disease statuses.

1.5.3.5 Race/Ethnicity

An individual's race/ethnicity is also associated with different intestinal microbes. A study in a multi-ethnic Dutch cohort from Amsterdam found that ethnicity was the strongest determinant of within-individual and between-individual microbial diversity, even when accounting for diet, area of residence in Amsterdam, lifestyle factors, or metabolic conditions (69). However, given that most non-Dutch participants in the study were first-generation immigrants, the authors suggest that these microbial differences may also be a factor of the individuals' previous environments and

a lack of microbial adaptation to the new environment (69). Another study combining data from the Human Microbiome Project and the American Gut Project found that self-identified ethnicity had slightly stronger associations than BMI, age, and sex, and that many of the taxa which significantly varied across ethnic groups also have been previously identified as highly-heritable taxa, including Christensenellaceae (70). Gut bacterial functions may also differ by race/ethnicity: one example is the production of soy isoflavone daidzein to equol by intestinal bacteria, a phenotype which was more prevalent in Korean American women and girls compared to Caucasian Americans and was not associated with soy or meat intake (236). Though many of these analyses could be confounded by lifestyle characteristics, geography, and immigration, they together suggest that there may be some impact of race/ethnicity on microbial structure and function.

1.5.3.6 Diet

Diet has a profound impact on the intestinal microbiome makeup. A study in U.S. adults demonstrated that administration of a plant-based diet versus an animal-based diet rapidly (within a few days) altered the microbiome composition and metabolic activity, and that foreign microorganisms were introduced through the differing food intakes (39). These dietary influences may even override other considerations such as the effect of genetics, as different inbred strains of mice showed reproducible differences in microbial changes from dietary intervention (237), and geography, as diet exchanges between Africans and African Americans showed reciprocal changes in microbial function associated with colon cancer risk (38). While these are rapid changes, how long they last is still not well understood. A controlled feeding study found detectable changes in the microbiome after one day of an administered diet, though after 10 days individuals on the same diet still had similar intersubject variations as they did prior to the feeding study (238). This same

study also noted that long-term dietary intake was more closely associated with *enterotypes* (proposed robust signature clusters of dominant bacteria in the gut (216)) than short-term dietary intake (238).

Changes have been noted by overall dietary pattern, nutrient sources, and amount of nutrients themselves, as reviewed by Dong and Gupta (225). Diets can also affect microbial richness at taxonomic or gene levels and is associated with changes in function of the microbiome (239). While individual-level differences are likely to exist, more consistent associations have been seen when comparing agrarian-style diets with western-style diets, where the agrarian-style diets are associated with more *Prevotella* and the western with more *Bacteroides* (239).

1.5.3.7 Physical Activity

Exercise can exhibit multiple different influences on the intestines, including reduced blood flow, changes to vagal tone, and less consistently with changes in intestinal motility, digestion, and absorption (240). Rodent studies suggest physical activity may impact the microbiome, though these are often impacted by differences in rodent breed and differences in methodology (240). Still, human studies do suggest that there may be a link between physical activity level and microbial profiles. A study among older men with objectively measured physical activity found some associations between physical activity and the relative abundance of particular taxa, though level of physical activity was not associated with within-individual diversity (241). Another study in college students found that self-reported high moderate-to-vigorous physical activity was associated with specific bacterial phylotypes and with group-level differences in bacteria, but these group-level differences were only seen when also comparing groups by low/high fiber intake (242).

Studies have also compared the microbiomes of extreme athletes and more sedentary individuals. Structural and functional differences were seen between the microbiomes of rugby players and healthy controls, with athletes having greater diversity and differences in multiple metabolic pathways (243). A recent study in marathon runners identified *Veillonella* genus bacteria and their conversion of systemically-derived lactate into propionate as a mechanism by which the microbiome can affect athletic performance (244). However, differences in microbial structure and function due to physical activity may depend on obesity status and can be reversed with a return to a sedentary lifestyle (245). Thus, the effects of physical activity, similar to diet, may be dependent on the length of participation in the lifestyle habit and differ according to other personal attributes.

1.5.3.8 Medications

Another factor which may influence gut bacterial composition, and potentially complicate associations with disease states, is medication use. A study combining data from Belgian and Dutch microbiome cohorts found a wide variety of medications were associated with microbial composition (antibiotics, antihistamines, antidepressants, hormones, and laxatives, among others), and that these medications accounted for most of the variation in microbiome composition (232). One would reasonably anticipate that antibiotics would have an impact on the intestinal microbiome, but the effects of non-microbial targeted drugs on microbial composition are intriguing. Potential effects could be through different modes of drug action: a study looking at non-steroidal anti-inflammatory drugs (NSAIDs) found that the type of drug, rather than the number of medications used, had a larger effect on diversity (246). For relevance to our Tobago study, metformin use was shown to be associated with compositional changes in the intestinal microbiome, and these changes were associated with some of the beneficial and adverse effects of

metformin use (247). Drugs that target humans may also have off-target effects on bacteria that influence their survival, and similarities in drug structural components may be associated with similar effects on microbial composition (248). These similarities in antibiotic/non-antibiotic drug structure, targets, and bactericidal properties may also contribute to increased antibiotic resistance (248).

1.5.3.9 Infections/Immune System

The intestines house the largest portion of the immune system, and regional differences in immune structures and functions can be found along the length of the intestines (249). Multiple mechanisms allow for the regulation of commensal bacteria and prevention of infection in the intestinal tract, including mucus production, excretion of antimicrobial proteins, and the "sampling" of bacteria by dendritic cells and the coating of commensals with immunoglobulin A (IgA) to prevent penetration of and association with epithelial cells (250). Breaches of the intestinal barrier can also result in innate and adaptive immune responses to eliminate the threatening pathogens (250).

Microbiota can interact with each other and with the immune system to help prevent colonization by pathogenic bacteria (251). Alternatively, some pathogens have adapted to use commensal microbiota as a mechanism to increase infectivity (251). Acute infections are associated with shifts in microbiota (such as increased proteobacteria) that can result in greater inflammation and tissue damage (251). These changes in intestinal bacteria and immune response can also have more distal effects through changes to immune system activity and increases in systemic inflammation, impacting both the regular state of immunity as well as the tone of immune responses (251).

1.5.3.10 Differences along the GI tract

The gastrointestinal tract is extremely varied in its morphology and function, allowing for digestion and maximal absorption of nutrients. However, these differences also affect the types of bacteria which may grow there. Moving along the gastrointestinal tract shows differences in nutrient absorption and availability, pH, oxygen tension, all of which may impact the bacteria which can reside there (252). Further differences can also be seen cross-sectionally, as different bacteria may be located within the lumen or at different points in the mucosa, though these differences are thought to be small in comparison to those along the intestinal tract (252).

Other aspects of morphology also differ as one moves along the intestines. As reviewed in Mowat and Agace (249), differences in the structure of the intestinal wall itself are found along the GI tract. The small intestine is characterized by larger villi, a smaller diameter, and longer length, allowing for maximum nutrient absorption. The large intestine has no villi, is larger in diameter, and houses most of the commensal bacteria. Mucus-producing cells increase along the GI tract and create an outer mucus layer (where bacteria are typically found) and an inner mucus layer.

1.5.4 Links Between the Intestinal Microbiome and Type 2 Diabetes

Given that the intestinal microbiome is impacted by many of the same factors that serve as risk factors for T2D (section **1.5.3**), it is not surprising that associations have been found between gut bacteria and the presence of T2D. A metagenome-wide association study using shotgun metagenomics in a case-control Chinese population found that individuals with T2D could be characterized by moderate gut dysbiosis, decreasing butyrate-producing bacteria, and increasing opportunistic pathogens (44); this suggests that microbial functional differences are seen in T2D.

Another study of shotgun metagenomics sequencing, but in European women, found both compositional and functional differences between individuals with T2D and normal glucose tolerance, though discriminatory gene clusters in this study were different than those identified in the previous Chinese study (45). A Japanese study of T2D patients and controls found structural differences, with T2D individuals having a decrease in obligate anaerobes and an increase in facultative anaerobes in fecal samples, as well as an increase in intestinal Gram-positive bacteria found in the bloodstream (253). While these studies used different populations and methods, collectively they suggest a relationship between the structure and function of the intestinal microbiome and the prevalence of T2D.

The link between intestinal bacteria and T2D may be through modulation of insulin resistance. The colonization of germ-free mice with bacteria from conventionally-raised mice showed an increase in body fat and insulin resistance (254). In addition to the usual caveat that "mice are not humans", another study using different strains of mice found diabetes-related differences in gut microbial composition after a dietary challenge depended on mouse strain, but could be partially repeated with microbial transplants into other mouse strains (255). Still, in a longitudinal study of humans Zhou et al. (256) showed microbes and some microbial-associated metabolites were associated with fasting SSPG and found that intra-individual correlations of microbes differed by insulin resistance status, suggesting that differences in microbes may be associated with insulin resistance in humans as well.

Multiple mechanisms may link the gut microbiota with insulin resistance. A study in Danish individuals found branched-chain amino acids were differentiated between those with insulin resistance and those without, that a functional shift in the microbiome associated with insulin resistance was independent of BMI, and that a few detected species were largely driving

these associations by increasing serum branched-chain amino acids (257). Alternative explanations include gut-derived lipopolysaccharides (LPS) from intestinal Gram-negative bacteria, which can bind to toll-like receptor 4 (TLR4) pro-inflammatory receptors and induce cellular insulin resistance, as well as modulation of bile acid pathways (258, 259). Previously, our group has demonstrated in the Tobago Health Study that baseline serum levels of LPS-binding protein, an acute-phase protein which facilitates the shuttling of LPS to the TLR4 receptor and serves as a surrogate marker of gut bacterial-derived LPS, was associated with increased trunk fat, increased HOMA-IR, and increased odds of incident impaired fasting glucose after 6 years of follow-up (51). Thus, there is some biological plausibility for the mechanistic link between intestinal bacteria and insulin resistance.

1.6 Statistical Analysis of Compositional Data

1.6.1 Compositional Data: Definition and Geometry

Compositional data are any data which sum to an arbitrary whole; thus, components are necessarily positive values which are constrained to somewhere between 0%-100% of the total measured sample. Because components sum to a whole, they have an inherent structural relationship with each other. Essentially, if one component were to increase in size, at least one other component must decrease as a result. This collinearity is not the result of redundancy in measured variables, but rather due to the special sample space in which compositional data lie. This sample space, referred to as a *simplex*, is a hyperplane of the real Euclidean sample space. For a composition made up of D components, the simplex space is made up of D vertices in D-1

dimensions; thus, a composition of three components would be represented by a two-dimensional triangle (**Figure 1.2**).

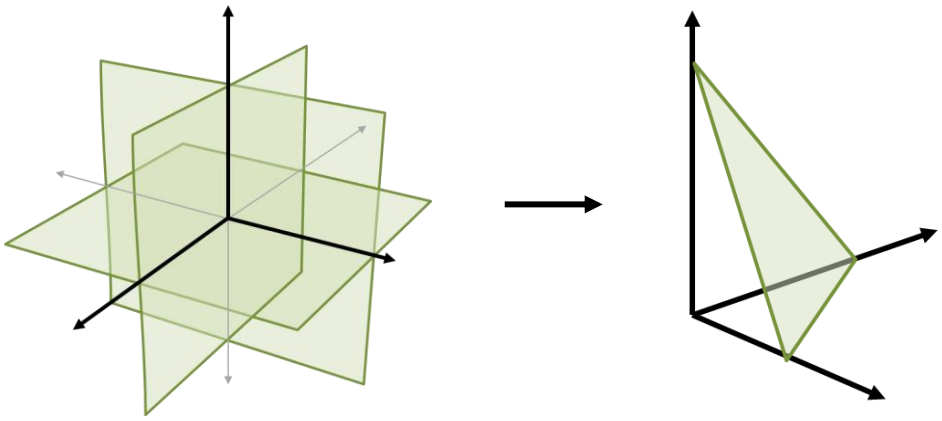

Figure 1.2 Comparison of a 3 dimensional Euclidean space to a 2 dimensional simplex space

Importantly, different sample space geometries require different statistical modeling approaches. Specifically, in compositional data, distances in the hyperplane are not equivalent to distances in the Euclidean space; thus, it is inappropriate to apply traditional Euclidean statistics (such as ordinary least squares regression) to data in the simplex.

1.6.2 Compositional Data Analysis

In logistic regression, outcomes range from 0 to 1 (or 0% to 100%), and in order to apply Euclidean methods to such data, we first must transform the probability data with a logit transformation. Compositional data is also constrained to being positive (0 or greater, and can be thought of ranging from 0% to 100%); similarly, transformations can be applied to compositional data which then allow Euclidean distance-based methods to be applied. Compositional Data Analysis (CoDA) was established by Aitchison (260) who developed the methods for transforming simplexed data to Euclidean sample spaces.

One such method is the additive log ratio (ALR) transformation, where simplexed data is represented as the sum of log ratios of components such that one component is used as the ratio denominator for all remaining components (creating *S-1* terms). If used as predictors, these ratios are interpreted with respect to the referent component, such that for any $\log_n$ term, the coefficient is "for an *n*-fold increase in the ratio of the numerator component to the denominator component". If used as an outcome, these would be interpreted similarly to a multinomial logistic regression. These transformations in CoDA result in three unique properties (261): *scale invariance*, which states that information obtained from the ratios of component parts is not impacted by specimen size; *permutation invariance*, that the ordering of components in a model shouldn't impact results; and *subcompositional coherence*, which states that missing components in a reduced dataset should not impact associations between component ratios present in both reduced and full datasets.

1.6.3 The Microbiome and Body Composition as Compositional Data

The importance of the simplex structure of compositional data is becoming increasingly recognized in the health sciences. There are increasing calls for data such as the high-throughput sequencing output of the microbiome (262), physical activity measures (263), and diet (264) to be viewed through a compositional lens. Body composition data derived from imaging methods should be thought of as compositional data. That measured tissues within a single image must sum to a whole (ex. the total area measured in a single-slice CT image) indicates that the different tissues may be seen as components to be used in a CoDA analysis.

In currently executed body composition analyses, issues of collinearity among body tissues or between body tissues and overall body size are often dealt with by exclusions of some imaged tissues to focus on tissues of primary interest (which may lead to information loss), or the use of

ratios, such as taking the tissue as a percentage of the total scanned area (ex. % fat located in the trunk), or using the ratio of two different tissue measurements (ex. VAT/SAT ratio). While investigating the relative amount of tissues might reduce the collinearity between different tissues or between tissues and a whole, the use of ratios is known to be problematic, resulting in spurious findings (265) and biased estimates (266). Thus, previous analyses which use untransformed measured values of some or all tissues, and analyses which use ratios or percent totals of tissues, are applying Euclidean distance-based methods to simplexed data, which may increase spurious findings. However, no study to date has utilized CoDA methods to look at associations of body composition tissues with health outcomes.

1.7 Summary and Public Health Significance

1.7.1 What is Known

The summary of the connection of risk factors contributing to T2D can be visualized in **Figure 1.3** below. Environmental and lifestyle factors, such as diet, exercise, and smoking, can act on both the intestinal microbiome and directly on particular organs and tissues. The microbiome, in turn, is structurally and functionally altered, resulting in changes in energy harvest, intestinal barrier integrity, and release of pro-inflammatory products. Together these all act to influence changes in body composition and immune activation. This culminates in the development and progression of cardiometabolic diseases such as T2D.

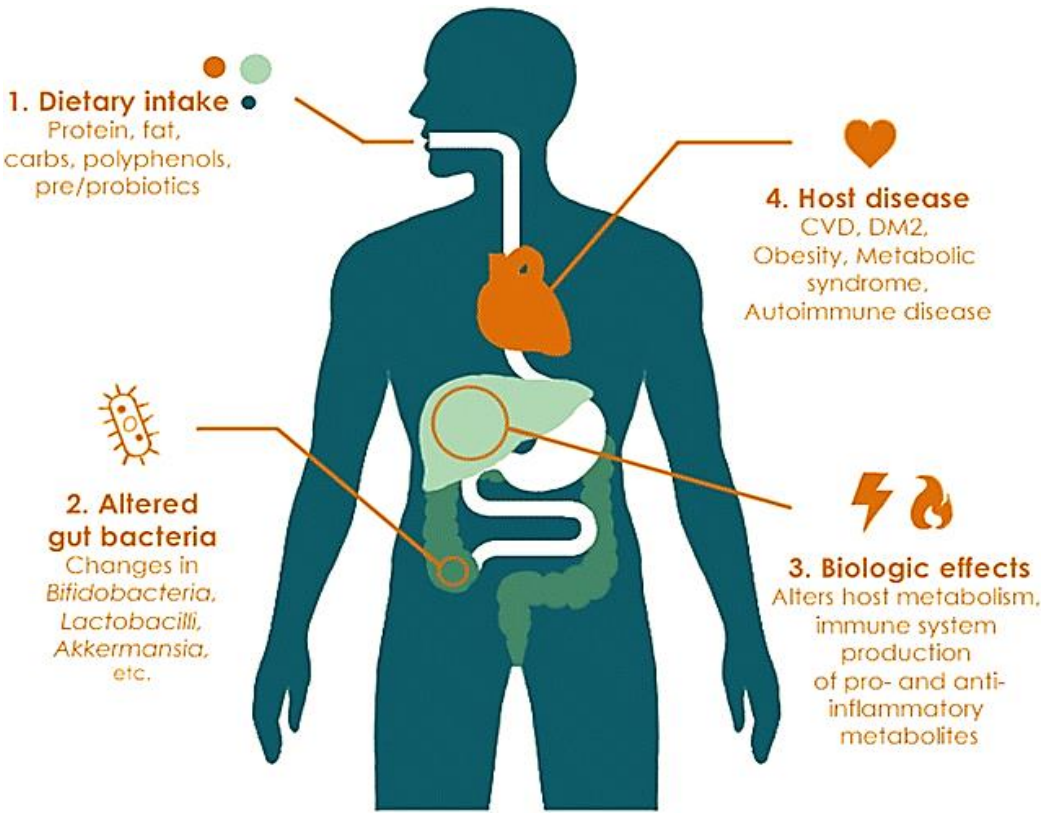

Figure 1.3 Impact of diet on the gut microbiome and human health

Figure from "Influence of diet on the gut microbiome and implications for human health" by Singh et al (267). Creative Common License: (http://creativecommons.org/licenses/by/4.0/)

1.7.2 Knowledge Gaps

Despite the wealth of knowledge in T2D risk factors, the knowledge of mechanisms linking these risk factors to T2D are still lacking. Disentangling the relationships between more proximate risk factors, such as body composition and the microbiome, is needed in order to understand what kind of lifestyle interventions may be most effective in preventing or ameliorating T2D. While body composition is a well-studied risk factor for T2D, there are many questions regarding the use of body composition imaging data. The use of AT radiodensity from CT images shows promise as a novel risk factor for T2D; however, its use has thus far been limited to abdominal AT depots, and it remains unclear whether or not AT radiodensity represents anything more than traditionally-

measured AT volumes. Current body composition analyses also suffer from improper statistical methodology, which may distort associations between metabolically active tissues and T2D, and a lack of inclusion of specific tissue types in different anatomical locations. Even greater unknowns exist in associations of the less-studied intestinal microbiome with T2D, with associations being impacted by a slew of intrinsic and extrinsic factors.

Further, greater representation of racial/ethnic minorities in body composition and microbiome research is needed, as they are disproportionately burdened by T2D and may have different risk factor profiles. This is especially true in Caribbean islands like Tobago, where T2D rates are exceptionally high and where lifestyle characteristics differ from other geographic locations.

1.7.3 Public Health Significance

Obesity and T2D epidemics are heavily burdening Caribbean peoples and health systems. While lifestyle factors such as poor-quality dietary intake are understood to be important risk factors driving these epidemics, information on downstream mechanisms such as adiposopathy and alterations of the microbiome are lacking. This dissertation will allow for the elucidation of the relationships between the microbiome, fat distribution and quality, and biomarkers of T2D in an African Caribbean population. This contribution is significant because it can be utilized to enhance and further develop targeted interventions for T2D. This study will also establish the first data repository for the microbiome in Tobago, aiding in future cardiometabolic research in the Caribbean. This work may help to turn the tide against rising rates of obesity and T2D, both in the Caribbean and worldwide.

1.8 Specific Aims

The goals of this dissertation are to investigate the associations of body composition imaging data (radiodensity and distribution) and the intestinal microbiome with T2D. My focus is on individuals of African Caribbean descent, as African Ancestry individuals are underrepresented in both body composition and microbiome studies. To address these goals, I will be utilizing cross-sectional data from the Tobago Health Study, a cohort of middle-aged and older men who predominantly identify as African Caribbean. At the most recent study visit (2014-2018), fasting glucose and medication use were ascertained to help identify individuals with T2D. CT was used to collect body composition measures in the abdomen and thigh, allowing for assessment of tissue radiodensities as well as tissue distributions. Using this data, we can investigate associations of adipose tissue radiodensity and upper and lower body compositions with T2D in African Caribbean men. Additionally, a subset of study participants was called back to participate in a pilot study for fecal sample collection and microbiome assessment; thus, we can also explore associations of the intestinal microbiome with T2D.

Paper 1: Associations of Thigh and Abdominal Adipose Tissue Radiodensity with Glucose and Insulin in Nondiabetic African-Ancestry Men

Aims: To determine the associations of abdominal (SAT and VAT) and peripheral (IMAT) adipose tissue radiodensities with glucose, insulin, and insulin resistance in African Caribbean men in Tobago

Hypothesis: Less radiodense adipose tissue in any depot will be associated with worse glucose, insulin, and insulin resistance measures

Paper 2: Associations of Upper and Lower Body Composition with Type 2 Diabetes in African Caribbean Men

Aims: To determine differential associations of adipose and muscle tissues in the abdomen and thigh with type 2 diabetes using a compositional data analytic approach

Hypothesis: Upper body VAT and SAT, and lower body IMAT, will be positively associated with prevalence of type 2 diabetes; lower body SAT and muscle will be inversely associated with type 2 diabetes

Paper 3: Associations of the Intestinal Microbiome with Type 2 Diabetes in African Caribbean Men

Aims: To perform exploratory analyses investigating different levels of intestinal microbial structure (cluster analysis, diversity metrics, and operational taxonomic units) with type 2 diabetes

2.0 Manuscript 1: Adipose Tissue Radiodensity with Serum Glucose and Insulin

Title: Associations of Thigh and Abdominal Adipose Tissue Radiodensity with Glucose and Insulin in Nondiabetic African-Ancestry Men

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2.1 Abstract

Objective: Decreased radiodensity of adipose tissue (AT) located in the visceral AT (VAT), subcutaneous AT (SAT), and intermuscular AT (IMAT) abdominal depots is associated with hyperglycemia, hyperinsulinemia, and insulin resistance independent of AT volumes. These

associations were sought in African-ancestry men, who have higher risk for type 2 diabetes and have been underrepresented in previous studies.

Methods: This cross-sectional analysis included 505 nondiabetic men of African-Caribbean ancestry (median age: 61 years; median BMI: 26.8 kg/m2) from the Tobago Health Study. AT volumes and radiodensities were assessed using computed tomography, including abdominal (VAT and SAT) and thigh (IMAT) depots. Associations between AT radiodensities were assessed with fasting serum glucose and insulin and with insulin resistance (updated homeostatic model assessment of insulin resistance, HOMA2-IR).

Results: Higher radiodensity in any AT depot was associated with lower log-insulin and log-HOMA2-IR (β range: -0.16 to -0.18 for each; all $P < 0.0001$). No AT radiodensity was associated with glucose. Thigh IMAT radiodensity associations were independent of, and similar in magnitude to, VAT radiodensities. Model fit statistics suggested that AT radiodensities were a better predictor for insulin and insulin resistance compared with AT volumes in individuals with overweight and obesity.

Conclusions: AT radiodensities at multiple depots are significantly associated with insulin and insulin resistance in African-ancestry men.

2.2 Study Importance Questions

What is already known?

- Abdominal adipose tissue radiodensity (visceral, subcutaneous, and intermuscular) is inversely associated with glucose and insulin levels, with most studies conducted in predominantly Caucasian cohorts.

What does this study add?

- Adipose tissue radiodensities, including thigh intermuscular adipose tissue, are inversely associated with fasting insulin and insulin resistance in African-ancestry individuals.
- Adipose tissue radiodensities may be more informative predictors of insulin resistance than adipose tissue volumes.

2.3 Introduction

Despite the fact that obesity is a major driver of type 2 diabetes (T2D), T2D also depends on the distribution of adipose tissue (AT) throughout the body, especially the amount of fat around and within non-AT organs (known as ectopic AT) (268). The size of non-ectopic AT depots such as the subcutaneous AT (SAT) and visceral AT (VAT) depots are associated with worse glucose and insulin levels, with the VAT depot being more strongly associated than SAT (269-271). Additionally, ectopic AT depots such as abdominal and thigh intermuscular AT (IMAT) volume are also associated with impaired glucose and insulin levels (270, 272, 273).

In addition to AT distribution, novel surrogate markers of AT biology, such as computed tomography (CT)-derived average AT radiodensity, may indicate more pathogenic AT. Biopsy studies performed in rodents and small human trials suggest that AT radiodensity may capture other AT attributes such as cell size (17), lipid content (18), and vascularity (19). Indeed, AT radiodensity is emerging as a marker of increased risk for cardiometabolic disease independent of tissue volume (22-26). Several reports using the Framingham cohort observed lower abdominal VAT and SAT radiodensity associated with worse cardiometabolic profiles, including higher

HOMA-IR (24), increased odds of impaired fasting glucose (23, 24), and increased glucose concentrations (22-24). Studies from the Multi Ethnic Study of Atherosclerosis (MESA) have also reported that individuals with lower vs. higher abdominal VAT, SAT, and IMAT radiodensity had greater levels of glucose and diabetes (25, 26).

These studies were performed in predominantly Caucasian cohorts or were not stratified by race; thus, there remains a paucity of data on associations of AT radiodensity with biomarkers of T2D risk in non-Caucasian racial/ethnic groups. This is an important area of inquiry, as African Ancestry individuals, who are at a higher risk of T2D independent of overall adiposity (53-55), also exhibit different ectopic AT distributions compared to their Caucasian counterparts, such as having lower VAT (56-59), higher abdominal SAT (56, 58, 59), and higher total IMAT (61). Additionally, data on non-abdominal IMAT radiodensity, such as in the thigh, is very sparse. Skeletal muscle, which is insulin sensitive, is found in larger quantities in the thigh compared to the abdomen (274). The location of IMAT next to skeletal muscle suggests a role for IMAT in insulin resistance; indeed, IMAT-secreted factors were shown to reduce insulin sensitivity in myotubes in vitro (181), and thigh IMAT volume is positively associated with both insulin resistance and risk of T2D independent of overall obesity (62, 147, 270). There are also racial/ethnic differences in regional IMAT distribution, with studies reporting greater thigh and calf IMAT in African Ancestry individuals compared to Caucasians (60, 62, 275) as opposed to similar (275, 276) or lower (277) levels of abdominal IMAT.

Thus, our primary objective was to determine if lower AT radiodensity, including thigh IMAT radiodensity, was associated with higher fasting serum levels of glucose and insulin, as well as insulin resistance, in middle-aged and older African Ancestry men without T2D. We hypothesized that less-dense AT would be associated with worse glucose and insulin levels.

2.4 Methods

2.4.1 Study Population

All men in this analysis were from the Tobago Health Study, which has been previously described (161). Briefly, the Tobago Health Study is a population-based, prospective cohort study of community-dwelling men aged 40 years and older, residing on the Caribbean island of Tobago, Trinidad and Tobago. Men from Tobago are of homogeneous African ancestry with low European admixture (<6%) (278). Participants in the Tobago Health Study were recruited without regard to health status and men were eligible if they were ambulatory, not terminally ill, and without a bilateral hip replacement. The baseline visit occurred from 2004-2007 and recruited 2,482 men; of these, a random subset (N=1,725) attended the first follow-up visit from 2010-2014. Men used in the current analysis attended an ancillary study visit from 2014-2018, when a convenience sub-sample of N=768 participants from the prior visit had computed tomography (CT) scans of the chest, abdomen, and mid-thigh for ectopic AT assessment. Exclusion from the current analysis included missing CT scans in the abdomen or in one or both thighs (N=33), having T2D (N=174), missing covariate data (N=12), non-African Caribbean ethnicity by self-report (N=43), and non-fasting serum samples (N=1). Individuals with T2D were excluded to better reflect potential associations of AT radiodensity on glucose and insulin levels without confounding effects of the later disease process. The final analytical sample included 505 individuals. Written informed consent was obtained from each participant using forms and procedures approved by the University of Pittsburgh Institutional Review Board, the U.S. Surgeon General's Human Use Review Board, and the Tobago Division of Health and Social Services Institutional Review Board. This study was completed in accordance with the Declaration of Helsinki.

2.4.2 Computed Tomography Scans

Abdominal and thigh volumes and radiodensities were assessed on 3 mm thick slices and 500 mm display field of view from scans acquired using a GE dual slice, high-speed NX/I CT scanner (GE Medical Systems, Waukesha, WI) with 120 KVp, 250 mA, 0.7 second gantry speed, and pitch of 1.5:1. For participants with body weight greater than 200 lbs, the mA was increased to 300. CT contrast was not used. Only one CT scanner was used, and a single individual collected the scans for all participants. Scans were electronically transmitted to the central CT reading center at Vanderbilt University where image analysis and quality control were performed.

Image analysis was performed using methods as previously described (276, 279, 280). Briefly, images were analyzed using a dedicated imaging processing workstation with custom-programmed subroutines (OsiriX, Pixmeo, Geneva, Switzerland) and a dedicated pen computing display (Cintiq, Wacom Technology Corporation, Vancouver, WA, USA). A radiologist-trained analyst manually traced anatomical boundaries (skin, muscular fascia, muscle, and peritoneum) in CT scans. Tissue attenuation thresholds of −190 to −30 Hounsfield Units (HU) were used to distinguish AT voxels in these defined regions. For each tissue, the volume (mm^3) and mean tissue attenuation (referred to here as AT radiodensity, in HU) were calculated.

Abdominal VAT and SAT were measured from CT scans of 3 contiguous slices of 3mm thickness centered at L4-L5. A lateral scout image was used to determine the z-axis location of the L4-L5 intervertebral space and that location and the slice immediately above and the slice immediately below were used to reconstruct a 9-mm thick single block of images. VAT was defined as AT located within the peritoneal cavity; SAT was defined as AT located beneath the skin and superficial to the abdominal muscular fascia.

Thigh IMAT was measured from CT scans of 10 contiguous slices of 3mm thickness at the mid-thigh level in both legs. An anterior-posterior scout scan of the entire femur was used to localize the mid-thigh position, and that location and the four slices immediately above and five slices immediately below were used to reconstruct a 30-mm thick single block of images. IMAT was defined as AT located within thigh muscle groups. IMAT volume was defined as the total IMAT summed across both thighs, and IMAT radiodensity was the average AT attenuation across both thighs.

Intrareader technical error (TE) in re-analysis of a 5% oversampling of blinded scans was 0.6% for total abdominal volume, 1.4% for SAT volume, and 4.8% for VAT volume whereas TE for abdominal radiodensities were 0.7% for SAT and 0.5% for VAT. For thigh measures, TE was 1.5% for total thigh volume, 2.7% for IMAT volume, and 1.0% for IMAT radiodensity.

2.4.3 Anthropometric Measurements

Standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight was recorded to the nearest 0.1 kg without shoes on a balance beam scale. BMI was calculated from body weight and standing height (kg/m^2). BMI categories were defined as normal weight ($18.5 \leq BMI < 25$), overweight ($25 \leq BMI < 30$), and obese ($BMI \geq 30$). Waist circumference was measured at the narrowest point of the waist using an inelastic tape. If there was no narrowest point, waist circumference was measured at the umbilicus.

2.4.4 Glucose and Insulin Measures

Fasting serum glucose and insulin measures were measured at the Advanced Research and Diagnostics Laboratory (ARDL), University of Minnesota. Fasting serum glucose was measured using an enzymatic procedure (interassay CV: 1.3-1.8%) and fasting serum insulin was measured using a sandwich immunoassay procedure (interassay CV: 3.1%) (assays manufacturer: Roche Diagnostics, Indianapolis, IN). Glucose was assessed in mg/dL units that were converted to SI units (mmol/L) by dividing by 18. Insulin was assessed in pmol/L units. The degree of insulin resistance was estimated by homeostasis model assessment (HOMA2-IR) and calculated using fasting glucose and insulin values and the HOMA2 Calculator v2.2.3 available from http://www.dtu.ox.ac.uk/homacalculator/ (281).

2.4.5 Other Measures

Information on lifestyle habits (current smoking [yes/no], number of hours walked per week, watching 14 or more hours of television per week [yes/no], current intake of alcohol of more than 4 drinks per week [yes/no]), ethnic self-identification (dichotomized as African-Caribbean Ethnicity [yes/no]), and medication use were assessed using standardized interviewer-administered questionnaires. Self-reported information on walking was recorded as walking is the predominant form of physical activity on the island of Tobago. Men were asked to bring all prescription medications taken in the past 30 days to their clinic visit. T2D was defined as currently taking an antidiabetic medication, regardless of fasting serum glucose level, or having a fasting serum glucose level of ≥7 mmol/l; all men with T2D were excluded from the current analyses.

2.4.6 Statistical Analyses

Men were stratified by median AT radiodensity, and comparisons of their baseline characteristics were made using two-sample t-test or Wilcoxon rank-sum test for continuous variables and Chi-square tests for categorical variables. Age-adjusted partial Spearman correlations were performed between AT radiodensities and outcomes/covariates.

Multiple linear regression models were performed separately for each outcome (glucose, insulin, HOMA2-IR), and separately for each predictor of interest (VAT radiodensity, SAT radiodensity, IMAT radiodensity). Insulin and HOMA2-IR were log-transformed to make residuals normal. Model covariates included age, weight, height, hours walked per week for exercise, watching 14+ hours of television per week, current smoking, and drinking 4+ alcoholic drinks per week. Identification of significant curvilinear relationships between AT volume and radiodensity (**Figure 2.1**) suggested that mutual adjustment for these variables would be inappropriate; thus, models including both AT volume and radiodensity were not included in our main analyses. A series of three models were run sequentially:

Model 1: model covariates + a specific AT radiodensity

Model 2: model covariates + VAT and SAT radiodensities

Model 3: model covariates + VAT and IMAT radiodensity

Multicollinearity was assessed using condition indices and was not found to be an issue in any of these models; however, multicollinearity was an issue for models including both SAT and IMAT radiodensities, so models which included simultaneous addition of both of these depots were not reported, but can be found in Supplementary Materials (**Appendix B Supplementary Tables, p.157**). Sensitivity analyses for regression models included further adjustment for respective total thigh and abdominal scan volumes (obtained from CT images), adjustment for respective AT depot

volume, and adjustment for respective AT depot volume after stratification by BMI category. In the BMI-stratified analyses, four individuals were excluded for having a BMI below 18.5 kg/m^2.

Changes in model fit statistics (Akaike Information Criteria [AIC] and Bayesian Information Criteria [BIC]) were assessed when all three AT radiodensity or all three AT volumes were added to covariate-adjusted models; though collinearity can affect the estimates of individual predictors, overall model fit is not affected, allowing simultaneous inclusion of multiple AT depots. A change in fit $\leq$ -2 for either AIC or BIC was regarded as representing greater model fit. Interactions between radiodensity and age were assessed.

Statistical significance was based on an $\alpha = 0.05$, and analyses were performed using SAS 9.4 software (SAS Institute, Inc., Cary, NC). Graphical 3-Dimensional Scatterplot was generated using the package scatterplot3d (282) in R version 3.5.2 (283).

2.5 Results

2.5.1 General Baseline Characteristics

Men had a median age of 61 years and a median BMI of 26.8 kg/m^2 (**Table 2.1**). There were low rates of reported physical activity and high reported sedentary behavior, but other lifestyle factors of current smoking and alcohol intake were relatively low. We next stratified our sample characteristics by median AT radiodensities (**Supplementary Table Appendix B Table 1**). Overall, individuals with AT radiodensities above the median in either the VAT, SAT, or IMAT depots had significantly lower anthropometric measures (weight, BMI, and waist circumference), lower AT depot volumes, and higher AT radiodensities in any depot (all $p < 0.0001$).

2.5.2 Association of AT Depot Attenuation with Anthropometric Measures and Levels of Glucose and Insulin

Table 2.2 shows interrelationships of AT radiodensities with anthropometric, CT-based, and glucose/insulin variables. In general, AT radiodensities were strongly positively correlated with each other and strongly negatively correlated with anthropometry and AT volume measures, with stronger associations seen between VAT and SAT radiodensity (all $p < 0.0001$). AT radiodensity in any depot was strongly negatively correlated with insulin levels and HOMA2-IR ($p < 0.0001$), while only VAT and SAT radiodensity were significantly (though weakly) associated with glucose.

The relationship between each AT depot's radiodensity and corresponding volume was explored visually using a scatterplot (**Figure 2.1**). Relationships followed an inverse association, where tissues at higher radiodensities tended to be of lower volume while tissues at lower radiodensities tended to have much higher volumes. Though the IMAT depot followed a similar curvilinear relationship as the VAT and SAT depots, it increased in volume at a much higher radiodensity as compared to the other depots, and did not reach the levels of low radiodensity that the other depots did.

The results from multiple linear regression analyses are shown in **Table 2.3**. Multiple linear regression model building indicated high collinearity between SAT and IMAT radiodensities; when regressing on one another, the model R^2 for SAT and IMAT radiodensities was ~0.70. Given these strong interrelationships, reported regression models did not include additional adjustment for AT volume, nor did they include mutual adjustment for SAT and IMAT radiodensities (though sensitivity analyses including these adjustments can be found in **Supplementary Tables Appendix B Table 2-4**).

After adjustment for age, weight, height, alcohol intake, smoking, hours walked per week, and television watching, higher radiodensity in any tissue was associated with significantly lower serum insulin and lower HOMA2-IR (all $p < 0.0001$). SAT and IMAT radiodensity associations persisted even after VAT radiodensity adjustment. To provide context using HOMA2-IR results, the smallest reported effect size of $\beta=-0.10$ (for VAT radiodensity, after adjustment for SAT radiodensity) indicates a 9.5% lower HOMA2-IR for every 1 SD increase in VAT radiodensity; in contrast, the largest effect size of $\beta=-0.18$ (for SAT adjustment alone) indicates a 16.5% lower HOMA2-IR for every 1 SD increase in SAT radiodensity. Notably, no AT radiodensity was statistically significantly associated with glucose, though there was a tendency towards lower glucose at higher AT radiodensities. Results remained similar in a number of sensitivity analyses including adjustment for all 3 AT radiodensities, adjustment for respective AT volumes, and adjustment for total abdominal and thigh CT scan volume (**Supplementary Tables Appendix B Table 2-4**).

We additionally explored the potential interactions of AT radiodensity-by-age in the main analysis models. No significant AT radiodensity-by-age interactions were identified for any outcome.

2.5.3 Model Fit Statistics

Changes in model fit statistics (AIC and BIC) were evaluated for the linear regression models (**Table 2.4**), where covariate-adjusted models were further adjusted for either all three AT radiodensities or all three AT volumes. For linear regression models, the simultaneous addition of VAT, SAT, and IMAT radiodensities to log-insulin and log-HOMA2-IR models greatly improved model fit by both AIC and BIC criteria. Compared to volume-alone or volume- and radiodensity-

adjusted models, the radiodensity-alone models accounted for a greater increase in model fit. Glucose models had worsening model fit by inclusion of AT radiodensities and/or volumes. Changes in model AIC and BIC for individual AT radiodensities and volumes remained similar to simultaneous AT inclusion (Supplementary Table **Appendix B Table 5**).

2.5.4 Models Stratified by BMI-Category

To further disentangle the relationship between AT volume, radiodensity, and T2D risk factors, we investigated the consistency of these relationships within each by BMI category. Models adjusted for covariates age, hours walking for exercise, TV watching, smoking, drinking, and a specific AT depot's volume and radiodensity together. The regression coefficients for each depot's radiodensity and volume are plotted in **Supplementary Figures Appendix B Figure 1-3**. Briefly, an overall trend was observed in which, after adjustment for AT volume, coefficients for AT radiodensity were significantly and inversely associated with log-insulin and log-HOMA2-IR in individuals with overweight and obesity, but not in individuals with normal weight status. Conversely, AT volumes were significantly and positively associated with log-insulin and log-HOMA2-IR in individuals with normal weight status, but not in individuals with overweight or obesity, after adjustment for AT radiodensity. Only lower VAT radiodensity and higher SAT volume were significantly associated with small increases in glucose, and only in individuals with normal weight status. These results were supported by assessing changes in model fit statistics (**Supplementary Table Appendix B Table 6**).

2.6 Discussion

In this population study of non-diabetic middle-aged and older African ancestry men, we identified relationships between higher AT radiodensity and better levels of insulin and insulin resistance. These relationships were overall similar but not entirely consistent with those seen in predominantly Caucasian or race -adjusted cohorts. Our study included the novel addition of thigh IMAT radiodensity, an AT depot in peripheral skeletal muscle, which showed associations that were independent of and as strong as VAT radiodensities. Importantly, insulin and HOMA2-IR model fits were largely improved with the addition of AT radiodensities, demonstrating that AT radiodensity may be most closely linked to insulin compared to glucose levels in these men.

AT radiodensity is a CT-derived measure indicating the tissue's opacity to X-rays. As such, it is not a direct measure of AT biology, which can only be assessed through invasive techniques such as biopsies. Nonetheless, studies indicate that AT radiodensity can reflect structural aspects of AT (17-19) and is associated with metabolic health (22-26). Importantly, there is a lack of information on AT radiodensity in African Ancestry populations, who are at higher risk for development of T2D independent of overall obesity (53-55). This is an important knowledge gap, as there are known racial/ethnic differences in AT distribution which may impact T2D risk, and suggestions of racial/ethnic differences in AT radiodensity as well (25). While cohort studies of Framingham and MESA have laid the groundwork for the associations of AT radiodensity with cardiometabolic health, further studies of these associations in African Ancestry individuals, specifically, and focusing additionally on relevant non-abdominal AT depots are needed to demonstrate consistency of findings in this high-risk racial/ethnic group.

While explorations of AT radiodensity and diabetes-related biomarkers are relatively novel, we are the first study to report on thigh IMAT radiodensity. MESA found that levels of

glucose and prevalence of diabetes increased across decreasing quartiles of abdominal IMAT radiodensity (independent of age, gender, and race/ethnicity) (26), indicating that IMAT radiodensity may play a role in diabetes risk. In contrast, our study found no associations between lower thigh IMAT radiodensity and fasting glucose, but did find significant associations with higher insulin and insulin resistance, even after adjustment for VAT radiodensity. It is important to note that regional IMAT accumulation may differentially impact the relationship between IMAT and metabolic health (284). Comparisons of regional distribution of IMAT are not well studied; however, results from Ruan et al (275) suggest that the thigh may have a greater amount of IMAT compared to the waist. Additionally, studies suggest that IMAT in African Ancestry individuals tends to be found in larger amounts in the thigh (60, 275) but not in the abdomen (275-277) when compared to Caucasian individuals. These differences in IMAT distribution and their metabolic consequences suggest a need for greater understanding of non-abdominal IMAT radiodensity and T2D risk factors.

Another interesting finding regarding IMAT radiodensity was its high collinearity with SAT, but not VAT, radiodensity. IMAT volume increases with increasing total AT, and racial/ethnic differences suggest that African American individuals have a greater IMAT increase at higher levels of adiposity compared to Asian and Caucasian individuals (61). We and others have demonstrated that while IMAT increases with aging, the rate of IMAT accumulation depends on weight gain status, with greater accumulation in weight-gainers and lesser accumulation in weight-losers (272, 285); this suggests that IMAT accumulation is influenced by overall adiposity, a large proportion of which is comprised of SAT. The origins of IMAT adipocytes is hypothesized to be derived from muscle-based mesenchymal progenitor cells (286); however, a recent study in mice demonstrated the ability of SAT-derived adipocyte progenitors to be released from SAT and

take up residence in muscle as IMAT in response to nutrient overload (287). People with obesity have a five times higher level of circulating progenitor cells (288). We report higher IMAT volumes at relatively higher radiodensities compared to SAT, perhaps indicating an increase in cell number rather than cellular hypertrophy as the driving force behind increased IMAT volume. Further research into human IMAT cellularity and origins is warranted.

Our findings of associations between abdominal AT radiodensities and glucose and insulin were similar, but not entirely consistent, with cross-sectional reports in men from the Framingham study (24). Notably, we reported stronger associations between SAT radiodensity than Framingham after adjustment for weight and height; additionally, our associations with glucose were not statistically significant and were markedly smaller than those reported in Framingham. These differences may be due to systematic differences between measurement collections, as Framingham scanned a much larger abdominal area; or they may also be attributable to analyzed population differences, given the overall differences between the cohorts (Caucasian vs. African Ancestry, ~10 year average older age in our study) and our exclusion of individuals with T2D.

In addition to examining the relationship between AT radiodensities and diabetes-related biomarkers, we were also able to determine the relative importance of AT radiodensity compared to AT volume, measures which are both derived from the same CT scan and reflect different aspects of tissue structure. We found that any specific AT depot's volume and radiodensity was strongly and inversely associated and followed a curvilinear pattern, similar to previous reports (25, 289). Given the strength of the curvilinear relationships in our study sample, we felt that mutual adjustment for AT radiodensity and volume would not be appropriate. However, to get at the relative importance of AT radiodensity compared to volume, we compared changes in model fit statistics. We report consistent and large improvements of model fit with the addition of AT

radiodensities to our models of insulin and HOMA2-IR. Importantly, these improvements in model fit were remarkably larger than those seen in models using AT volumes, and they weren't substantially improved by simultaneous addition of AT radiodensities and volumes. However, stratification by BMI category revealed a pattern whereby AT volume was a more significant and informative marker in individuals with normal weight but not in individuals with overweight or obesity, and AT radiodensity exhibited the opposite pattern. That the associations with radiodensity were present only in individuals with overweight and obesity after volume adjustment may indicate dysfunctional AT growth in these individuals.

Our study has several potential limitations. Given our use of an all-male cohort, we were unable to examine associations in women. Another limitation is the use of self-reported physical activity, which may not be the most accurate assessment of physical activity (290). However, our study also has several strengths. The use of multi-slice CT at multiple anatomical locations allowed us to obtain and compare volumetric and radiodensity data across multiple AT depots. Additionally, the use of an African-ancestry cohort provides information on an understudied and high-risk ethnic group.

In conclusion, higher AT radiodensities are significantly associated with lower insulin and HOMA2-IR levels in a cohort of African-ancestry men. These associations are independent of adjustment of other depot radiodensities. Our novel thigh IMAT findings highlight the importance of this depot's radiodensity in diabetes risk. Future studies investigating changes in IMAT radiodensity with insulin metabolism and mechanistic studies of AT radiodensity are warranted.

2.7 Tables and Figures

Table 2.1 Characteristics of African-Caribbean men (N=505)

Variable	Median (IQR), Mean (SD), or N (%)
Age (years)	61.0 (56.0, 68.0)
Weight (kg)	82.5 (73.4, 93.1)
Height (cm)	175.6 (6.7)
BMI (kg/m^2)	26.8 (24.2, 29.9)
Underweight [BMI < 18.5] (%)	4 (0.8%)
Normal Weight [18.5 ≤ BMI < 25] (%)	159 (31.5%)
Overweight [25 ≤ BMI < 30] (%)	219 (43.4%)
Obese [BMI ≥ 30] (%)	123 (24.4%)
Waist Circumference (cm)	95.9 (89.0, 104.0)
Lifestyle and Comorbidities	
Current Smoker (%)	36 (7.1%)
Drinks Alcohol 4+/week (%)	67 (13.3%)
Hours Walked per Week	2.0 (0.0, 5.0)
Watches television ≥ 14 hours/week (%)	242 (47.9%)
CT-Derived Measures	
VAT Volume (cm^3)	78.3 (49.0, 118.0)
SAT Volume (cm^3)	174.4 (118.6, 234.1)
IMAT Volume (cm^3)	108.0 (84.4, 140.5)
VAT Radiodensity (HU)	-89.6 (-94.7, -82.1)
SAT Radiodensity (HU)	-99.7 (-103.3, -94.0)
IMAT Radiodensity (HU)	-70.9 (-73.3, -68.0)
Total Abdominal Volume (cm^3)	563.3 (465.1, 668.0)
Total Thigh Volume (cm^3)	1625.3 (1396.4, 1849.4)
Glucose and Insulin Metabolism	
Glucose (mmol/L)	4.8 (4.4, 5.2)
Insulin (pmol/L)	52.0 (34.0, 81.0)
HOMA2-IR	1.0 (0.6, 1.5)

Results reported as Median (IQR) or Mean (SD) for continuous variables and N (%) for categorical.
Abbreviations: VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue

Table 2.2 Interrelationship of all adiposity and metabolic measures used in our analyses (age-adjusted Spearman correlations)

Variable	VAT Radiodensity	SAT Radiodensity	IMAT Radiodensity
Weight (kg)	-0.57‡	-0.60‡	-0.39‡
BMI (kg/m^2)	-0.62‡	-0.68‡	-0.44‡
Waist Circumference (cm)	-0.64‡	-0.72‡	-0.47‡
VAT Volume (cm^3)	-0.82‡	-0.70‡	-0.47‡
SAT Volume (cm^3)	-0.64‡	-0.84‡	-0.57‡
IMAT Volume (cm^3)	-0.52‡	-0.61‡	-0.57‡
Total Abdominal Scan Volume (cm^3)	-0.67‡	-0.74‡	-0.48‡
Total Thigh Scan Volume (cm^3)	-0.55‡	-0.67‡	-0.49‡
VAT Radiodensity (HU)	--	0.74‡	0.48‡
SAT Radiodensity (HU)	0.74‡	--	0.66‡
IMAT Radiodensity (HU)	0.48‡	0.66‡	--
Glucose (mmol/L)	-0.14†	-0.13†	-0.08
Insulin (pmol/L)	-0.58‡	-0.63‡	-0.47‡
HOMA2-IR	-0.58‡	-0.63‡	-0.47‡

†= <0.05, ‡= <0.0001

Abbreviations: VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue

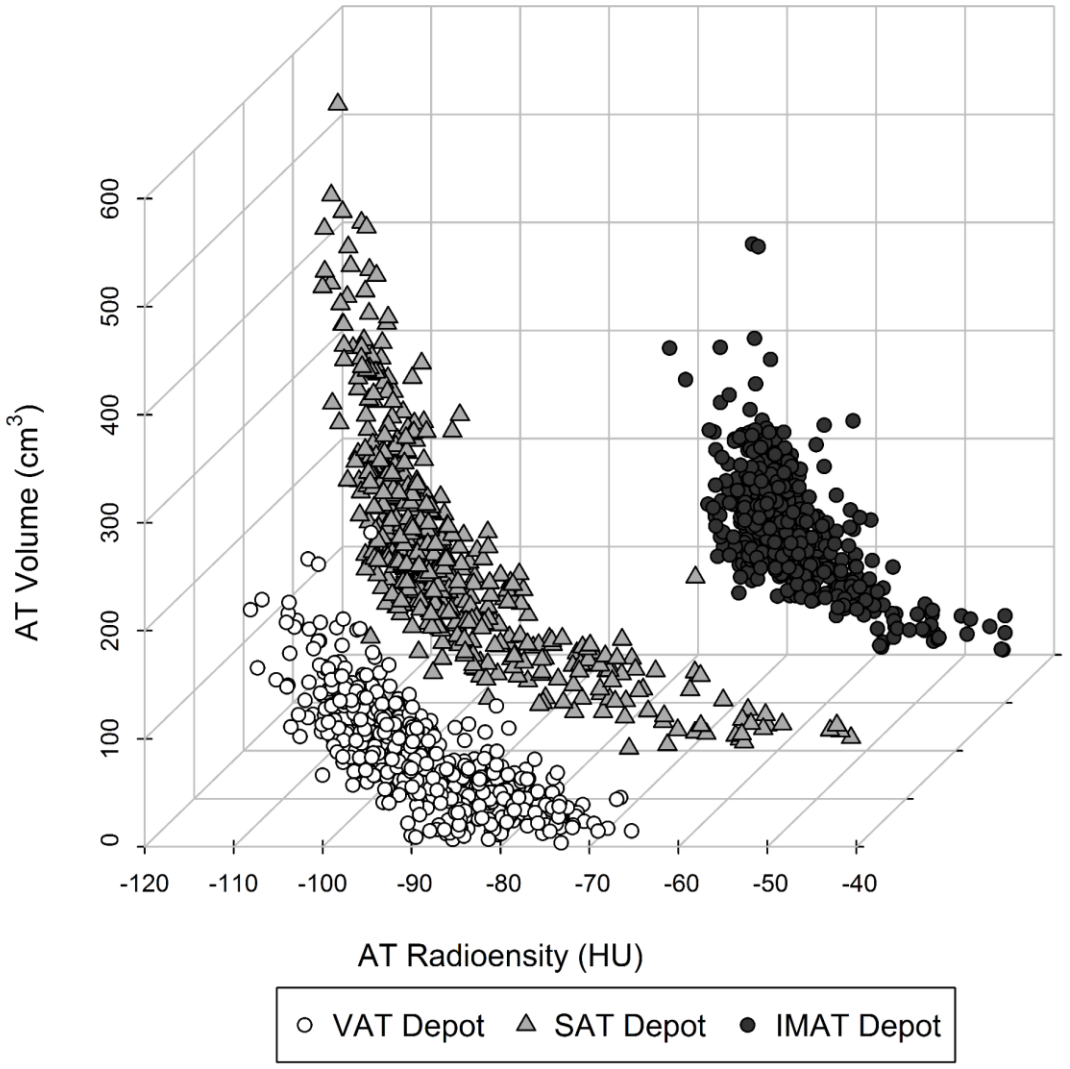

Figure 2.1 Scatterplot of AT radiodensity by volume, per tissue depot

Abbreviations: AT = Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue

Table 2.3 Difference in fasting glucose and insulin levels and HOMA2-IR per 1 SD (95% CI) increase in AT radiodensity

Outcome	Model	VAT Radiodensity (SD = 8.44 HU)	SAT Radiodensity (SD = 10.71 HU)	IMAT Radiodensity (SD = 5.70 HU)
Glucose (mmol/L)	M + Depot	-0.04 (-0.11, 0.03)	-0.05 (-0.12, 0.02)	-0.01 (-0.07, 0.05)
	M + VAT + SAT	-0.03 (-0.10, 0.05)	-0.03 (-0.11, 0.04)	--
	M + VAT + IMAT	-0.04 (-0.11, 0.03)	--	-0.00 (-0.07, 0.07)
Log Insulin (pmol/L)	M + Depot	-0.16 (-0.21, -0.11) ‡	-0.18 (-0.24, -0.13) ‡	-0.16 (-0.21, -0.11) ‡
	M + VAT + SAT	-0.10 (-0.16, -0.05) †	-0.14 (-0.20, -0.08) ‡	--
	M + VAT + IMAT	-0.12 (-0.17, -0.07) ‡	--	-0.13 (-0.18, -0.08) ‡
Log HOMA2-IR	M + Depot	-0.16 (-0.21, -0.11) ‡	-0.18 (-0.24, -0.13) ‡	-0.16 (-0.20, -0.11) ‡
	M + VAT + SAT	-0.10 (-0.16, -0.05) †	-0.14 (-0.20, -0.08) ‡	--
	M + VAT + IMAT	-0.12 (-0.17, -0.07) ‡	--	-0.13 (-0.18, -0.08) ‡

M = age, weight, height, alcohol intake, smoking, hours walked/week, and television watching ≥ 14 hours/week

Significant P-values: † = <0.05, ‡ = <0.0001

Abbreviations: AT = Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue

Table 2.4 Change in multiple linear regression model fit statistics after inclusion of all AT depot radiodensities

Outcome	Δ AIC			Δ BIC		
	All Radiodensities	All Volumes	Radiodensities & Volumes	All Radiodensities	All Volumes	Radiodensities & Volumes
Glucose (mmol/L)	2.77	3.07	3.89	3.00	3.30	4.43
Log-Insulin (pmol/L)	-57.45 †	-29.73 †	-58.95 †	-57.21 †	-29.50 †	-58.41 †
Log-HOMA2-IR	-58.01 †	-29.47 †	-59.48 †	-57.78 †	-29.24 †	-58.94 †

† = improvement of fit (Δ ≤ -2) compared to covariate-alone model

Compares inclusion/exclusion of (1) all 3 AT radiodensities simultaneously, (2) all three AT volumes simultaneously, or (3)all 3 AT radiodensities AND volumes, to the base covariate model [age, weight, height, alcohol intake, smoking, hours walked/week, and television watching ≥ 14 hours/week]

Abbreviations: AT=Adipose Tissue

3.0 Manuscript 2: Upper and Lower Body Composition with Type 2 Diabetes

Title: Relative Associations of Upper and Lower Body Composition with Diabetes in African Caribbean Men

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3.1 Abstract

Objective: Both upper and lower body tissue compositions are associated with type 2 diabetes; however, simultaneous adjustment of regional body compositions as measured by detailed imaging methods are lacking. We assessed the associations of upper and lower body adipose tissue (AT) and muscle tissues with type 2 diabetes categories in African Caribbean men.

Methods: This cross-sectional analysis included 610 men (median age: 62 years; mean BMI: 27.8 kg/m2) from the Tobago Health Study. Diabetes categories (normal glucose, impaired fasting glucose, type 2 diabetes) were defined by fasting glucose and antidiabetic medication use. Abdominal and thigh computed tomography scans were obtained. The abdomen was divided into

three components: subcutaneous AT (ASAT), visceral AT (VAT), and remaining "Other tissues"; the thigh was divided into four components: subcutaneous AT (TSAT), intermuscular AT (IMAT), muscle, and bone. A log2 ratio transformation was applied to each region to create two abdominal component ratios (ASAT:Other and VAT:Other) and three thigh component ratios (TSAT:Bone, IMAT:Bone, and Muscle:Bone). Linear regressions were used to predict glucose, insulin, and HOMA-IR in unmedicated participants. Partial proportional odds models were used to predict diabetes category in all participants.

Results: A two-fold higher ASAT:Other ratio was associated with significantly higher log-transformed glucose, insulin, and HOMA-IR (β's range 0.07-0.19), and with higher odds of being in a higher diabetes category (OR: 1.95, 95% CI:1.14-3.34). A two-fold higher thigh Muscle:Bone ratio was strongly associated with lower odds of being in a higher diabetes category (OR: 0.36, 95%CI: 0.13-0.98). Other tissues showed differential but nonsignificant associations with continuous risk factors, and none showed associations with diabetes category.

Conclusions: ASAT and thigh muscle were the strongest determinants of diabetes category, with little contributions from other body tissues. The simultaneous inclusion of upper and lower body compositions may yield greater information on differential risk of metabolically active tissues.

3.2 Introduction

Obesity, and the regional growth of adipose tissue (AT), is a major risk factor for cardiometabolic diseases such as hypertension and type 2 diabetes. Simultaneous comparisons of the effects of upper- and lower-body compartments with type 2 diabetes indicate harmful effects of upper-body AT accumulation and protective effects of lower-body AT accumulation (63).

Imaging methods such as computed tomography (CT) can identify distinct metabolically active tissues within these regions, such as AT and muscle, and demonstrate further differential associations with cardiometabolic disease. Abdominal subcutaneous AT (ASAT) and visceral AT (VAT) are generally positively associated with type 2 diabetes and related biomarkers, with most studies indicating a stronger association with VAT (22, 23, 269, 291, 292). Within the lower body, results are more mixed and depend on tissue type, studied population, and modeled covariates. After adjustment for upper-body adiposity, thigh subcutaneous AT (TSAT) appear to be protective against or unassociated with type 2 diabetes and related biomarkers (64, 65, 67, 293-295). In contrast, lower-body intermuscular AT (IMAT) is generally positively associated (62, 64, 65, 147, 270, 272, 293). Thigh muscle may be beneficial or harmful, with these differential associations seemingly due to effect modification by obesity status (67, 296).

The collection and analysis of both upper and lower-body CT scans for type 2 diabetes risk assessment is relatively uncommon (64-67) (293-295). African Ancestry individuals, who are at greater risk for type 2 diabetes independent of overall adiposity (53-55) and exhibit different AT distributions (greater ASAT (56, 58, 59) and IMAT (61), lower VAT (56-59)) compared to their Caucasian counterparts, are underrepresented in this literature. Further, previous analyses of upper and lower-body composition do not analytically treat body imaging data as compositional data. Compositional data are defined as components which sum to a whole, and as such, they have an inherent correlation structure so that an increase in one component must come at the expense of at least one other component. Such data are more appropriately modeled using a compositional data analysis (CoDA) approach (260); however, while health sciences fields such as high-throughput sequencing (262), physical activity measures (263), and diet (264) are applying compositional approaches to their data, the body composition field has not.

This analysis uses a cohort of African Caribbean men that has both abdominal and thigh CT scans. Using a CoDA approach, we compare the simultaneous and relative effects of upper (ASAT and VAT) and lower (TSAT, thigh IMAT, and thigh muscle) tissues with type 2 diabetes prevalence.

3.3 Methods

3.3.1 Study Population

All men in this analysis were from the Tobago Health Study, which has been previously described (161). Briefly, the Tobago Health Study is a population-based, prospective cohort study of community-dwelling men aged 40 years and older, residing on the Caribbean island of Tobago, Trinidad and Tobago. Men from Tobago are of homogeneous African ancestry with low European admixture (<6%) (278). Participants in the Tobago Health Study were recruited without regard to health status and men were eligible if they were ambulatory, not terminally ill, and without a bilateral hip replacement. The baseline visit occurred from 2004-2007 and recruited 2,482 men; of these, a random subset (N=1,725) attended the first follow-up visit from 2010-2014. Men used in the current analysis attended an ancillary study visit from 2014-2018, when a convenience sub-sample of N=768 participants from the prior visit had CT scans of the chest, abdomen, and mid-thigh for ectopic AT assessment. Exclusion from the current analysis included non-African Caribbean ethnicity by self-report (N=67), missing CT scans in the abdomen or in one or both thighs (N=31), missing covariate data (N=53), being underweight (N=4), and non-fasting serum samples (N=1). Two individuals were also excluded for improper serum handling that led to

glucose degradation. The final analytical sample included 610 individuals. Written informed consent was obtained from each participant using forms and procedures approved by the University of Pittsburgh Institutional Review Board, the U.S. Surgeon General's Human Use Review Board, and the Tobago Division of Health and Social Services Institutional Review Board. This study was completed in accordance with the Declaration of Helsinki.

3.3.2 Computed Tomography Scans

Abdominal and thigh volumes were assessed on 3 mm thick slices and 500 mm display field of view from scans acquired using a GE dual slice, high-speed NX/I CT scanner (GE Medical Systems, Waukesha, WI) with 120 KVp, 250 mA, 0.7 second gantry speed, and pitch of 1.5:1. For participants with body weight greater than 200 lbs, the mA was increased to 300. CT contrast was not used. Only one CT scanner was used, and a single individual collected the scans for all participants. Scans were electronically transmitted to the central CT reading center at Vanderbilt University where image analysis and quality control were performed.

Image analysis was performed using methods as previously described (276, 279, 280). Briefly, images were analyzed using a dedicated imaging processing workstation with custom-programmed subroutines (OsiriX, Pixmeo, Geneva, Switzerland) and a dedicated pen computing display (Cintiq, Wacom Technology Corporation, Vancouver, WA, USA). A radiologist-trained analyst manually traced anatomical boundaries (skin, muscular fascia, muscle, bone, and peritoneum) in CT scans. Tissue attenuation thresholds of −190 to −30 Hounsfield Units (HU) were used to distinguish AT voxels in these defined regions and tissue attenuations of -29 to 160 HU were used to distinguish lean muscle voxels. For each tissue, the volume (mm^3) was calculated.

Abdominal VAT and ASAT were measured from CT scans of 3 contiguous slices of 3mm thickness centered at L4-L5. A lateral scout image was used to determine the z-axis location of the L4-L5 intervertebral space and that location and the slice immediately above and the slice immediately below were used to reconstruct a 9-mm thick single block of images. VAT was defined as AT located within the peritoneal cavity; ASAT was defined as AT located beneath the skin and superficial to the abdominal muscular fascia. The remaining non-VAT and non-ASAT tissues were not separately measured, but were combined to form an "Other" tissue group.

TSAT, thigh IMAT, and thigh muscle were measured from CT scans of 10 contiguous slices of 3mm thickness at the mid-thigh level in both legs. An anterior-posterior scout scan of the entire femur was used to localize the mid-thigh position, and that location and the four slices immediately above and five slices immediately below were used to reconstruct a 30-mm thick single block of images. Hand-drawn boundaries were traced at the medulla, cortex, thigh muscles, fascia, and skin in three of the ten slices; boundaries were imputed over the remaining slices and verified for accuracy by the trained analyst. Bone volume was identified as the cortical volume. Lean muscle volume was defined as the sum of the adductors, hamstrings, and quadriceps muscles across both thighs. TSAT was defined as AT located between the skin and the muscle fascia, and IMAT was defined as AT located within thigh muscle groups. TSAT and IMAT volumes were defined as the total AT type summed across both thighs.

3.3.3 Generation of Compositions and Additive Log Ratio Transformation

As the regional distribution of tissues is of primary interest, two separate compositions were created: abdominal and thigh. The abdominal composition was comprised of VAT, ASAT,

and the 'Other' remaining abdominal tissues. Similarly, thigh composition was comprised of TSAT, IMAT, muscle, and bone.

The additive log ratio (ALR) transformation is described in greater detail elsewhere (260). Briefly, for a composition made up of D components (x_1, x_2, …, x_D), the ALR transformation generates D-1 terms where each term is the log of the ratio of each component to a referent component, ex. $\log(x_1/x_D)$, $\log(x_2/x_D)$, …, $\log(x_{D-1}/x_D)$. When a composition is used as an independent variable in a regression model, all D-1 components are entered into the model as covariates, and the interpretation of the regression coefficient is determined as the log base-fold increase in the ratio of x_i to x_D, holding all other component ratios constant.

For the abdominal composition, the 'Other' tissue component was used as the referent; for the thigh composition, the bone was used as a referent. A $\log_2$ transformation was applied to these ratios so that interpretation of coefficients is for a two-fold increase in the ratio numerator compared to the denominator.

3.3.4 Outcome Definition: Type 2 Diabetes Categories

Fasting serum glucose and insulin measures were measured at the Advanced Research and Diagnostics Laboratory (ARDL), University of Minnesota. Fasting serum glucose was measured using an enzymatic procedure (interassay CV: 1.3-1.8%). Diabetes categories were defined based on American Diabetes Association (ADA) criteria (92). Individuals taking antidiabetic medications were classified as "Type 2 Diabetes" regardless of measured fasting glucose.

3.3.5 Other Measures

Standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight was recorded to the nearest 0.1 kg without shoes on a balance beam scale. BMI was calculated from body weight and standing height (kg/m^2).Information on current smoking [yes/no], number of hours walked per week, watching 14 or more hours of television (TV) per week [yes/no], current intake of alcohol of more than 4 drinks per week [yes/no], ethnic self-identification (dichotomized as African-Caribbean Ethnicity [yes/no]), family history of hypertension or diabetes [yes/no] and medication use were assessed using standardized interviewer-administered questionnaires. Self-reported information on walking was recorded as walking is the predominant form of physical activity on the island of Tobago. Men were asked to bring all prescription medications taken in the past 30 days to their clinic visit.

3.3.6 Statistical Analyses

Population characteristics were reported overall and stratified by obesity status; p-values for linear trend were reported, with linear contrasts used for continuous variables and Cochrane-Armitage trend test used for categorical variables. Ternary plots for abdominal and thigh compositions were generated using the package 'compositions' (297) in R version 3.5.2 (283), and the mean compositions for each hypertension and diabetes category was plotted over the population distribution. Age-adjusted Pearson correlations were reported between the ALR-transformed components and BMI. Linear regression (for log glucose, log insulin, and log HOMA-IR outcomes) and partial proportional odds regression (for ordinal diabetes category outcome) models were performed adjusting for age, BMI, family histories of diabetes, drinking 4+ alcoholic

drinks per week, current smoking, watching TV ≥ 14 hours per week, hours walked per week for exercise, taking lipid-modifying medications, and the ALR-transformed abdominal and thigh compositions; linear regressions for continuous biomarkers were additionally adjusted for antidiabetic medication use. Partial proportional odds models with unequal slopes for lipid-modifying medications was chosen after rejection of the score test and empirical cumulative logit plots indicated that this variable was the only one violating the proportional odds assumption in these models. Interactions of tissues with respective total abdominal or thigh volumes, or with BMI, were also visualized and assessed using the PROCESS macro (298). Statistical significance was based on $\alpha = 0.05$, and analyses were performed using SAS 9.4 software (SAS Institute, Inc., Cary, NC).

3.3.6.1 Sensitivity analysis

Regression analyses which use only the abdominal compositions and total abdominal volume or only the thigh compositions and total thigh volumes are presented in **Supplementary Tables Appendix C Table 2** and **Appendix C Table 3**, respectively.

Our abdominal composition in main analyses was divided into three components: the ASAT, VAT, and "Other tissue" components; these were chosen as only the total abdominal volume, the ASAT, and the VAT were accurately traced and measured (as described in Methods above), while other abdominal tissues were not separately quantified. Given that the "Other" component is thus comprised of various abdominal tissues (organs, bone and bone marrow, muscle, and IMAT), with muscle and IMAT being of key interest in our analyses, as a sensitivity analysis we estimated the abdominal muscle and IMAT without manual tissue tracing in the area between the peritoneal cavity and the muscular fascia using attenuation tissue thresholds defining AT (-190 to -30 HU) and lean muscle (-29 to 160 HU). Using this method, estimates of abdominal

IMAT are likely to include spinal bone marrow AT, and thus abdominal IMAT would be overestimated. We previously accurately measured abdominal VAT, ASAT, muscle, and IMAT during a previous study visit of the Tobago Health Study (N=301, men not part of this main analysis), and correlations of accurately measured abdominal muscle and IMAT with VAT, ASAT, and total abdominal volume in the previous visit were similar to correlations obtained within our sensitivity analysis estimates (**Supplementary Table Appendix C Table 4**); thus, our estimates of muscle and IMAT in the abdomen should serve as good surrogates for the true measures. Abdominal compositions in the sensitivity analyses now consisted of ASAT, VAT, abdominal IMAT, abdominal muscle, and remaining "Other", such that two new logratio terms (IMAT:Other and Muscle:Other) were included in models. Models were otherwise constructed as indicated in the main analyses.

Other sensitivity analyses included the addition of thigh muscle attenuation (**Supplementary Table Appendix C Table 5**), a qualitative measure representing intramyocellular lipid accumulation (299, 300), which was defined as the average HU across measured thigh volumes, where a lower average HU indicates greater fatty infiltration.

3.4 Results

3.4.1 General Baseline Characteristics

Overall population characteristics and characteristics stratified by obesity status are displayed in **Table 3.1**. Men were slightly older and overweight, with a median age of 62 and mean

BMI of 27.7 kg/m^2. About 23% of the men had type 2 diabetes, with a majority (~76%) being on an antidiabetic medication.

Ternary plots (**Figure 3.1-Figure 3.4**) were constructed to show overall abdominal and thigh composition distributions in the population, as well as the mean compositions for each of the diabetes categories. Ternary plots are read such that the closer an individual is plotted towards a particular corner, the greater that individual's composition is comprised of that component (with a corner being completely 100% that composition). In the abdominal compositions (**Figure 3.1**), individuals in higher diabetes categories appeared to have a greater %ASAT, and a slight shift to having a greater %VAT. In the thigh compositions (**Figure 3.2-Figure 3.4**), individuals in higher diabetes categories appeared to have greater %TSAT and %IMAT, while having lesser %Bone and %Muscle.

3.4.2 Association of Tissue Depots with Anthropometric Measures and Diabetes Categories

Age-adjusted Pearson correlations (**Table 3.2**) were performed to investigate associations between ALR-transformed AT components, BMI, and continuous risk factor measures. BMI was most strongly correlated with ASAT and TSAT, and showed similar correlations with VAT and IMAT components. Interrelationships among all of the components were high, with some of the highest correlation coefficients being between ASAT and either of the thigh AT components. Despite these higher correlations, multicollinearity among these variables was not identified when investigating condition indices and variance proportions in regression models.

We next performed linear regression models (for log glucose, log insulin, and log HOMA-IR) and partial proportional odds regression models (for diabetes categories) (**Table 3.3**). For upper-body composition, after adjustment for risk factors, antidiabetic medication use, and lower

body composition, higher ASAT ratios were statistically significantly associated with higher log glucose and log HOMA-IR ($\beta = 0.06$ and 0.15, respectively; both $p < 0.05$), while non-significantly associated with higher log insulin ($\beta = 0.09$, $p=0.10$); meanwhile, higher VAT ratios were only significantly associated with higher log insulin and log HOMA-IR (both $\beta = 0.10$, $p < 0.05$). For lower-body compositions, no component was statistically significantly associated with glucose, insulin, or HOMA-IR; however, TSAT and muscle were inversely associated with log glucose and positively associated with log insulin, while IMAT was inversely associated with both log insulin and log HOMA-IR.

When looking at diabetes categories, only ASAT (OR: 1.81, 95% CI: 1.06-3.10) and thigh muscle (OR: 0.37, 95%CI: 0.14-1.01) were associated with odds of being in a higher diabetes category. Though neither the VAT, IMAT, or TSAT components reached statistical significance, the point estimates and confidence intervals for TSAT (OR: 0.71, 95%CI: 0.43-1.18) suggested a potentially protective effect against diabetes.

3.4.3 Interactions

Interactions between tissue ratios and the overall volume of the respective scanned region were assessed. There were no interactions of component ratios by their respective total abdominal or thigh volumes in continuous glucose, insulin, or insulin resistance models. However, in models with a dichotomous diabetes status outcome, significant interactions were identified for ASAT and TSAT. In models with ASAT interactions, as total abdominal size increased, the harmful association of higher ASAT with greater odds of type 2 diabetes was attenuated and then reversed. In contrast, as total thigh size increased, the protective association of higher TSAT with lower odds of type 2 diabetes grew stronger.

If looking at interactions by BMI instead of by regional size, similar results were obtained as with the regional volume interactions. However, some additional interactions with thigh IMAT were seen, whereby at higher BMIs, higher thigh IMAT was associated with lower log-insulin, and at lower BMIs, higher thigh IMAT was associated with greater odds of type 2 diabetes.

3.4.4 Sensitivity Analyses

Models which used only abdominal CT scans (**Supplementary Table Appendix C Table 2**) or only thigh CT scans (**Supplementary Table Appendix C Table 3**) showed somewhat different results when compared to main analysis models. Models which included only upper-body measures had slightly attenuated effects for ASAT and slightly larger effects for VAT than those in the combined upper and lower-body models. For models which included only lower-body measures compared to main analysis models, results were more mixed. For TSAT, results for log-insulin and log-HOMA-IR were much stronger in the lower-body alone models, while the association with diabetes category was attenuated. For IMAT, the log-insulin and log-HOMA-IR effects were more attenuated in the lower-body alone models. And for thigh muscle, associations with glucose and with diabetes category were stronger in the lower-body alone models.

Main analysis results remained consistent in sensitivity analyses where abdominal muscle and IMAT were estimated and included as additional independent component ratios (**Supplementary Table Appendix C Table 4**) and in models with adjustment for thigh muscle density (**Supplementary Table Appendix C Table 5**).

3.5 Discussion

We show that upper-body ASAT, but not VAT, is the primary AT depot associated with diabetes in this African-ancestry population. Additionally, we show that inclusion of thigh muscle with other body tissues is also important for assessing associations with type 2 diabetes. Our findings underscore the importance of including both upper and lower-body tissues in analyses of diabetes risk and development, and indicate that the role of SAT in diabetes may be underappreciated in this population.

Previous studies which have included both abdominal and thigh tissues together (64-67) (293-295) tended to exclude some tissues within the fully-adjusted models and did not take into account total abdominal or thigh areas. Given the structural nature of compositional data and the non-CoDA approaches used in previous analyses, collinearity issues may have impacted the ability to simultaneously adjust for all tissue types and/or adjust for total abdominal and thigh volumes in addition to body mass. By using a CoDA approach, we are able to overcome structural collinearity issues within body composition data, allowing for adjustments of multiple tissue types and total regional sizes.

The most surprising finding in our study is the strong positive association of ASAT with diabetes, while there was no association of VAT with diabetes. Our findings may differ from other published results for multiple reasons. First, while abdominal imaging scans are more common in body imaging studies than thigh imaging scans, simultaneous inclusion of abdominal and thigh scans are less common. In our sensitivity analyses looking at only upper or only lower body composition, we found that the addition of the lower body tissues attenuated the effects of VAT while increasing those of ASAT, thus indicating the importance of including multiple regional body compositions in determining specific tissue effects. Second, the use of a compositional data

analytic method allows for the investigation of tissue effects independent of overall compositional sizes. This in effect reduces the collinearity between tissues and markers of overall mass (such as BMI or total abdominal volume), which may be biasing estimates in other analyses. Third, our study is in an African ancestry population, who typically have lower levels of VAT (56-59) and higher levels of ASAT (56, 58, 59) compared to Caucasian populations. Thus, it is possible that with our population's relatively lower levels of VAT compared to ASAT, that the effects of ASAT on diabetes was easier to capture than the effects of VAT. This scenario has some plausibility, as our interaction analysis found that higher ASAT storage was harmful in individuals with smaller abdominal sizes but was protective in individuals with larger abdominal sizes.

We report that higher thigh muscle was inversely associated with glucose and diabetes categories. Muscle is an important glucose sink, and multiple studies have demonstrated inverse associations of muscle with diabetes risk (67, 296, 301-304). The use of CT derived measures of muscle size, however, is still complicated by the fact that increasing muscle can alternatively indicate greater fatty infiltration of the muscle or an adaptation to help support heavier weights in obesity. Because our analyses utilized a compositional data analytic approach, we are able to better disentangle some of the different effects of changes in both muscle and AT with increasing body size. To address the first scenario, we included a sensitivity analysis which further adjusted models for a qualitative measure of intramyocellular lipid accumulation (thigh muscle CT attenuation) and found that this did not significantly impact our estimates. For the second scenario, the use of a compositional data analysis methodology gives estimates independent of total composition (i.e. thigh) size, and further investigations of interactions did not find our muscle estimates to vary across increasing thigh volumes or across increasing BMI. Thus, independent of upper and lower

body AT and body size, the increase or maintenance of skeletal muscle tissue may be an important consideration for diabetes prevention.

We found relatively high correlations between our transformed ASAT and TSAT components. That ASAT and TSAT are highly correlated, and that they comprise the greatest percentage of abdominal and thigh AT compartments, speaks to the theorized role of subcutaneous white AT as the primary storage site for triglycerides (171). Despite these shared roles, there are functional and metabolic differences between ASAT and TSAT which may contribute to their opposing roles in cardiometabolic health (63). Our findings that these tissues have opposing associations with fasting glucose are consistent with previous reports from Health ABC (65). However, both ASAT and TSAT were similarly positively associated with insulin in our regression models, though only ASAT was associated with insulin resistance. A few studies suggest that having more TSAT is associated with better insulin sensitivity (67, 293-295); while we did not observe an inverse relationship between TSAT and insulin resistance, this may be in part to differences in insulin resistance/sensitivity estimations, or may also be due to racial/ethnic differences such that African ancestry individuals tend to have higher HOMA-IR compared to hyperinsulinemic clamp-matched European ancestry individuals (305).

Interestingly, while IMAT was strongly correlated with ASAT and TSAT, its associations with insulin and HOMA-IR were opposite of the subcutaneous ATs. The high correlation between subcutaneous AT and IMAT is not unexpected; previous studies (272, 285) demonstrated that increases in IMAT mirror increases in total adiposity, of which SAT is a major component. However, the role of IMAT in insulin resistance remains understudied. IMAT can secrete factors which induce insulin resistance in neighboring muscles (181), indicating that it could play a direct role in insulin resistance pathology. We previously demonstrated that lower IMAT radiodensity, a

marker of increased tissue lipid accumulation, was associated with greater insulin resistance (306), and that increased calf IMAT volume is associated with incident diabetes (272). In this study, we also show that while thigh IMAT is not associated with diabetes categories in the overall population, it is positively associated with diabetes in individuals with lower BMIs. Still, it may be that IMAT is only a marker of overall adiposity and metabolic dysfunction (307). It is also possible that the location of IMAT can impact its relationship with local and global insulin sensitivity (284), and thus our findings of thigh IMAT and insulin resistance would not be generalizable to other anatomical locations.

Our study has a few limitations. First, we did not have accurate measures of abdominal muscle and IMAT available in our dataset, and instead collapsed them with other tissues into a singular 'other' variable. However, in sensitivity analyses which estimated abdominal muscle and IMAT, we found that these additional abdominal components did not substantially change results. Second, our analyses are cross-sectional in nature, and so causality cannot be determined. Still, the use of partial proportional odds models allows for some stronger evidence for our reported effects. Third, our analyses are limited to men, who have different body compositions (174) compared to their female counterparts. Our study also has several strengths. This study is novel in its use of both upper and lower body CT imaging data to assess tissue associations with type 2 diabetes, overcoming the previous limitations of upper and lower body composition studies that could not precisely measure different tissues. We also utilized a compositional data analytic approach, allowing us to incorporate multiple tissue types as well as total sizes of imaged areas without suffering collinearity issues.

In conclusion, simultaneous assessment of upper and lower body compositions demonstrated deleterious effects from higher levels of ASAT, and protective effects of thigh

muscle, with respect to type 2 diabetes in African Caribbean men. These findings indicate the importance of incorporating regional body compositions when assessing cardiometabolic risk.

3.6 Tables and Figures

Table 3.1 Population Characteristics, Overall and by Obesity Status

Variable	Mean (SD), Median (IQR), or N(%)				
	Overall (N=610)	Normal (N=177)	Overweight (N=266)	Obese (N=167)	P-value
Demographic and Lifestyle Factors					
Age (years)	62.0 (57.0, 68.0)	63.0 (58.0, 71.0)	62.0 (57.0, 69.0)	60.0 (56.0, 65.0)	0.0003
Weight (kg)	85.5 (15.4)	70.6 (7.0)	84.2 (7.9)	103.4 (12.3)	<.0001
Height (cm)	175.5 (6.7)	176.0 (6.6)	175.4 (6.9)	174.9 (6.3)	0.1186
BMI (kg/m^2)	27.8 (4.7)	23.2 (21.7, 24.2)	27.4 (26.2, 28.3)	32.9 (30.9, 35.4)	<.0001
Current Smoker [N(%)]	44 (7.2%)	16 (9.0%)	19 (7.1%)	9 (5.4%)	0.1906
Drinks 4+ alcoholic beverages per week [N(%)]	75 (12.3%)	20 (11.3%)	36 (13.5%)	19 (11.4%)	0.9699
Watches TV ≥ 14 hours per week [N(%)]	294 (48.2%)	84 (47.5%)	128 (48.1%)	82 (49.1%)	0.7609
Walking for exercise (hours per week) [N(%)]	1.9 (0.0, 5.0)	1.5 (0.0, 4.5)	2.1 (0.0, 5.0)	1.5 (0.0, 5.0)	0.5095
On lipid-modifying medications [N(%)]	79 (13.0%)	18 (10.2%)	33 (12.4%)	28 (16.8%)	0.0696
Has family history of Diabetes [N(%)]	340 (55.7%)	90 (50.8%)	148 (55.6%)	102 (61.1%)	0.0564
Cardiometabolic Disease Measures					
Fasting Glucose (mg/dL)	89.0 (81.0, 102.0)	87.0 (79.0, 97.0)	89.0 (82.0, 102.0)	93.0 (83.0, 115.0)	0.0008
Fasting Insulin (μU/mL)	9.0 (5.8, 14.0)	5.7 (4.0, 7.7)	9.0 (6.3, 13.2)	15.0 (11.5, 19.8)	<.0001
HOMA-IR	2.2 (1.3, 3.5)	1.3 (0.9, 1.8)	2.1 (1.4, 3.1)	3.7 (2.5, 5.5)	<.0001

Table 3.1 Continued

Type 2 Diabetes Categories [N(%)] Normal Glucose Impaired Fasting Glucose Type 2 Diabetes	 401 (65.7%) 70 (11.5%) 139 (22.8%)	 136 (76.8%) 13 (7.3%) 28 (15.8%)	 176 (66.2%) 31 (11.7%) 59 (22.2%)	 89 (53.3%) 26 (15.6%) 52 (31.1%)	<.0001
Antidiabetic Medication Use [N(%)]	106 (17.4%)	23 (13.0%)	46 (17.3%)	37 (22.2%)	0.0251
Body Composition Tissue Measures					
ASAT Volume (cm^3)	181.8 (129.2, 245.7)	101.8 (49.3)	188.2 (50.9)	308.3 (100.3)	<.0001
VAT Volume (cm^3)	86.1 (52.3, 125.0)	44.6 (26.7, 68.4)	92.4 (39.6)	138.6 (56.6)	<.0001
Other Abdominal Volume (cm^3)	312.8 (46.6)	288.9 (37.8)	308.5 (40.6)	345.2 (46.3)	<.0001
Total Abdominal Volume (cm^3)	581.5 (485.6, 690.6)	442.8 (69.8)	590.0 (79.4)	792.0 (139.7)	<.0001
TSAT Volume (cm^3)	341.2 (229.9, 485.0)	204.0 (111.9)	361.6 (133.7)	587.1 (228.3)	<.0001
IMAT Volume (cm^3)	118.4 (50.5)	80.3 (36.8)	120.4 (37.4)	143.6 (118.5, 187.7)	<.0001
Thigh Muscle Volume (cm^3)	1068.4 (172.8)	951.1 (139.6)	1079.3 (144.0)	1175.2 (171.4)	<.0001
Thigh Bone Volume (cm^3)	44.3 (41.6, 47.9)	42.8 (40.5, 45.4)	44.6 (5.1)	46.3 (4.8)	<.0001
Total Thigh Volume (cm^3)	1608.9 (326.0)	1278.2 (176.0)	1605.9 (174.8)	1964.2 (254.4)	<.0001

Continuous p-values: linear regression predicting the characteristic (for parametric), or Joncheere-Terpstra Test (for nonparametric). Categorical p-values: Cochrane-Armitage trend test for binary variables, or Mantel-Haenszel Chi-square test for ordinal variables.

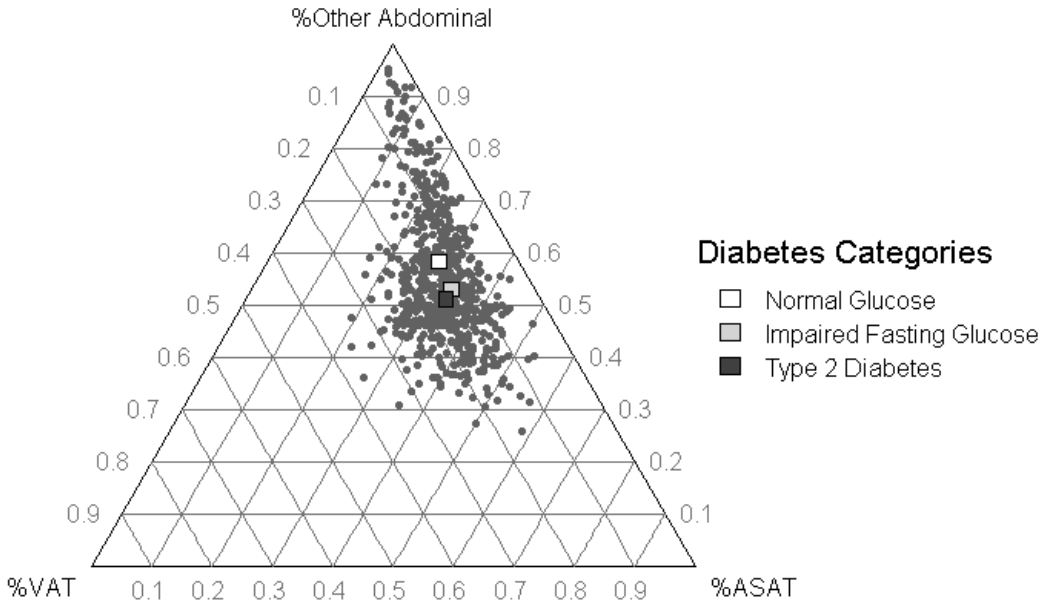

Figure 3.1 Ternary Plot of Population Abdominal Compositions (VAT, ASAT, and Other Tissue), with Mean Composition by Diabetes Category

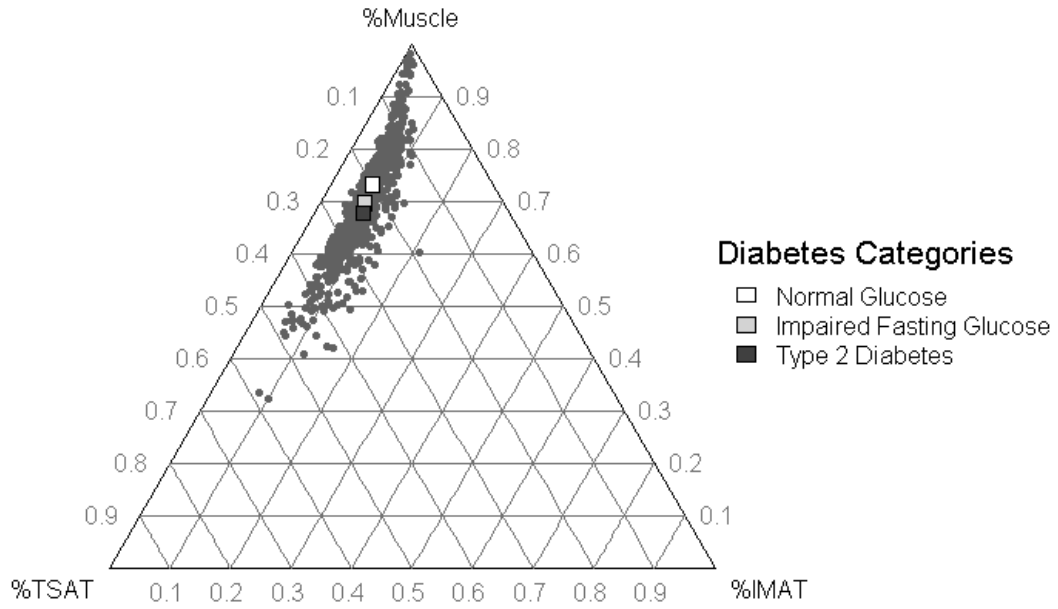

Figure 3.2 Ternary Plot of Population Thigh Compositions (Muscle, TSAT, and IMAT), with Mean Composition by Diabetes Category

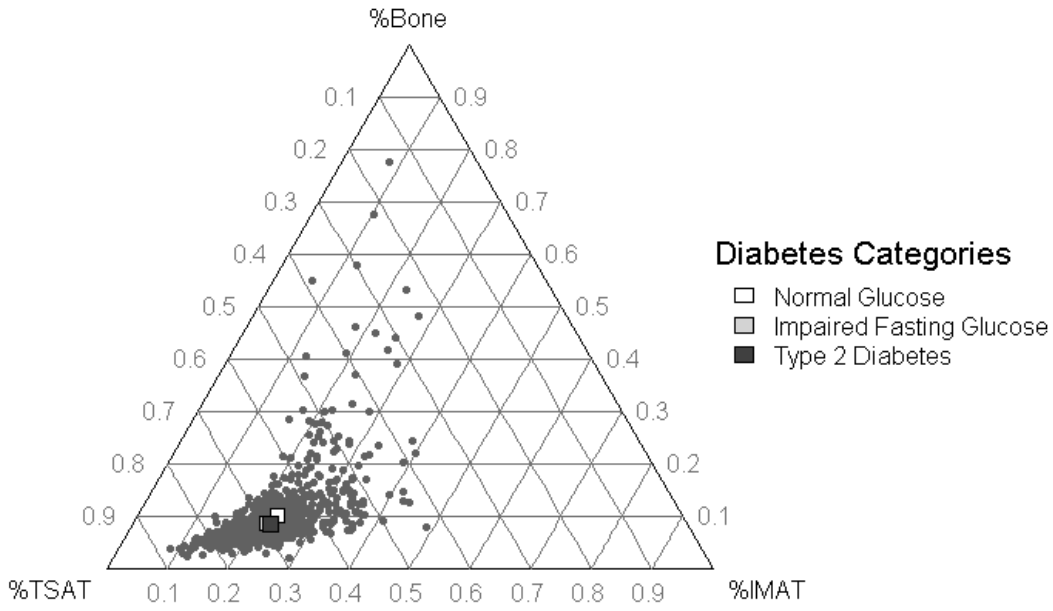

Figure 3.3 Ternary Plot of Population Thigh Compositions (Bone, TSAT, and IMAT), with Mean Composition by Diabetes Category

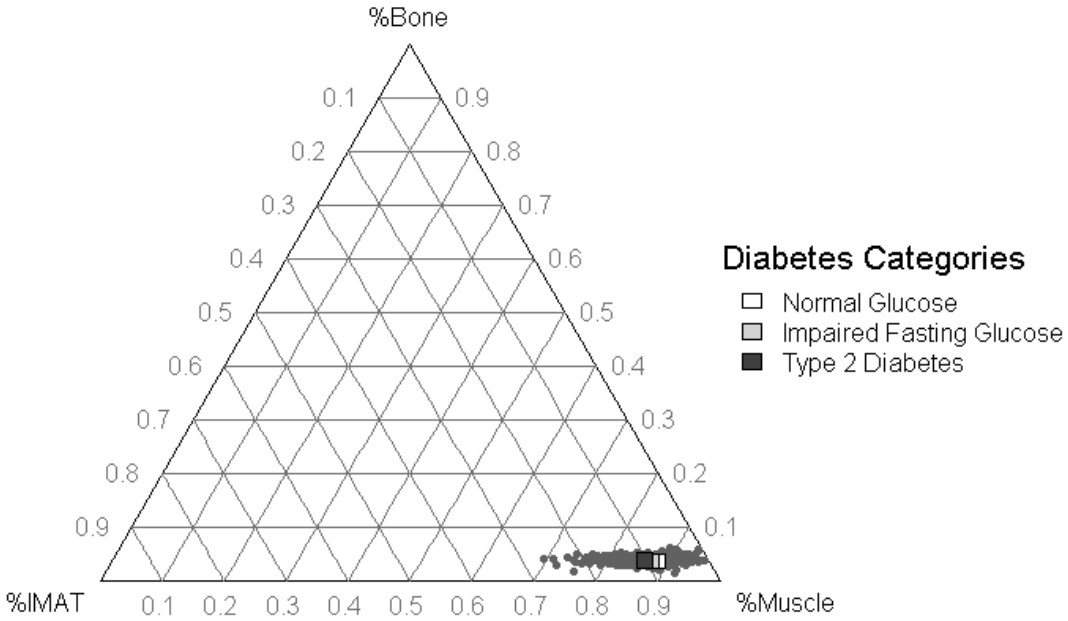

Figure 3.4 Ternary Plot of Population Thigh Compositions (Bone, IMAT, and Muscle), with Mean Composition by Diabetes Category

Table 3.2 Age-Adjusted Pearson Partial Correlation Coefficients for ALR-Transformed Components, BMI, and Glucose and Insulin

	ASAT[a]	VAT[a]	TSAT[a]	Thigh IMAT[a]	Thigh Muscle[a]	BMI (kg/m2)
ASAT[a]	1	0.73 ‡	0.89 ‡	0.80 ‡	0.14 †	0.69 ‡
VAT[a]	0.73 ‡	1	0.64 ‡	0.61 ‡	0.13 †	0.58 ‡
TSAT[a]	0.89 ‡	0.64 ‡	1	0.82 ‡	0.20 ‡	0.70 ‡
Thigh IMAT[a]	0.80 ‡	0.61 ‡	0.82 ‡	1	0.29 ‡	0.61 ‡
Thigh Muscle[a]	0.14 †	0.13 †	0.20 ‡	0.29 ‡	1	0.27 ‡
BMI (kg/m2)	0.69 ‡	0.58 ‡	0.70 ‡	0.61 ‡	0.27 ‡	1
Log Glucose	0.16 ‡	0.16 †	0.09 †	0.06	-0.04	0.18 ‡
Log Insulin	0.60 ‡	0.54 ‡	0.55 ‡	0.46 ‡	0.22 ‡	0.66 ‡
Log HOMA-IR	0.58 ‡	0.53 ‡	0.52 ‡	0.42 ‡	0.15 †	0.64 ‡

†: $p < 0.05$, ‡: $p < 0.0001$

a: Log_2 Transformed AT Depot

Table 3.3 Multivariable-Adjusted Regressions for Body Composition Tissues with Continuous Risk Factors (Top) and Ordinal Risk Factors (Bottom)

Risk Factor	VAT	ASAT	TSAT	Thigh IMAT	Thigh Muscle
Log Glucose *	0.001 (-0.03, 0.03)	**0.06 (0.02, 0.11)**	-0.03 (-0.07, 0.02)	-0.002 (-0.05, 0.04)	-0.07 (-0.16, 0.03)
Log Insulin *	**0.10 (0.04, 0.17)**	0.09 (-0.02, 0.19)	0.06 (-0.04, 0.16)	-0.07 (-0.17, 0.03)	0.15 (-0.06, 0.36)
Log HOMA-IR *	**0.10 (0.02, 0.19)**	**0.15 (0.04, 0.27)**	0.03 (-0.08, 0.15)	-0.07 (-0.18, 0.04)	0.08 (-0.19, 0.35)
Type 2 Diabetes Categories	0.95 (0.70, 1.28)	**1.81 (1.06, 3.10)**	0.71 (0.43, 1.18)	1.07 (0.67, 1.71)	0.37 (0.14, 1.01)

Data are reported as the multivariable adjusted β (95% CI) for continuous risk factor data and OR (95% CI) for Ordinal categorical data.

All models adjusted for age, BMI, drinking 4+ drinks per week, current smoker, watching television ≥14 hours/week, hours walked per week for exercise, lipid-modifying medication, family history of diabetes, log ratios of abdominal tissues (with "Other" tissue as referent component), log ratios of thigh tissues (with bone volume as referent component), and total abdominal and thigh volumes.

* Additionally adjusted for antidiabetic medication use

4.0 Manuscript 3: The Intestinal Microbiome with Type 2 Diabetes

Title: Association of the Gut Microbiome with Type 2 Diabetes in African Caribbean Men

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4.1 Abstract

Objectives: To determine associations of the intestinal microbiome with type 2 diabetes and related risk factors in an African Caribbean population.

Methods: Fecal samples were collected from 253 men from the Tobago Health Study (median age: 60 years, mean BMI: 28.2 kg/m^2), and the V4 region of the bacterial 16s rRNA was extracted and amplified. Clinical characteristics, computed tomography scans of the abdomen and thighs, and diet were collected and organized into groups of variables based on similarity. Diabetes status was investigated using dichotomized (yes/no) or categorized (normal, impaired fasting glucose,

type 2 diabetes) definitions based on fasting glucose and antidiabetic medication, and associations with continuous glucose and insulin were also assessed. Associations of the microbiome within each variable grouping were assessed after adjustment for sociodemographic covariates. PERMANOVA and hierarchical clustering analysis using Manhattan distances were used to investigate natural and data-driven cluster separations. Alpha diversity metrics (Tail, Shannon, Simpson, Reciprocal Simpson, and Evenness) and logratio transformed taxa were investigated as potential predictors or outcomes of group variables using regression analyses.

Results: Across variable groupings, sociodemographic factors were the main variables driving both clustering and alpha diversity. Clustering based on various diabetes status definitions and antidiabetic medication shared similar influential taxa, including *Akkermansia*, *Enterobacteriaceae*, and several taxa from the *Clostridiales* order. *Faecalibacterium* was inversely associated with glucose and borderline with diabetes categories. Inclusion of microbial taxa did not improve diabetes models' adjusted R^2 beyond sociodemographic factors, though diversity metric predictors minorly improved glucose models.

Conclusions: In this sample, sociodemographic factors are the biggest influencers of microbial clustering and diversity, and the addition of the microbiome as a predictor did not significantly improve diabetes models. Based on these preliminary analyses, while some relationships may exist between the microbiome and various type 2 diabetes risk factors, traditional risk factors may still be a more influential target for diabetes intervention.

4.2 Introduction

The intestinal microbiome is increasingly recognized as playing a role in the pathophysiology of type 2 diabetes. Metagenome-wide association studies have determined some structural differences in individuals with type 2 diabetes, including lower amounts of butyrate-producing bacteria such as *Roseburia* and higher amounts of *Clostridiales* and *Lactobacillus*, as well as functional differences involving energy metabolism, butyrate production, and oxidative stress resistance (44, 45). Studies have also demonstrated changes in the microbiome following metformin use (247, 308, 309), indicating that the microbiome may serve as a mediating and therapeutic target for diabetes intervention.

The microbiome can differ by a variety of factors, including geographical location (68), race/ethnicity (70), and lifestyle factors such as diet (39) and exercise (310). Studies of the intestinal microbiome rarely include populations in the Caribbean region, where rates of diabetes are very high (3); of those performed in Caribbean populations, only one (311) has assessed associations of the microbiome with type 2 diabetes. Additionally, few studies on the microbiome and type 2 diabetes in general include large numbers of African Ancestry individuals (73, 311, 312).

This microbiome analysis uses stool samples from a study of men from the Caribbean island of Tobago, Trinidad and Tobago. We first investigate the associations of sociodemographic, lifestyle, medication, diet, and anthropometric and body composition measures with intestinal microbial diversity and the relative abundance of taxa. We then report on the associations of the microbiome with diabetes in this population.

4.3 Methods

4.3.1 Study Population

All men in this analysis were from the Tobago Health Study, which has been previously described (161). Briefly, the Tobago Health Study is a population-based, prospective cohort study of community-dwelling men aged 40 years and older, residing on the Caribbean island of Tobago, Trinidad and Tobago. Men from Tobago are of homogeneous African ancestry with low European admixture (<6%) (278). Participants in the Tobago Health Study were recruited without regard to health status and men were eligible if they were ambulatory, not terminally ill, and without a bilateral hip replacement. The baseline visit occurred from 2004-2007 and recruited 2,482 men; of these, a random subset (N=1,725) attended the first follow-up visit from 2010-2014. A convenience subsample of N=768 participants were invited back to an ancillary visit in 2014-2018 to receive CT scans of the chest, abdomen, and thigh for body composition assessment. Clinical and lifestyle characteristics were also obtained at this ancillary study.

Beginning in June 2017, men who participated in the body composition ancillary study were invited back to participate in a microbiome pilot study for fecal sample collection. A convenience sample of 262 men returned to the clinic for additional interview and sample collection; of these, 259 men donated a fecal sample, and 253 of these samples were able to have DNA extracted, resulting in a final sample size of N=253 for analysis. The time difference between clinic visit date and microbiome pilot study visit date was a median of 2.5 years, with a minimum of 1.1 years and a maximum of 3.4 years.

4.3.2 Fecal Sample Collection and Processing

Fecal samples were collected at home by participants using a Zymo Research DNA/RNA Shield Fecal Collection Tube (Zymo Research, catalog No. R1100-9-T). Participants were instructed to collect 1 spoonful of feces, invert several times in the collection tube, and to store in a refrigerator until the specimen could be brought to the Calder Hall medical clinic in Tobago. Returned specimens were stored at -80°C and shipped on dry ice to the University of Pittsburgh (GSPH), where the specimens were again stored at –80°C. Samples were later thawed and aliquoted into 1.5 mL tubes. Of the 259 fecal samples returned, six of them were unable to be aliquoted, resulting in a final sample size of 253 fecal samples.

DNA extraction was performed using Qiagen PowerSoil DNA Isolation kits (MO BIO Laboratories) at the University of Pittsburgh Center for Medicine & the Microbiome. PCR was performed using barcoded amplicons of the V4 variable region of the 16S rRNA. Samples were then purified using magnetic bead size selection (AMPure XP, Beckman Coulter, US) according to manufacturer instructions. Purified samples were combined into a pooled sample for sequencing using an Illumina MiSeq (Illumina, San Diego, CA, USA). Reads were de-multiplexed using standard Illumina software. An in-house software pipeline was used for quality control of reads. Reads meeting quality control cutoffs were merged and processed using an in-house Mothur-dependent pipeline to generate taxonomically classified reads.

4.3.3 Outcome Definitions: Glucose, Insulin, and Diabetes

Fasting serum glucose and insulin measures were obtained during the clinic visit (2014-2017) and measured at the Advanced Research and Diagnostics Laboratory (ARDL), University

of Minnesota. Fasting serum glucose was measured using an enzymatic procedure (interassay CV: 1.3-1.8%) and fasting serum insulin was measured using a sandwich immunoassay procedure (interassay CV: 3.1%) (assays manufacturer: Roche Diagnostics, Indianapolis, IN). Glucose was assessed in mg/dL units. Insulin was assessed in pmol/L units.

Antidiabetic medication use was assessed at both the clinic visit and at the microbiome fecal sample collection visit; as some individuals newly began antidiabetic medications between visits, antidiabetic medication use was defined using the medication reporting at the fecal sample collection visit. Type 2 diabetes was defined as currently taking an antidiabetic medication, regardless of fasting serum glucose level, or having a fasting serum glucose level of ≥ 126 mg/dL.

4.3.4 Covariate Definitions

4.3.4.1 Dietary Intake

Long-term dietary intake was assessed using a 146-item semi-quantitative monthly food frequency questionnaire (FFQ) developed specifically for the Trinidad and Tobago population (153). Items from this questionnaire were linked to food items in the United States Department of Agriculture (USDA) Food Composition Database, Standard Reference Release 28 (SR28) (313). This database has commonly been used in other studies to estimate nutrition of foods in the Caribbean (150, 314, 315), and Caribbean nutrition tables are based on nutritional databases from the United States and the United Kingdom (316). Additionally, many foods in Trinidad and Tobago are imported from the US, and with the exception of one food item (the fruit *pommecythere*) almost all foods from our questionnaire had representation in the USDA database. Various mixed dishes from Trinidad and Tobago have previously had nutrients calculated using USDA databases and rigorously-obtained recipes (150), and these values were linked to for energy

intake instead. Dietary intakes were considered to be missing if individuals were missing ≥10% of food items from the questionnaire or if unreasonable energy intakes were reported (<600kcal/day or >5.000 kcal/day).

Questionnaire items were broken down into representative food groups, and these food groups were further categorized into high-fiber containing foods (vegetables, whole fruits, whole grains, nuts, and legumes) and low-fiber containing foods (remaining food groups). Given the compositional nature of dietary data, an additive log ratio transformation with a log base 2 was used to create a high-fiber-to-low-fiber variable.

4.3.4.2 Medication Use

Men were asked to bring all prescription medications taken in the past 30 days prior to their fecal sample collection visit, as well as antibiotics used within the past two weeks. Medications considered in this analysis include antidiabetic medications, lipid-modifying medications, and antibiotic medications, all dichotomized as [yes/no].

4.3.4.3 Demographics, Lifestyle Factors, and Anthropometry

Demographic characteristics (ethnic self-identification and education status) were obtained at the baseline visit (2004-2007) using interviewer-administered questionnaires. Given the age for study inclusion at baseline was 40 years and older, it is not thought that education status will have changed from the baseline visit until now. Ethnic self-identification was dichotomized as African-Caribbean Ethnicity [yes/no]. Education status was categorized into four groups: primary (1^{st}-5^{th} Standard, ages 5-11), secondary (high school, Form 1^{st} thru 5^{th}, ages 11-16) / technical vocational training, some university / associate degree, and university graduate or higher degree.

Information on age and lifestyle habits (current smoking [yes/no], number of hours walked per week, watching 14 or more hours of television per week [yes/no], and current intake of alcohol of more than 4 drinks per week [yes/no]) were assessed using standardized interviewer-administered questionnaires at the clinic visit. Self-reported information on walking was recorded as walking is the predominant form of physical activity on the island of Tobago.

Standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight was recorded to the nearest 0.1 kg without shoes on a balance beam scale. BMI was calculated from body weight and standing height (kg/m^2).

4.3.4.4 Computed Tomography

Abdominal and thigh volumes were assessed on 3 mm thick slices and 500 mm display field of view from scans acquired using a GE dual slice, high-speed NX/I CT scanner (GE Medical Systems, Waukesha, WI) with 120 KVp, 250 mA, 0.7 second gantry speed, and pitch of 1.5:1. For participants with body weight greater than 200 lbs, the mA was increased to 300. CT contrast was not used. Only one CT scanner was used, and a single individual collected the scans for all participants. Scans were electronically transmitted to the central CT reading center at Vanderbilt University where image analysis and quality control were performed.

Image analysis was performed using methods as previously described (276, 279, 280). Briefly, images were analyzed using a dedicated imaging processing workstation with custom-programmed subroutines (OsiriX, Pixmeo, Geneva, Switzerland) and a dedicated pen computing display (Cintiq, Wacom Technology Corporation, Vancouver, WA, USA). A radiologist-trained analyst manually traced anatomical boundaries (skin, muscular fascia, muscle, bone, and peritoneum) in CT scans. Tissue attenuation thresholds of −190 to −30 Hounsfield Units (HU)

were used to distinguish AT voxels in these defined regions and tissue attenuations of -29 to 160 HU were used to distinguish lean muscle voxels. For each tissue, the volume (mm^3) was calculated.

Abdominal VAT and ASAT were measured from CT scans of 3 contiguous slices of 3mm thickness centered at L4-L5. A lateral scout image was used to determine the z-axis location of the L4-L5 intervertebral space and that location and the slice immediately above and the slice immediately below were used to reconstruct a 9-mm thick single block of images. VAT was defined as AT located within the peritoneal cavity; ASAT was defined as AT located beneath the skin and superficial to the abdominal muscular fascia. The remaining non-VAT and non-ASAT tissues were not separately measured, but were combined to form an "Other" tissue group.

TSAT, thigh IMAT, and thigh muscle were measured from CT scans of 10 contiguous slices of 3mm thickness at the mid-thigh level in both legs. An anterior-posterior scout scan of the entire femur was used to localize the mid-thigh position, and that location and the four slices immediately above and five slices immediately below were used to reconstruct a 30-mm thick single block of images. Hand-drawn boundaries were traced at the medulla, cortex, thigh muscles, fascia, and skin in three of the ten slices; boundaries were imputed over the remaining slices and verified for accuracy by the trained analyst. Bone volume was identified as the cortical volume. Lean muscle volume was defined as the sum of the adductors, hamstrings, and quadriceps muscles across both thighs. TSAT was defined as AT located between the skin and the muscle fascia, and IMAT was defined as AT located within thigh muscle groups. TSAT and IMAT volumes were defined as the total AT type summed across both thighs.

Given the scans were performed in two areas, two separate compositions were created: abdominal and thigh. The abdominal composition was comprised of VAT, ASAT, and the 'Other' remaining abdominal tissues. Similarly, thigh composition was comprised of TSAT, IMAT,

muscle, and bone. An additive log ratio transformation with a log base 2 was applied to each composition. For the abdominal composition, the 'Other' tissue component was used as the referent; for the thigh composition, the bone was used as a referent.

4.3.5 Statistical Methods

In the following statistical analyses, non-microbiome variables were categorized as either base covariates (age, BMI, Caribbean ethnicity, education, smoking status, antibiotic use, lipid-modifying medication use, and the time difference between the clinic interview date and the fecal sample donation) or grouped variables (other metadata, organized into the following groups: diet [energy intake and fiber-containing food ratio], body composition [VAT, ASAT, TSAT, Muscle, Thigh IMAT, and time difference between CT scan and fecal sample collection], physical activity [hours walked per week for exercise and television watching ≥ 14 hours/week], or diabetes [glucose, insulin, HOMA-IR, diabetes status, and antidiabetic medication use]). A conceptual model for the associations of these variables with diabetes is provided in **Appendix D Figure 1**. The below listed analyses were performed several times using an in-house pipeline, with each round resulting in removal of uninformative variables.

4.3.5.1 Distance-Based Analyses

Inter-sample distances were visualized using multidimensional scaling (MDS) plots of Manhattan distances. Distances were assessed in three methods.

The first method was the application of PERMANOVA tests, which were used to identify factors associated with distancing between samples.

The second method involved hierarchical cluster analysis with multinomial logistic regression (HCAMLR). Using Manhattan distances, hierarchical clusters, k, were computed using Ward's minimum variance method. Starting from the root node (k=1), at each cut lower in the hierarchical cluster tree (increasing cluster size by 1), log linear models were fit to model the probability of an individual being in a particular cluster; the iteration with the smallest p-value for that predictor was determined to be the "optimal cluster cutoff" for that variable. Contrastingly, an overall optimal cluster stopping level for the entire hierarchical cluster tree was identified using the distance-based pseudo-F statistic from Calinski-Harabasz criteria.

The third method was identification of cluster influencers, which are the pairwise comparisons between clusters that identify taxonomic differences which explain cluster separation. Clusters were identified either using the Manhattan distance/Ward's minimum variance method, or using "natural" categorical variable clusters within the data. ANOVA models were used to quantify the amount of variation a particular taxon was contributing to intra- and inter-cluster membership. The $\log_{10}$ ratio of the R^2 for a model without a particular taxon compared to the model with that particular taxon can be used to identify taxa which are influential in separating pairwise clusters, where a more negative R^2 $\log_{10}$ ratio indicates greater separation.

4.3.5.2 Distribution-Based and Abundance-Based Analyses

Regression analyses were used to assess associations of group variables with the microbiome. Models were constructed with the microbiome as an outcome (with base covariates and group variables as predictors), or with the group variables as an outcome (with base covariates and the microbiome as predictors). The $\log_{10}$ ratio of the p-value for a microbiome component as a predictor to the p-value for that microbiome component as a response was used to determine if the component was more strongly associated as a predictor or as a response. A combined p-value

score, cpvs = $(\log_{10}(\text{predictor p-value})^2 + \log_{10}(\text{response p-value})^2)^{1/2}$, was used to create a cutoff to remove spurious $\log_{10}$ p-value ratios.

The microbiome was assessed as either alpha diversity metrics (distribution-based) or as additive log ratio transformed taxa (abundance-based). Microbial alpha diversity was visualized using rank-abundance plots and stacked bar plots. Multiple diversity indices (Tail, Shannon, Simpson, Reciprocal Simpson, and Evenness) were considered. Taxonomically classified reads were transformed using an additive log ratio.

4.4 Results

4.4.1 Sample Characteristics

Study sample characteristics are presented in **Table 4.1**. Missing data existed for many variables, with a majority of missing data originating in the CT scans. There were 222 participants with no missing data. The sample is mostly older, with a median age of 60, and overweight with a mean BMI of 28.2. A majority of participants identified as African Caribbean (91.6%). Diabetes rates were high with a prevalence of 22.5%; of individuals with type 2 diabetes, a majority of them (>73%) were treated with an antidiabetic medication.

Figure 4.1 shows the mean taxon abundance for 251 of the participants. On average, participants in this sample had higher abundances of members of the *Bacteroidetes* phylum, with nearly 40% of the average fecal sample being comprised of *Prevotella* (~29%) or *Bacteroides* (~13%) bacteria. A large proportion of the remaining highly-abundant taxa were from the *Firmicutes* phylum, with many members also being from the order *Clostridiales*.

4.4.2 Distance-Based Analyses

4.4.2.1 PERMANOVA

Results of PERMANOVA analysis for each series of group variables is listed below in **Table 4.2**. Significant associations within each variable grouping were predominantly from the base covariates, with Caribbean ethnicity and BMI appearing in all models, and smoking status in nearly all models. Education was statistically significant only in glucose/insulin models, but while not statistically significant in other group variable models, was generally close to significant ($p \sim 0.1$). Within the diet grouping, total energy intake was also significant in PERMANOVA.

4.4.2.2 HCAMLR

Results of the HCAMLR for each series of group and base covariate variables was determined; an example showing associations at the Calinski-Harabasz optimal cutoff for each variable grouping is shown below in **Table 4.3**. In general, most models suggested 6 clusters as an optimal cutoff, though physical activity analyses suggested 2 clusters. Variables associated with clustering tended to be from the base covariates (predominantly Caribbean ethnicity and age), though some specific group-level variables for body composition, diet, and diabetes groupings were also significantly associated with clustering.

4.4.2.3 Cluster Influencers

Figure 4.2 depicts an example cluster influencing graph for taxa which are important for cluster membership at a 6-cluster cut-off. Taxa which served as cluster influencers for distance-based clusters were predominantly Bacteroides and Prevotella_9. While other taxa such as Ruminococcaceae_UCG-002, Lachnoclostridium, Faecalibacterium, and Akkermansia were

identified as cluster influencers, these tended to be less influential than Bacteroides and Prevotella_9.

Naturally-occurring clustering within grouped variables included the physical activity (watching TV ≥ 14 hours/week) and diabetes (antidiabetic medication, diabetes status, and diabetes category) groups (**Table 4.4**). Among diabetes-related clusters, there was significant overlap between clusters formed by antidiabetic medication usage and diabetes status or categories. These included many Ruminococcaceae taxa, as well as Akkermansia, Blautia, Pseudobutyrivibrio, Enterobacteriaceae_uncl, Eubacterium_coprostanoligenes_grp, and Ruminiclostridium_6.

4.4.3 Distribution- and Abundance-Based Analyses

For group variables, the Evenness and Reciprocal Simpsons indices found microbial diversity to be a predictor of glucose, and the Simpson metric to be a predictor of energy intake; however, the relationship with energy intake did not hold up with the combined p-value score cut-off. No other group variables were significantly associated with alpha diversity metrics either as a predictor or response. In contrast, various sociodemographic factors (education, age, Caribbean ethnicity) were generally significant and independent positive predictors of alpha diversity, while BMI was a negative predictor, across multiple variable groupings (example using the Diabetes Category variable grouping in **Table 4.5**).

Results of abundance-based analyses are summarized for the microbiome as a predictor of group variables (**Table 4.6**) or as a responder of group variables (**Table 4.7**). Taxa were more likely to be a significant response to group variables rather than a significant predictor of them. Of note, lower *Faecalibacterium* was a positive predictor of glucose; *Faecalibacterium* was also

inversely associated with diabetes categories, but this did not surpass the combined p-value score cut-off. Some reciprocal relationships were identified. When the microbiome was seen as a response, for example, Ruminococcus_1 was inversely associated with thigh IMAT and positively associated with thigh muscle; Succinivibrio was inversely associated with eating a high-fiber food ratio, but positively associated VAT; and Pseudobutyrivibrio was inversely associated with glucose but positively associated with log-HOMA-IR. Similarities in relationships with taxa also existed: for example, higher in Eubacterium_coprostanoligenes_grp were associated with higher abdominal SAT and thigh IMAT, and higher Incertae_Sedis was associated with higher thigh muscle and eating a higher high-fiber food ratio.

We then looked at the explanatory power of including alpha diversity metrics or ALR-transformed taxa as predictors of diabetes status (dichotomous or categories), glucose and insulin measures, and antidiabetic medication use. In all models, addition of microbial taxa resulted in a decreased model adjusted R^2, though none of these were statistically significant decreases (all $p > 0.4$). However, for multiple diversity metrics, significant minor increases in model explanatory power were detected if diversity was included as a predictor for glucose (**Table 4.8**).

4.5 Discussion

These analyses are a first step at building a model that uses the microbiome to predict type 2 diabetes. By looking at the microbiome at different levels (clusters, diversity, and relative abundance) and by looking at the microbiome as both a predictor and a response, we can piece together which variables may explain most of the variance in the microbiome, and consequently build an appropriate and parsimonious model for type 2 diabetes. In these preliminary analyses,

we demonstrated that some of the most significant factors impacting microbial clustering were ethnicity, BMI, and smoking status, while other lifestyle factors or body composition measures had little effect. We found consistent cluster-influencing bacteria across diabetes definitions and antidiabetic medication usage. At the alpha diversity level, the microbiome was only a predictor of fasting glucose, and was more affected as a response to sociodemographic factors. And at a relative abundance level, only *Faecalibacterium* showed a potential inverse association with glucose and diabetes categories.

The role of the microbiome with type 2 diabetes is complex and likely bidirectional. Previous studies indicate functional changes in the microbiome between diabetic and non-diabetic states (44, 45). Our group previously reported that a surrogate marker of gut-bacterial derived inflammation was associated with incident impaired fasting glucose and alterations in body composition (51). Thus, the actions of intestinal bacteria may promote insulin resistance and diabetes through multiple pathways. However, adoption of lifestyle changes (such as diet and exercise) or antidiabetic medication usage as a consequence of a diabetes diagnosis may also have impacts on microbial structure and function. Thus, teasing apart these relationships is essential to understanding not only the role of the microbiome in diabetes pathology, but also the potential impacts of therapeutic interventions on the microbiome.

We report that clustering was predominantly explained by ethnicity, BMI, and smoking status, and borderline with education, and similarly that sociodemographic variables were associated with bacterial alpha diversity. In the Hispanic Community Health Study/Study of Latinos, authors reported that factors related to migration, acculturation, and sociodemographics were strong influencers of intestinal microbiome diversity, and that the degree of adoption of a Westernized lifestyle was associated with a lower *Prevotella*/*Bacteroides* ratio (317). Similarly, a

study in the Netherlands reported that many sociodemographic factors were also associated with between-individual diversity, most notably ethnicity, and that *Prevotella* was higher among some ethnic groups who migrated from countries with high fiber intake (69). While Trinidad and Tobago as a nation is considered a high-income country (131), Tobago itself is less socioeconomically advantaged. While Tobagonians in our sample report relatively high-fiber intake, the population is also undergoing Westernization. Given that our study population is middle-aged and older and likely experienced a less Westenized lifestyle for most of their lives, it is possible that their gut microbiomes may be less impacted by these sociocultural changes, similar to the dampened impact of acculturation in older first-generation Latinx migrants (317); this may contribute to some of these similarities noted between our population and those from the Hispanic and Dutch migrant populations.

The association between alpha diversity and health is complex. Studies reporting alpha diversity metrics in relation to disease have been conflicting, which may be the result not only of different diversity metrics used, but also of incorrect assumptions that diversity metrics reflect stability, compositionality, or community interactions of intestinal microbiomes (318). Reports by Fei et al. showed that a lower alpha diversity was associated with higher cardiometabolic risk factors among African Ancestry individuals, but that these associations (and the particular risk factors that were associated with diversity) differed across the studied countries (311). In contrast, we report that alpha diversity was a positive predictor of fasting glucose. These differences not only highlight that associations may differ between geographical locations, but also further question the role of diversity metrics alone as a predictor of health-related outcomes.

At a relative abundance level, we report that individuals with worse diabetes profiles tended to share many bacteria from the *Firmicutes* phylum, especially from the *Clostridiales* order.

Many *Firmicutes* are known short chain fatty acid (SCFA) producers (319), and recent work has demonstrated that greater fecal excretion of SCFAs was associated with obesity and cardiometabolic dysfunction (320); while we do not have circulating or excreted levels of SCFA measured, it is possible that there is greater potential of SCFA production in our individuals with worse diabetes/glucose profiles given the higher *Clostridiales* presence. We also report that lower *Faecalibacterium* was associated with higher glucose and borderline with diabetes. While *Faecalibacterium* is also a SCFA producer, it is known for production of butyrate, which is thought to be beneficial for colonic health (319). It is also interesting to note that some associations were also observed with risk factors such as diet, physical activity, and body composition, and that some of the same taxa were associated with multiple of these measures. This may speak to the complex interrelatedness of lifestyle factors with the microbiome. Given that inclusion of microbial taxa as a predictor in models did not improve model explanatory power, this may also suggest that while these risk factors are all interrelated, targeting of modifiable traditional risk factors may still provide more benefit.

Our analyses have some limitations worth mentioning. First, this analytic sample originated from an ancillary pilot study of the microbiome in the Tobago Health Study population. The smaller sample size limits our statistical power and ability to thoroughly investigate some information such as diet. Second, this ancillary study also occurred a period of time after clinical variable collection. While we did adjust for time differences between clinical variable collection and fecal sample donation, and attempted to update diabetes status through additional medication collection, it is possible that changes in lifestyle habits or diabetes status may have occurred over the time difference period. Third, our analysis is limited only to men who were middle aged and older; thus, we are unable to generalize our results to younger populations or to women. Still, our

study has several strengths. This is the first reporting of the intestinal microbiome in the Lesser Antilles region of the Caribbean, and one of the few samples of predominantly African ancestry. We also had detailed collection of important confounders, including diet and medication, as well as unique measures including upper and lower body composition.

In conclusion, while we found some significant associations between the intestinal microbiome structure and fasting glucose levels in Tobagonian men, most explanatory power for diabetes remained in traditional risk factor variables. Further, various lifestyle factor and anthropometric predictors were associated with different microbial profiles. Future directions should investigate the relative contributions of combined lifestyle and microbiome on type 2 diabetes risk in this population, and whether the microbiome may act as a mediator between traditional risk factors and type 2 diabetes.

4.6 Tables and Figures

Table 4.1 Sample Characteristics

Variable Grouping	Variable	N (253)	Mean (SD), Median (IQR) or N (%)
Base Covariates	Age (years)	253	60 (56, 68)
	BMI (kg/m^2)	253	28.2 (4.8)
	African-Caribbean Ethnicity [Y/N]	249	228 (91.6%)
	Education Status Primary Secondary/Technical Vocational Training Some University/Associate Degree University Graduate or Advanced Degree	247	 173 (70.0%) 55 (22.3%) 11 (4.5%) 8 (3.2%)
	Current Smoker [Y/N]	253	23 (9.1%)
	Antibiotic Medication Use [Y/N]	253	7 (2.8%)
	Lipid-Modifying Medication Use [Y/N]	253	20 (7.9%)
	Dry Season [Y/N]	253	120 (47.4%)
	Time difference between clinic visit and fecal sample collection (years)	253	2.5 (1.6, 2.6)
Diet	Energy Intake (kcal)	249	2419.6 (862.7)
	High Fiber Food Intake (g)	249	1181.4 (879.4, 1551.5)
	Low Fiber Food Intake (g)	249	902.4 (628.2, 1212.1)
Physical Activity	Hours Walked per Week	250	2.0 (0.0, 6.0)
	Watches Television ≥ 14 hours/week [Y/N]	251	116 (46.2%)
Body Composition	Abdominal SAT Volume (cm^3)	243	185.1 (133.7, 249.8)
	Abdominal VAT Volume (cm^3)	243	100.0 (56.8, 130.2)
	Abdominal Other Volume (cm^3)	243	312.2 (45.6)
	Total Abdominal Volume (cm^3)	243	596.7 (503.8, 685.3)
	Thigh SAT Volume (cm^3)	237	352.5 (238.5, 515.1)
	Thigh Muscle Volume (cm^3)	239	1079.8 (186.3)
	Thigh IMAT Volume (cm^3)	239	109.4 (45.2)
	Thigh Bone Volume (cm^3)	241	45.4 (5.2)
	Total Thigh Volume (cm^3)	237	1626.1 (350.7)
	Time difference between CT scan and fecal sample collection (years)	245	1.6 (1.0, 2.3)
Diabetes	Glucose (mg/dL)	251	89.0 (82.0, 108.0)
	Insulin (µU/mL)	251	9.7 (6.7, 14.8)
	HOMA-IR	251	2.4 (1.5, 3.6)
	Antidiabetic Medication Use [Y/N]	253	42 (16.6%)
	Type 2 Diabetes [Y/N]	253	57 (22.5%)
	Type 2 Diabetes Categories Normal Impaired Fasting Glucose Type 2 Diabetes	253	 169 (66.8%) 27 (10.7%) 57 (22.5%)

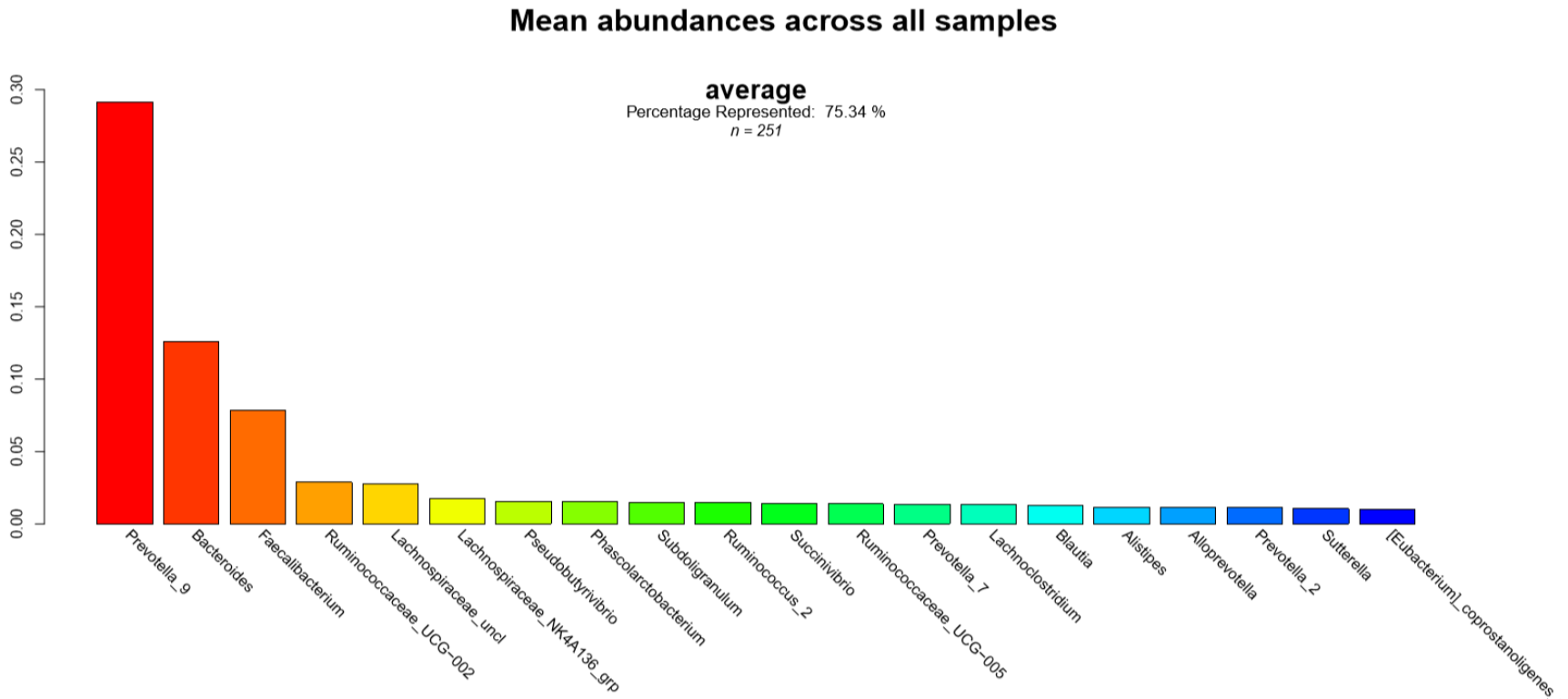


Figure 4.1 Mean taxon abundance across samples (N=251)

Table 4.2 PERMANOVA Significant Variables, by Group Variable Categories

Group Variable	Statistically Significant Variables
Body Composition	African Caribbean ethnicity and BMI
Diet	African Caribbean ethnicity, BMI, smoking status, and total energy intake
Physical Activity	African Caribbean ethnicity, BMI, and smoking status
Glucose/Insulin	African Caribbean ethnicity, BMI, smoking status, and education status
Diabetes [Y/N]	African Caribbean ethnicity, BMI, and smoking status
Diabetes Categories	African Caribbean ethnicity, BMI, and smoking status

Table 4.3 Cluster Cutoffs and Variables Associated with Clustering, by Group Variable Categories

Group Variable	Optimal Cluster Cutoff (Calinski-Harabasz criteria)	Variable Significantly Associated with Cluster	Cluster Association and Direction
Body Composition	6	African Caribbean Ethnicity	Cluster 3 (-)
		Age	Cluster 2 (+), Cluster 3 (-)
		Time difference between clinical visit and fecal sample collection	Cluster 1 (+)
		Time difference between CT scan and fecal sample collection	Cluster 1 (-), Cluster 6 (+)
		Total Abdominal CT Volume	Cluster 2 (-), Cluster 3 (+)
Diet	6	African Caribbean Ethnicity	Cluster 2 (-), Cluster 4 (-)
		BMI	Cluster 4 (+)
		Total Energy Intake	Cluster 2 (-), Cluster 4 (+)
Physical Activity	2	BMI	Cluster 1 (-), Cluster 2(+)
Glucose/Insulin	6	African Caribbean Ethnicity	Cluster 2 (-)
		Age	Cluster 4 (-)
		Smoking Status	Cluster 4 (+)
		Antidiabetic Medication	Cluster 4 (+)
Diabetes [Y/N]	6	African Caribbean Ethnicity	Cluster 2 (-)
		Age	Cluster 4 (-), Cluster 5 (+)
		Diabetes	Cluster 1 (+), Cluster 3 (-)
Diabetes Categories	6	African Caribbean Ethnicity	Cluster 2 (-)
		Age	Cluster 4 (-), Cluster 5 (+)
		Diabetes Category	Cluster 1 (+), Cluster 3 (-)

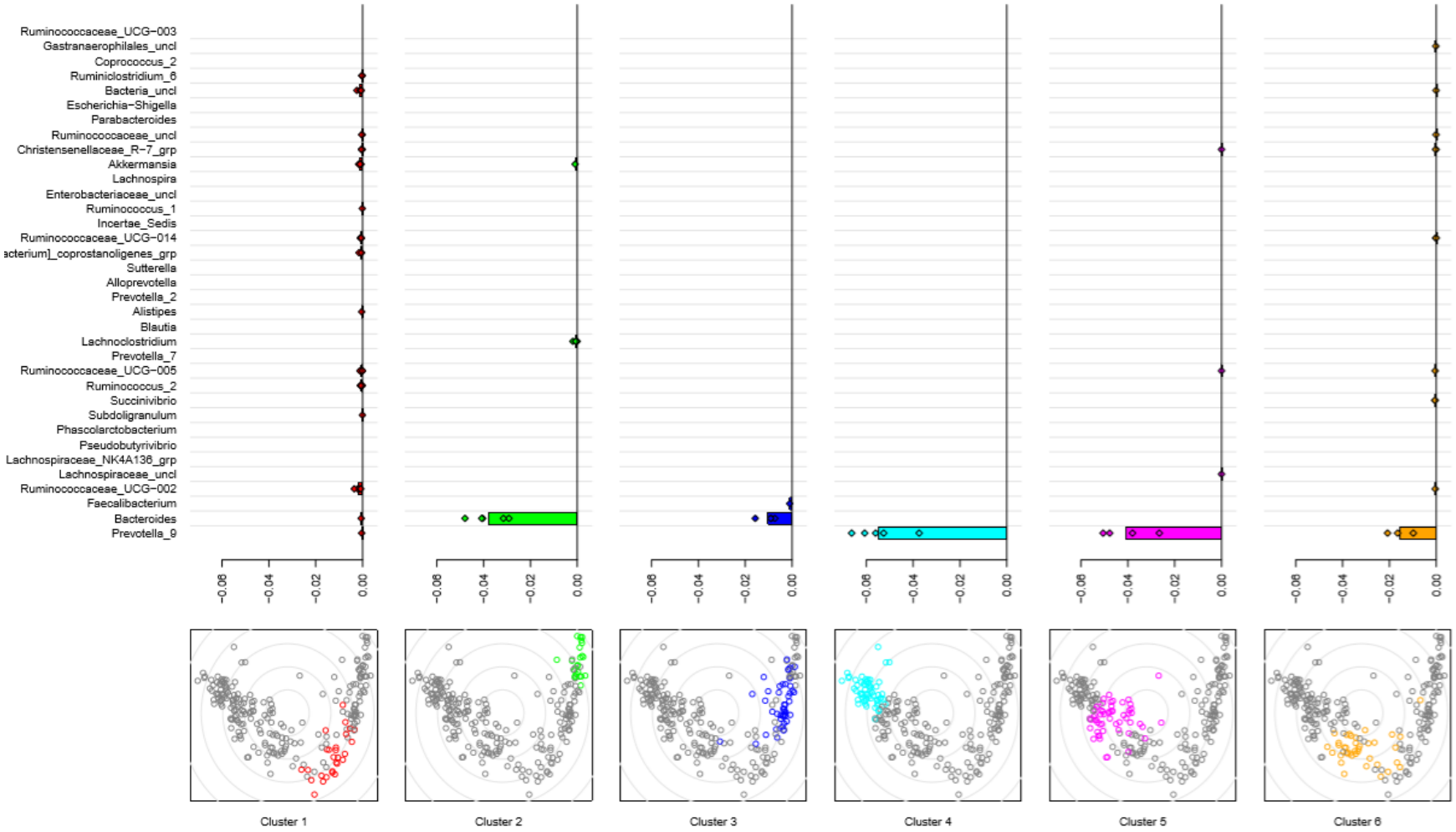


Figure 4.2 Cluster Unifiers in Diabetes Category Groupings

The Y-axis shows the top 35 abundant taxa, while the X-axis displays samples by cluster number. The bars on each line indicate the $\log_{10} R^2$ ratio for inclusion of that taxon, such that a larger bar indicates importance of that taxon in unifying cluster membership.

Table 4.4 Naturally-Occurring Cluster Influencing Taxa

Group Variable	Group	Cluster Influencers (Unifiers)
Physical Activity	Doesn't Watch TV ≥ 14 hours/week	Ruminococcaceae_UCG-002
	Watches TV ≥ 14 hours/week	Lachnospiraceae_NK4A136_grp, Succinivibrio, Alistipes, Alloprevotella, Prevotella_2, Sutterella, Ruminococcus_1, Incertae_Sedis, Enterobacteriaceae_uncl, Akkermansia, Christensenellaceae_r-7_grp, Parabacteroides, Ruminiclostridium_6, Gastranaerophilales_uncl
Glucose/Insulin	Antidiabetic Medication = No	Prevotella_9, Ruminococcaceae_UCG-002
	Antidiabetic Medication = Yes	Gastranaerophilales_uncl, Ruminococcaceae_UCG-003, Coprococcus_2, Ruminiclostridium_6, Baceria_uncl, Ruminococcaceae_uncl, Akkermansia, Enterobacteriaceae_uncl, Eubacterium_coprostanoligenes_grp, Blautia, Prevotella_7, Ruminococcus_2, small Sutterella, Pseudobutyrivibrio
Diabetes [Y/N]	Type 2 Diabetes = No	Coprococcus_2
	Type 2 Diabetes = Yes	Gastranaerophilales_uncl, Ruminococcaceae_UCG-003, Ruminiclostridium_6, Baceria_uncl, Ruminococcaceae_uncl, Akkermansia, Enterobacteriaceae_uncl, Ruminococcaceae_UCG-014, Eubacterium_coprostanoligenes_grp, Blautia, Succinivibrio, Pseudobutyrivibrio, Lachnospiraceae_NK4A136_grp, Ruminococcaceae_UCG-002, Sutterella, Alloprevotella
Diabetes Category	Normal Glucose	Lachnospiraceae_uncl, Coprococcus_2
	Impaired Fasting Glucose	Ruminoclostridium_6, Christensenellaceae_R-7_grp, Akkermansia, Enterobacteriaceae_uncl, Eubacterium_coprostanoligenes_grp, Prevotella_2, Ruminococcaceae_UCG-005, Pseudobutyrivibrio, Lachnospiraceae_ uncl, Prevotella_9, Ruminococcus_1, Incertae_Sedis, Alloprevotella, Lachnoclostridium, Sutterella
	Type 2 Diabetes	Ruminococcaceae_UCG-003, Ruminiclostridium_6, Bacteria_uncl, Ruminococcaceae_uncl, Akkermansia, Enterobacteriaceae_uncl, Ruminococcaceae_UCG-014, Eubacterium_coprostanoligenes_grp, Blautia, Succinivibrio, Pseudobutyrivibrio, Lachnospiraceae_NK4A136_uncl, Ruminococcaceae_UCG-002, Faecalibacterium

Table 4.5 Significant coeficients predicting Alpha Diversity metrics, adjusted for other covariates in Diabetes Category grouping

	Coefficient (p-value < 0.05)			
Alpha Diversity Metric	Age	BMI	Education	African Caribbean Ethnicity
Tail	0.08	--	--	--
Shannon	--	--	--	--
Simpson	--	-0.002	0.02	--
Reciprocal Simpson	--	-0.02	0.12	--
Evenness	--	--	0.02	0.04

Table 4.6 Taxa Relative Abundance as Predictors, within Group Variable Categories

Group	Variable	Taxa
Body Composition	Abdominal SAT	↑: Eubacterium_coprostanoligenes_grp
	Thigh IMAT	↑: Eubacterium_coprostanoligenes_grp
	Thigh Muscle	↑: Alistipes
Physical Activity	Hours Walked per Week	↑: Ruminococcaceae_UCG-005
		↓: Ruminococcus_1
Glucose/Insulin	Glucose	↓: Faecalibacterium

All taxa surpassed the combined p-value score cut-off

Table 4.7 Taxa Relative Abundance as Responders, within Group Variable Categories

Microbiome as a Response		
Group	Variable	Taxa
Body Composition	Abdominal SAT	↑: Ruminococcaceae_uncl
	Thigh IMAT	↓: Ruminococcus_1, Dialister
	Thigh Muscle	↑: Ruminococcus_1, Incertae_Sedis
	Abdominal VAT	↑: Succinivibrio
	Total Abdominal Volume	↓: Coprococcus_2
Diet	Energy Intake	↓: Bacteroides, Alistipes
	High Fiber/Low Fiber Food Ratio	↑: Lachnospira, Incertae_Sedis ↓: Succinivibrio
Physical Activity	Hours Walked per Week	↑: Lachnospiraceae_NK4A136_grp
Glucose/Insulin	Glucose	↓: Pseudobutyrivibrio
	Log(HOMA-IR)	↑: Pseudobutyrivibrio

All taxa surpassed the combined p-value score cut-off

Table 4.8 Change in adjusted R^2 comparing models with and without diversity metrics as a predictor of glucose

Diversity Metric	Δ in Adj. R^2	Difference ANOVA p-value
Tail	0.0063	0.1138
Shannon	0.0142	0.0362
Simpson	0.0158	0.0287
Reciprocal Simpson	0.0182	0.0205
Evenness	0.0178	0.0220

5.0 Overall Conclusions and Public Health Significance

5.1.1 Dissertation Results Summary

This dissertation showed the relationship of novel risk factors – adipose tissue radiodensity and the intestinal microbiome – with type 2 diabetes in an understudied racial/ethnic group. It also applied an approach used in microbiome research, compositional data analysis, to the field of body composition and demonstrated that abdominal subcutaneous adipose tissue is a major driver of diabetes in this population. Taken together, these papers provide new information in an understudied population, filling in multiple gaps in the literature

The results of Paper 1 showed that lower adipose tissue radiodensity in both abdominal and peripheral (IMAT) adipose tissues was associated with higher insulin and insulin resistance. This paper expands the knowledge of adipose tissue radiodensity to include non-abdominal IMAT, which was found to be independent of (but a similar magnitude to) abdominal VAT. Paper 1 also explored the relationship between adipose tissue radiodensity and volume, with results indicating that radiodensity may be a more informative marker of insulin resistance than volume.

Paper 2 further expanded on body composition analyses and the role of non-abdominal tissues in type 2 diabetes development. We found that, relative to non-VAT/SAT abdominal tissue, increases in abdominal SAT volume was positively associated with type 2 diabetes, while VAT was not associated; in contrast, increases thigh muscle (relative to thigh bone) was inversely associated with type 2 diabetes. We also confirmed previous findings that SAT in the abdomen and thigh have opposing relationships with diabetes, with lower body SAT being somewhat protective.

In both Papers 1 and 2, abdominal VAT and SAT were positively associated with insulin and insulin resistance; however, in Paper 2, thigh IMAT size was inversely associated with insulin and insulin resistance while IMAT radiodensity was positively associated in Paper 1. It is important to note that while Paper 1 demonstrated a strong relationship between adipose tissue radiodensity and volume, that the analysis in Paper 2 used an entirely different transformation of volume (looking at increases relative to another component), included more tissue types than Paper 1 did, and did not exclude individuals with type 2 diabetes as Paper 1 did. Still, further research will still be needed to determine if the structural qualities we believe tissue radiodensity is measuring (i.e. predominantly lipid accumulation) are consistent across tissue types and anatomical locations.

The results of Paper 3 begin to tie in the microbiome to the relationships between traditional risk factors, body composition, and type 2 diabetes. Our preliminary findings show that microbial clustering was driven primarily by sociodemographic and lifestyle factors. We also identified specific taxa that are associated with body composition, diet, physical activity, and diabetes-related variables. While the next steps will include building models predicting type 2 diabetes while simultaneously adjusting for many of the group factors together, our current assessments may hint at some of the bidirectional and mediating mechanisms by which the microbiome can influence diabetes risk. Of future research interest are the associations with body composition measures, which are themselves likely to be mediators in the relationship between the microbiome and type 2 diabetes.

5.1.2 Dissertation Public Health Significance

This dissertation has made three significant contributions with impacts in public health research. First, this research includes an underrepresented racial/ethnic group who are at increased risk of type 2 diabetes. As previous research in all of these aims have a noticeable lack of information in African Ancestry participants, these dissertation aims are providing crucial insights that improve our collective knowledge on the pathophysiology of type 2 diabetes and how it might differ across different groups and geographical locations.

Second, findings from this dissertation call for a change in body composition analytic methods. In my first aim, we demonstrate that when investigating adipose tissue radiodensity, it is likely inappropriate to adjust further for tissue volume given the strong curvilinear relationship between volume and radiodensity and given that this further adjustment does not improve model fit criteria. In my second aim, we apply a methodology (compositional data analysis) which is used in the microbiome field, and in doing so eliminate many of the concerns and analytic missteps in the body composition field. Importantly, this methodology can be used for a variety of imaging methods, and as such may have broader utility for body composition analysts as well as other studies utilizing imaging data for health-related research.

And third there are some results and products generated by this dissertation which may have immediate influence on research and health in Tobago. In the process of completing this dissertation, I have developed databases housing information on both diet and the microbiome in the Tobago Health Study. These databases are the first for this study and for the island of Tobago, and the applications for analyses and future visits within the Tobago Health Study cohorts will further aid in disentangling the complex interplay of the many diabetes risk factors as they exist in Trinidad and Tobago. Additionally, our strong findings relating subcutaneous adipose tissue

volume and radiodensity to diabetes may also have clinical applications for Tobago, as assessment of diabetes risk could be assessed through means such as waist circumference or skin-fold tests.

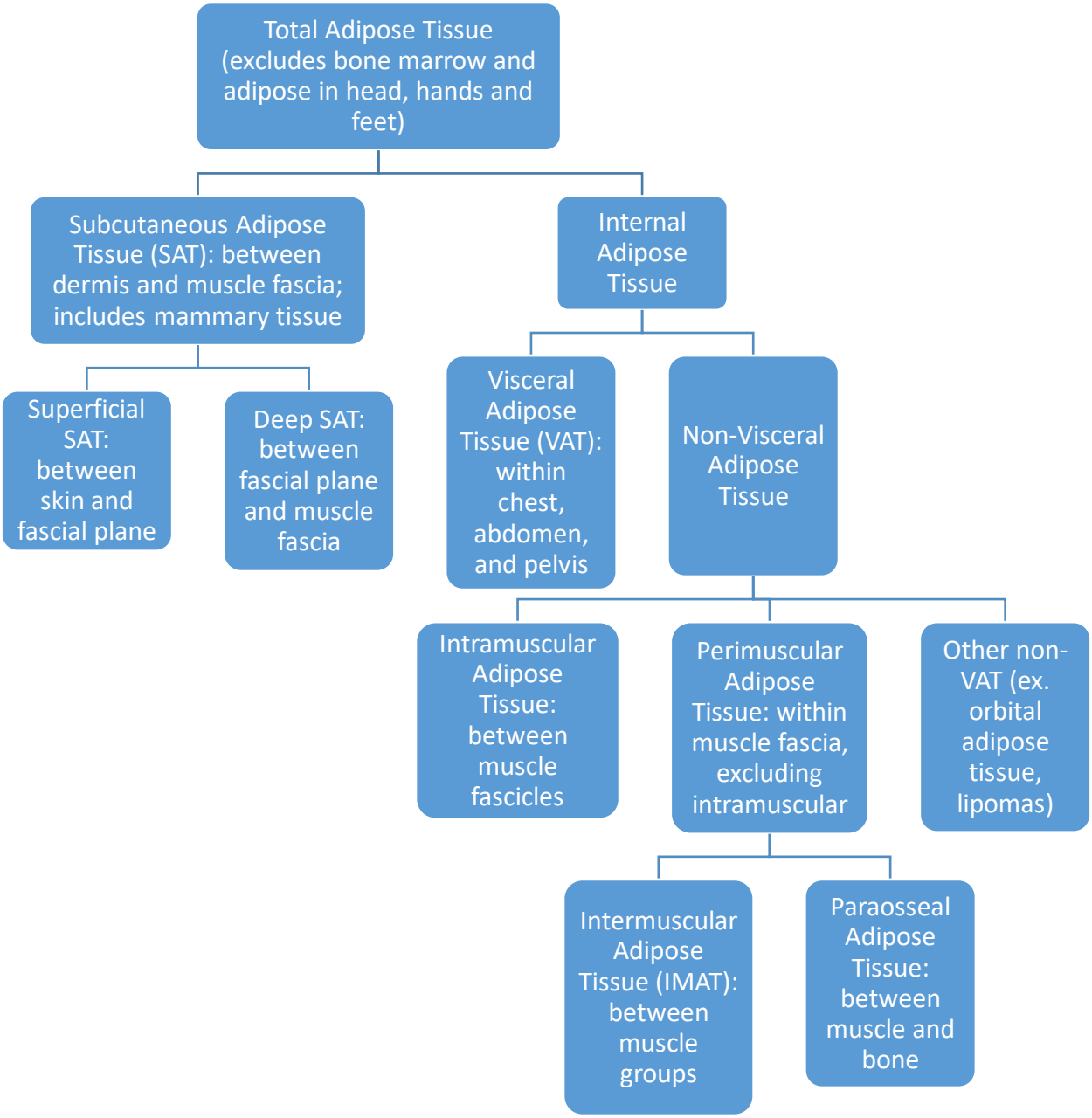
Appendix A Background Tables and Figures

Appendix A Table 1 Levels of Physical Activity Definitions

Activity Level	STEPS (148)	Tobago	
	Method: Self-report	Method: Self-Report	Method: Activity Monitor
High	• Vigorous-intensity activity on 3+ days (≥1,500 MET-minutes/week) OR • 7+ days of any combination of walking, moderate- or vigorous-intensity activities (≥3,000 MET-minutes per week)	How many hours were spent a day doing heavy physical activity? (Examples- heavy construction work, heavy farming, fishing with a net, etc.)	Percent of monitor wear time in vigorous activity, no sleep
Moderate	• 3+ days of vigorous-intensity activity of ≥20 minutes/day OR • 5+ days of moderate-intensity activity or walking ≥30 minutes/day OR • 5+ days of any combination of walking, moderate- or vigorous-intensity activities (≥600 MET-minutes/week)	How many hours were spent a day doing moderate physical activity? (Examples- Gardening, carrying light loads, strenuous housework, continuous walking, repairs, light construction work, e.g. plumbing, electrician, etc.)	Percent of monitor wear time in moderate activity, no sleep
Low/Light	Not meeting any of the above criteria	How many hours were spent a day doing light physical activity? (Examples- Sitting or reclining, standing, driving, light housework, slow leisure walking, etc.). TV-watching time was subtracted from this to try and remove "sitting or reclining" as a light physical activity.	Percent of monitor wear time in light activity, no sleep

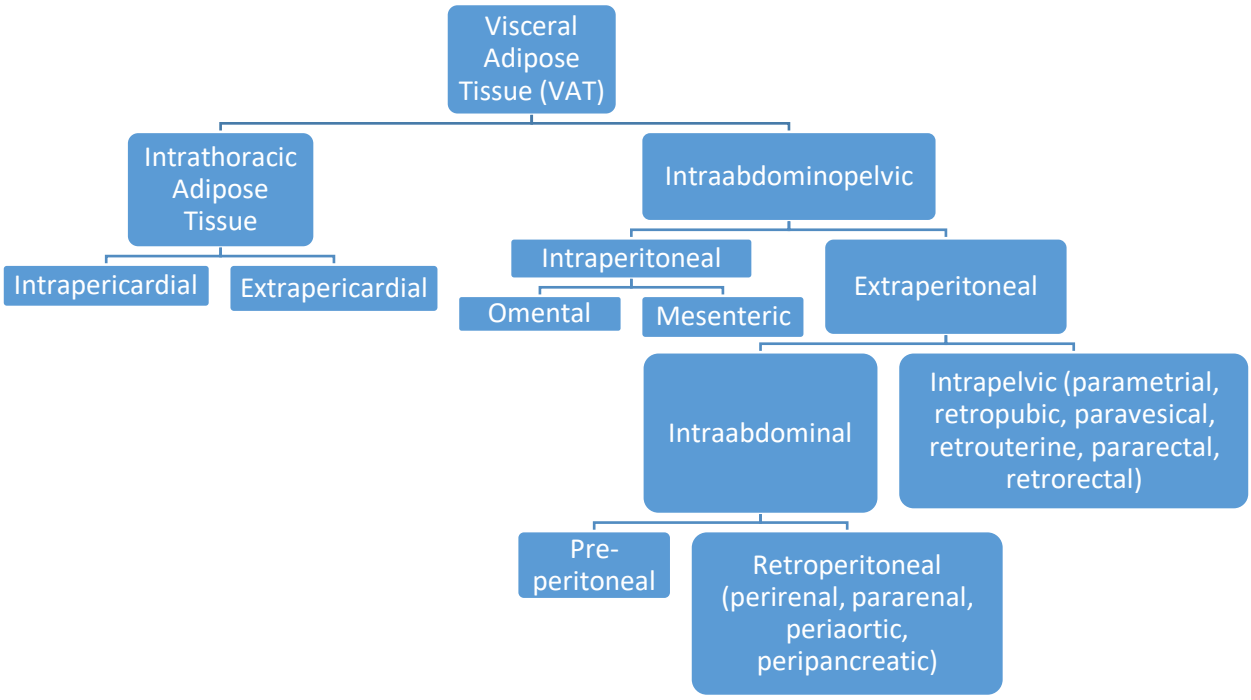
Appendix A Table 1 continued

Sedentary	Minutes/day spent in sedentary activities (sitting or reclining)	Hours/week watching television, averaged	Percent of monitor wear time in sedentary activity, no sleep



Appendix A Figure 1 Classification of Adipose Tissue Depots.

Adapted from Shen et al (1)



Appendix A Figure 2 Classification of Visceral Adipose Tissue Depots.

Adapted from Shen et al (1)

Appendix B Paper 1 Supplementary Tables/Figures

Appendix B Table 1 Study population characteristics, stratified by median value of AT depot radiodensity

Variable	Low VAT Radiodensity (n=253)	High VAT Radiodensity (n=252)	P-value	Low SAT Radiodensity (n=253)	High SAT Radiodensity (n=252)	P-value	Low IMAT Radiodensity (n=253)	High IMAT Radiodensity (n=252)	P-value
Age (years)	61.0 (56.0, 68.0)	61.0 (57.0, 68.0)	0.5516	61.0 (56.0, 68.0)	61.0 (56.0, 68.0)	0.8439	60.0 (56.0, 67.0)	61.5 (57.5, 70.0)	0.0050
Weight (kg)	90.2 (81.0, 98.0)	75.2 (67.9, 83.7)	<.0001	90.1 (79.0, 100.3)	75.5 (68.0, 84.5)	<.0001	86.5 (77.5, 95.9)	77.0 (68.4, 88.3)	<.0001
Height (cm)	175.6 (6.4)	175.5 (6.9)	0.8690	175.1 (6.5)	176.0 (6.8)	0.1556	175.7 (6.5)	175.5 (6.8)	0.7189
BMI (kg/m^2)	29.0 (26.7, 32.0)	24.6 (22.5, 26.9)	<.0001	29.0 (26.7, 32.2)	24.7 (22.5, 27.0)	<.0001	28.0 (25.9, 30.9)	25.2 (22.7, 28.1)	<.0001
Waist Circumference (cm)	102.1 (95.9, 109.1)	89.3 (84.1, 95.9)	<.0001	103.0 (95.3, 110.6)	89.4 (84.0, 96.0)	<.0001	99.1 (93.2, 107.1)	91.0 (84.1, 99.2)	<.0001
BMI Category *									
Normal Weight (%)	26 (10.3%)	133 (53.6%)		30 (11.9%)	129 (52.0%)		40 (15.8%)	119 (48.0%)	
Overweight (%)	126 (49.8%)	93 (37.5%)		118 (46.6%)	101 (40.7%)		129 (51.0%)	90 (36.3%)	
Obese (%)	101 (39.9%)	22 (8.9%)	<.0001	105 (41.5%)	18 (7.3%)	<.0001	84 (33.2%)	39 (15.7%)	<.0001
Lifestyle and Comorbidities									
Current Smoker (%)	14 (5.5%)	22 (8.7%)	0.1627	14 (5.5%)	22 (8.7%)	0.1627	18 (7.1%)	18 (7.1%)	0.9902
Drinks Alcohol 4+/week (%)	44 (17.4%)	23 (9.1%)	0.0062	38 (15.0%)	29 (11.5%)	0.2447	41 (16.2%)	26 (10.3%)	0.0511

Appendix B Table 1 Continued

Hours Walked per Week	2.0 (0.0, 5.3)	1.5 (0.0, 5.0)	0.7630	2.0 (0.0, 5.3)	1.5 (0.0, 5.0)	0.9246	2.0 (0.0, 5.0)	1.5 (0.0, 5.3)	0.7298
Watches TV ≥ 14/week (%)	123 (48.6%)	119 (47.2%)	0.7538	134 (53.0%)	108 (42.9%)	0.0230	125 (49.4%)	117 (46.4%)	0.5029
Impaired Fasting Glucose (%)	43 (17.0%)	33 (13.1%)	0.2203	48 (19.0%)	28 (11.1%)	0.0135	41 (16.2%)	35 (13.9%)	0.4666
CT-Derived Measures									
VAT Volume (cm^3)	116.3 (93.5, 141.7)	49.7 (31.6, 68.6)	<.0001	107.9 (78.3, 137.1)	51.7 (30.9, 77.4)	<.0001	98.1 (68.5, 128.7)	60.9 (31.9, 102.5)	<.0001
SAT Volume (cm^3)	209.8 (173.8, 273.0)	122.4 (78.2, 174.6)	<.0001	229.5 (183.0, 288.1)	119.4 (77.0, 162.4)	<.0001	203.9 (168.1, 266.3)	133.7 (79.1, 188.2)	<.0001
IMAT Volume (cm^3)	129.3 (103.4, 157.0)	89.9 (62.7, 113.5)	<.0001	129.3 (103.3, 156.2)	89.4 (61.8, 112.7)	<.0001	129.3 (101.3, 160.4)	91.8 (62.2, 116.0)	<.0001
VAT Radiodensity (HU)	-94.7 (-97.9, -92.5)	-82.0 (-86.1, -77.4)	<.0001	-94.3 (-97.8, -90.3)	-82.5 (-88.4, -77.4)	<.0001	-93.2 (-96.7, -88.5)	-84.6 (-90.5, -78.5)	<.0001
SAT Radiodensity (HU)	-102.5 (-105.2, -100.3)	-94.3 (-98.9, -84.4)	<.0001	-103.3 (-105.4, -101.5)	-93.9 (-97.8, -83.5)	<.0001	-102.1 (-104.9, -99.7)	-95.1 (-99.7, -84.1)	<.0001
IMAT Radiodensity (HU)	-72.2 (-74.0, -70.4)	-68.7 (-71.4, -63.6)	<.0001	-72.4 (-74.2, -70.9)	-68.4 (-70.8, -63.0)	<.0001	-73.3 (-74.7, -71.9)	-68.0 (-69.5, -63.0)	<.0001
Total Abdominal Volume (cm^3)	643.5 (565.3, 747.8)	473.4 (416.5, 555.5)	<.0001	645.1 (564.6, 754.6)	472.5 (411.5, 557.7)	<.0001	612.2 (531.5, 714.8)	501.7 (417.9, 612.2)	<.0001
Total Thigh Volume (cm^3)	1750.6 (1604.1, 1964.7)	1463.3 (1291.5, 1650.2)	<.0001	1774.9 (1625.3, 1992.5)	1460.3 (1288.0, 1623.4)	<.0001	1717.2 (1552.2, 1959.0)	1465.2 (1302.2, 1692.4)	<.0001
Glucose and Insulin Metabolism									
Glucose (mmol/l)	4.8 (4.4, 5.2)	4.7 (4.4, 5.3)	0.0641	4.9 (4.4, 5.3)	4.7 (4.4, 5.1)	0.0082	4.8 (4.4, 5.3)	4.8 (4.4, 5.2)	0.2355
Insulin (pmol/l)	72.0 (50.0, 101.0)	36.0 (27.0, 53.0)	<.0001	74.0 (50.0, 102.0)	36.0 (27.0, 53.5)	<.0001	68.0 (45.0, 95.0)	38.0 (28.0, 61.5)	<.0001

Appendix B Table 1 Continued

HOMA2-IR	1.33 (0.95, 1.86)	0.68 (0.51, 0.97)	<.0001	1.36 (0.93, 1.86)	0.67 (0.49, 0.97)	<.0001	1.24 (0.85, 1.75)	0.72 (0.51, 1.16)	<.0001

Results reported as Mean (SD) for normally distributed and Median (IQR) for non-normally distributed continuous variables, or N (%) for categorical. Statistical comparisons were made using two-sample t-test or Wilcoxon rank-sum test for continuous variables and Chi-square tests for categorical variables within an AT depot. Median VAT radiodensity was -89.6, median SAT radiodensity was -99.7, and median IMAT radiodensity was -70.9.

* = excludes 4 individuals who were underweight and all located in the high radiodensity groups for each AT depot (Fisher's Exact p for each: $p < 0.0001$)

Abbreviations: AT = Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue, HU=Hounsfield Unit

Appendix B Table 2 Sensitivity Analyses for Multiple Linear Regression Glucose Models β (95%CI), per SD Higher AT Radiodensity

Model 1: Adjusting for Covariates and a Specific AT Radiodensity	Outcome: Glucose		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.04 (-0.11, 0.03)	-0.05 (-0.12, 0.02)	-0.09 (-0.18, -0.01)
	SAT Radiodensity	(SD = 10.71 HU)	-0.05 (-0.12, 0.02)	-0.07 (-0.15, 0.01)	-0.03 (-0.11, 0.05)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.01 (-0.07, 0.05)	-0.02 (-0.09, 0.05)	-0.02 (-0.09, 0.05)
Model 2A: Adjusting for Covariates and VAT + SAT Radiodensities	Outcome: Glucose		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + Two AT Radiodensities	Covariates + Two AT Radiodensities	Covariates + Two AT Radiodensities
	VAT Radiodensity	(SD = 8.44 HU)	-0.03 (-0.10, 0.05)	-0.03 (-0.11, 0.05)	-0.08 (-0.17, 0.01)
	SAT Radiodensity	(SD = 10.71 HU)	-0.03 (-0.11, 0.04)	-0.06 (-0.14, 0.03)	-0.04 (-0.13, 0.05)

Appendix B Table 2 Continued

Model 2B: Adjusting for Covariates and VAT + IMAT Radiodensities	Outcome: Glucose		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + Two AT Radiodensities	Covariates + Two AT Radiodensities	Covariates + Two AT Radiodensities
	VAT Radiodensity	(SD = 8.44 HU)	-0.04 (-0.11, 0.03)	-0.05 (-0.12, 0.03)	-0.09 (-0.18, -0.00)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.00 (-0.07, 0.07)	-0.01 (-0.08, 0.06)	-0.01 (-0.09, 0.06)
Model 2C: Adjusting for Covariates and SAT + IMAT Radiodensities	Outcome: Glucose		Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + Two AT Radiodensities	Covariates + Two AT Radiodensities	Covariates + Two AT Radiodensities
	SAT Radiodensity	(SD = 10.71 HU)	-0.10 (-0.21, 0.02)	-0.12 (-0.23, 0.00)	-0.08 (-0.18, 0.03)
	IMAT Radiodensity	(SD = 5.70 HU)	0.06 (-0.04, 0.16)	0.05 (-0.05, 0.16)	0.05 (-0.06, 0.15)
Model 3: Adjusting for Covariates and VAT +	Outcome: Glucose		Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes

Appendix B Table 2 Continued

SAT + IMAT Radiodensities					
	Model		Covariates + All Three AT Radiodensities	Covariates + All Three AT Radiodensities	Covariates + All Three AT Radiodensities
	VAT Radiodensity	(SD = 8.44 HU)	-0.02 (-0.10, 0.05)	-0.03 (-0.11, 0.05)	-0.08 (-0.16, 0.01)
	SAT Radiodensity	(SD = 10.71 HU)	-0.08 (-0.21, 0.04)	-0.10 (-0.23, 0.02)	-0.08 (-0.20, 0.03)
	IMAT Radiodensity	(SD = 5.70 HU)	0.05 (-0.05, 0.16)	0.05 (-0.05, 0.16)	0.05 (-0.06, 0.16)
	Model reported in manuscript (adjusts for age, weight, height, alcohol intake, smoking, hours walked/week, and TV watching ≥ 14 hours/week)				
	Sensitivity analyses:	1) Mutual adjustment of SAT and IMAT radiodensities (row 22), or SAT, VAT, and IMAT radiodensities (row 28) 2) Additional adjustment for total abdominal volume and total thigh volume (column E) 3) Additional adjustment for respective AT depot volume			
Abbreviations: AT = Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue, HU=Hounsfield Unit					

Appendix B Table 3 Sensitivity Analyses for Multiple Linear Regression Log-Insulin Models β (95%CI), per SD Higher AT Radiodensity

Model 1: Adjusting for Covariates and a Specific AT Radiodensity	Outcome: Log-Insulin		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.16 (-0.21, -0.11)	-0.14 (-0.19, -0.08)	-0.15 (-0.22, -0.08)
	SAT Radiodensity	(SD = 10.71 HU)	-0.18 (-0.24, -0.13)	-0.16 (-0.22, -0.10)	-0.15 (-0.22, -0.09)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.16 (-0.21, -0.11)	-0.14 (-0.20, -0.09)	-0.18 (-0.24, -0.13)
Model 2A: Adjusting for Covariates and VAT + SAT Radiodensities	Outcome: Log-Insulin		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.10 (-0.16, -0.05)	-0.10 (-0.16, -0.04)	-0.11 (-0.18, -0.04)
	SAT Radiodensity	(SD = 10.71 HU)	-0.14 (-0.20, -0.08)	-0.13 (-0.19, -0.06)	-0.11 (-0.18, -0.04)

Appendix B Table 3 Continued

Model 2B: Adjusting for Covariates and VAT + IMAT Radiodensities	Outcome: Log-Insulin		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.12 (-0.17, -0.07)	-0.11 (-0.17, -0.06)	-0.12 (-0.19, -0.05)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.13 (-0.18, -0.08)	-0.12 (-0.17, -0.07)	-0.15 (-0.21, -0.10)
Model 2C: Adjusting for Covariates and SAT + IMAT Radiodensities	Outcome: Log-Insulin		Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	SAT Radiodensity	(SD = 10.71 HU)	-0.11 (-0.20, -0.03)	-0.09 (-0.18, 0.00)	-0.06 (-0.16, 0.03)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.08 (-0.16, -0.00)	-0.09 (-0.16, -0.01)	-0.12 (-0.20, -0.03)
Model 3: Adjusting for Covariates and VAT +	Outcome: Log-Insulin		Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes

Appendix B Table 3 Continued

SAT + IMAT Radiodensities					
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.11 (-0.17, -0.05)	-0.10 (-0.16, -0.05)	-0.11 (-0.18, -0.04)
	SAT Radiodensity	(SD = 10.71 HU)	-0.05 (-0.14, 0.04)	-0.04 (-0.13, 0.05)	-0.00 (-0.10, 0.10)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.09 (-0.17, -0.02)	-0.10 (-0.17, -0.02)	-0.13 (-0.21, -0.05)
	Model reported in manuscript (adjusts for age, weight, height, alcohol intake, smoking, hours walked/week, and TV watching ≥ 14 hours/week)				
	Sensitivity analyses:	1) Mutual adjustment of SAT and IMAT radiodensities (row 22), or SAT, VAT, and IMAT radiodensities (row 28) 2) Additional adjustment for total abdominal volume and total thigh volume (column E) 3) Additional adjustment for respective AT depot volume			
Abbreviations: AT = Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue, HU=Hounsfield Unit					

Appendix B Table 4 Sensitivity Analyses for Multiple Linear Regression HOMA2-IR Models β (95%CI), per SD Higher AT Radiodensity

Model 1: Adjusting for Covariates and a Specific AT Radiodensity	Outcome: Log-HOMA2-IR		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.16 (-0.21, -0.11)	-0.14 (-0.20, -0.09)	-0.15 (-0.22, -0.08)
	SAT Radiodensity	(SD = 10.71 HU)	-0.18 (-0.24, -0.13)	-0.17 (-0.22, -0.11)	-0.15 (-0.22, -0.09)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.16 (-0.20, -0.11)	-0.14 (-0.20, -0.09)	-0.18 (-0.23, -0.13)
Model 2A: Adjusting for Covariates and VAT + SAT Radiodensities	Outcome: Log-HOMA2-IR		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.10 (-0.16, -0.05)	-0.10 (-0.16, -0.04)	-0.11 (-0.18, -0.04)
	SAT Radiodensity	(SD = 10.71 HU)	-0.14 (-0.20, -0.08)	-0.13 (-0.19, -0.07)	-0.11 (-0.18, -0.05)

Appendix B Table 4 Continued

Model 2B: Adjusting for Covariates and VAT + IMAT Radiodensities	Outcome: Log-HOMA2-IR		Reported in Manuscript; Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.12 (-0.17, -0.07)	-0.11 (-0.17, -0.06)	-0.12 (-0.19, -0.06)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.13 (-0.18, -0.08)	-0.12 (-0.17, -0.07)	-0.15 (-0.20, -0.10)
Model 2C: Adjusting for Covariates and SAT + IMAT Radiodensities	Outcome: Log-HOMA2-IR		Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes
	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	SAT Radiodensity	(SD = 10.71 HU)	-0.12 (-0.20, -0.03)	-0.10 (-0.18, -0.01)	-0.07 (-0.16, 0.03)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.07 (-0.15, 0.00)	-0.08 (-0.16, 0.00)	-0.11 (-0.19, -0.03)
Model 3: Adjusting for Covariates and VAT + SAT + IMAT Radiodensities	Outcome: Log-HOMA2-IR		Excluding Individuals with Type 2 Diabetes	Excluding Individuals with Type 2 Diabetes, Adjusting for Total Abdominal and Thigh CT Scan Volumes	Excluding Individuals with Type 2 Diabetes, Adjusting for Respective AT Depot Volumes

Appendix B Table 4 Continued

	Model		Covariates + One AT Radiodensity	Covariates + One AT Radiodensity	Covariates + One AT Radiodensity
	VAT Radiodensity	(SD = 8.44 HU)	-0.11 (-0.17, -0.05)	-0.10 (-0.16, -0.05)	-0.11 (-0.18, -0.04)
	SAT Radiodensity	(SD = 10.71 HU)	-0.06 (-0.15, 0.03)	-0.05 (-0.14, 0.04)	-0.01 (-0.11, 0.09)
	IMAT Radiodensity	(SD = 5.70 HU)	-0.09 (-0.17, -0.01)	-0.09 (-0.17, -0.01)	-0.13 (-0.21, -0.04)
	Model reported in manuscript (adjusts for age, weight, height, alcohol intake, smoking, hours walked/week, and TV watching ≥ 14 hours/week)				
	Sensitivity analyses:	1) Mutual adjustment of SAT and IMAT radiodensities (row 22), or SAT, VAT, and IMAT radiodensities (row 28) 2) Additional adjustment for total abdominal volume and total thigh volume (column E) 3) Additional adjustment for respective AT depot volume			
Abbreviations: AT = Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue, HU=Hounsfield Unit					

Appendix B Table 5 Change in model AIC/BIC with inclusion of a single AT radiodensity or volume

Outcome	Depot	Δ AIC			Δ BIC		
		Depot Radiodensity	Depot Volume	Depot Radiodensity & Volume	Depot Radiodensity	Depot Volume	Depot Radiodensity & Volume
Glucose	VAT	0.68	1.38	-1.14	0.75	1.45	-0.99
	SAT	0.37	0.50	1.96	0.44	0.56	2.11
	IMAT	1.90	1.90	3.63	1.97	1.97	3.77
Log-Insulin	VAT	-32.95 †	-14.37 †	-31.17 †	-32.89 †	-14.30 †	-31.02 †
	SAT	-42.32 †	-22.89 †	-43.31 †	-42.25 †	-22.82 †	-43.17 †
	IMAT	-39.84 †	-0.42	-40.99 †	-39.77 †	-0.35	-40.84 †
Log-HOMA2-IR	VAT	-33.62 †	-13.88 †	-31.74 †	-33.55 †	-13.81 †	-31.59 †
	SAT	-43.12 †	-22.99 †	-44.03 †	-43.05 †	-22.92 †	-43.88 †
	IMAT	-39.55 †	-0.31	-40.82 †	-39.49 †	-0.24	-40.67 †

† = improvement of fit (Δ ≤ -2)

Compares inclusion/exclusion of (1) a specific AT radiodensity, (2) a specific AT volume, or (3) both specific AT radiodensity AND volume, to the base covariate model [age, weight, height, alcohol intake, smoking, hours walked/week, and TV watching ≥ 14 hours/week]

Abbreviations: AT = Adipose Tissue, AIC = Akaike information criterion, BIC = Bayesian information criterion

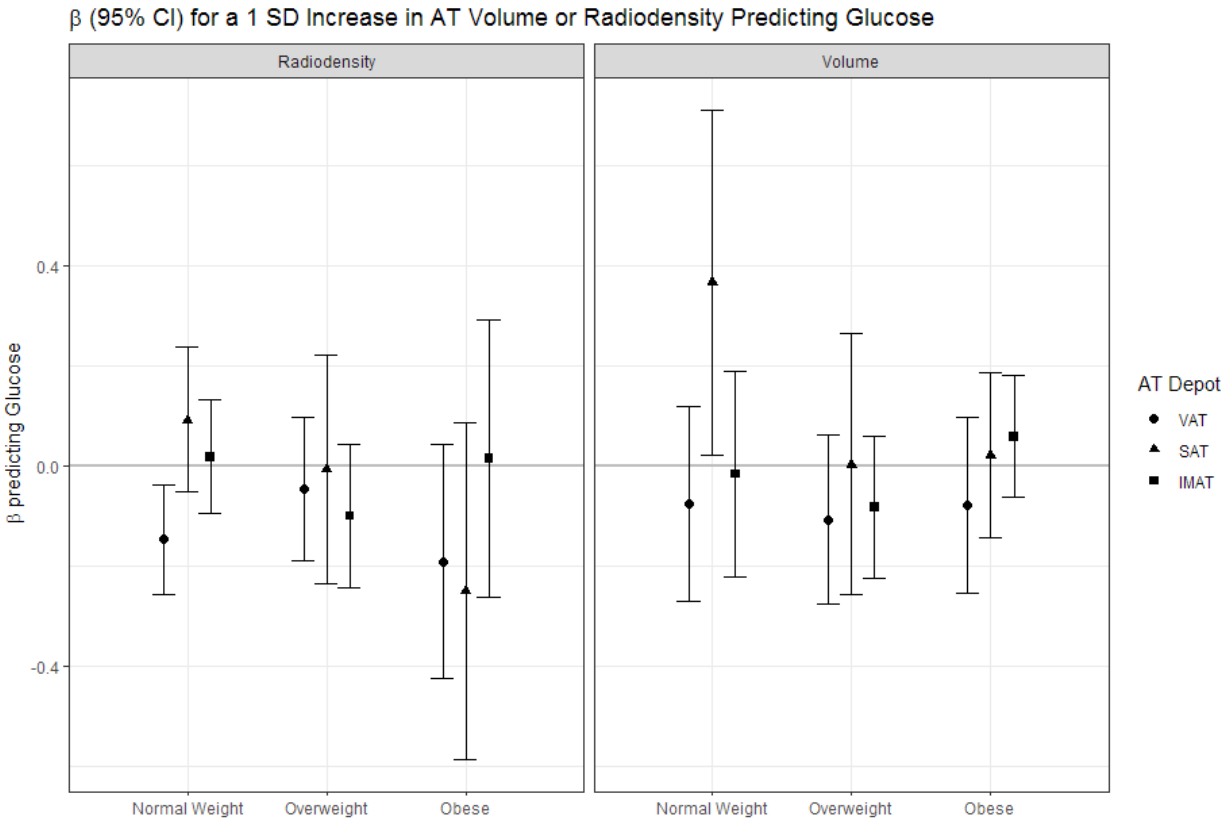
Appendix B Table 6 Change in multiple linear regression model fit statistics after inclusion of all AT depot radiodensities or volumes, stratified byBMIstatus

Outcome	Obesity Status	Δ AIC			Δ BIC		
		All Radiodensities	All Volumes	Radiodensities & Volumes	All Radiodensities	All Volumes	Radiodensities & Volumes
Glucose	Normal Weight	-3.08 †	-7.35 †	-7.23 †	-2.47 †	-6.74 †	-5.75 †
	Overweight	4.34	4.30	7.82	4.77	4.73	8.87
	Obese	-0.32	3.10	2.85	0.48	3.90	4.81
Log-Insulin	Normal Weight	-19.59 †	-31.46 †	-25.82 †	-18.99 †	-30.85 †	-24.34 †
	Overweight	-36.42 †	-24.02 †	-36.61 †	-35.99 †	-23.58 †	-35.56 †
	Obese	-34.38 †	-28.21 †	-33.83 †	-33.58 †	-27.42 †	-31.86 †
Log-HOMA2-IR	Normal Weight	-19.12 †	-31.70 †	-26.05 †	-18.52 †	-31.10 †	-24.57 †
	Overweight	-35.82 †	-23.21 †	-36.04 †	-35.39 †	-22.78 †	-34.99 †
	Obese	-36.84 †	-28.88 †	-36.02 †	-36.04 †	-28.08 †	-34.06 †

† = improvement of fit (Δ ≤ -2)

Compares inclusion/exclusion of (1) a specific AT radiodensity, (2) a specific AT volume, or (3) both specific AT radiodensity AND volume, to the base covariate model [age, weight, height, alcohol intake, smoking, hours walked/week, and TV watching ≥ 14 hours/week]

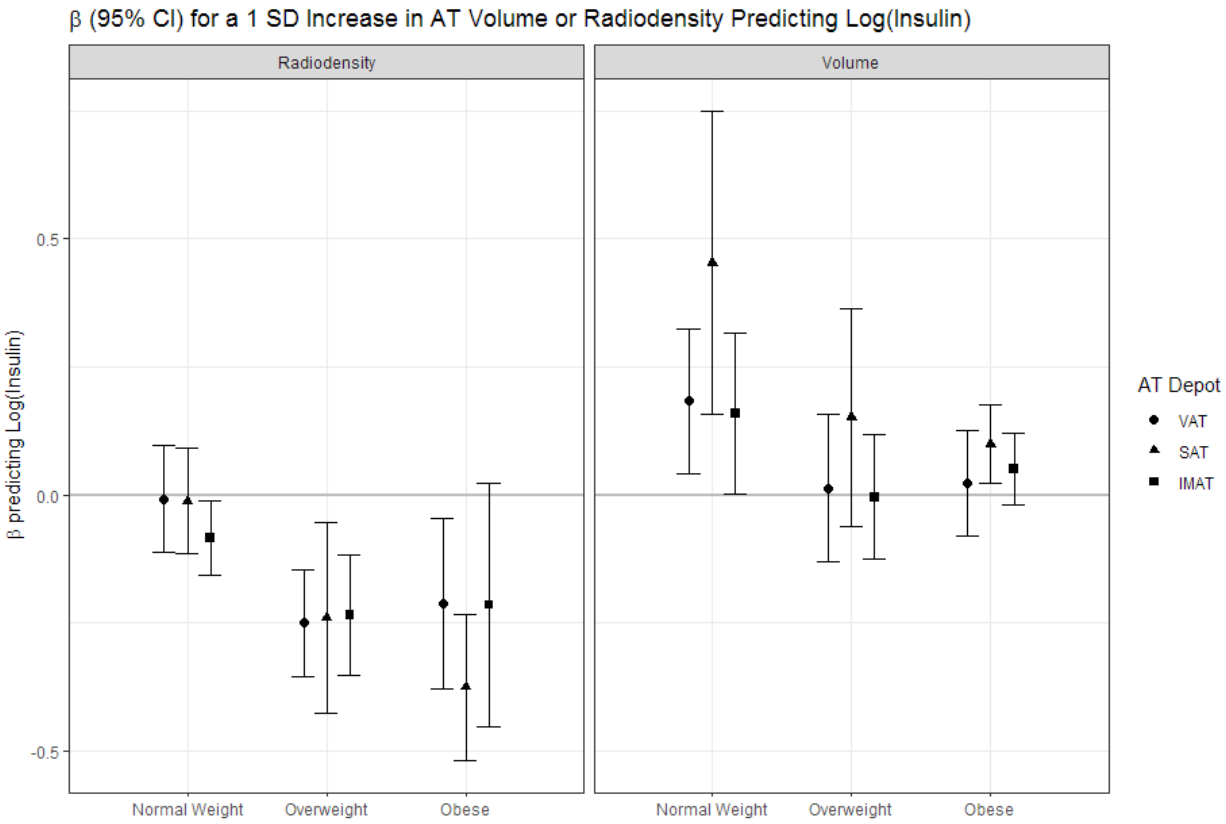
Abbreviations: AT=Adipose Tissue, AIC = Akaike information criterion, BIC = Bayesian information criterion



Appendix B Figure 1 Multiple linear regression coefficients (95% confidence intervals) for AT depot radiodensity and volume predicting glucose levels, stratified by obesity status.

Models adjusted for age, alcohol intake, smoking, walking, TV watching, AND the simultaneous adjustment of a specific adipose tissue depot's volume and radiodensity. Models did not adjust for multiple AT depots. CI's above or below the 0.0 line are statistically significant.

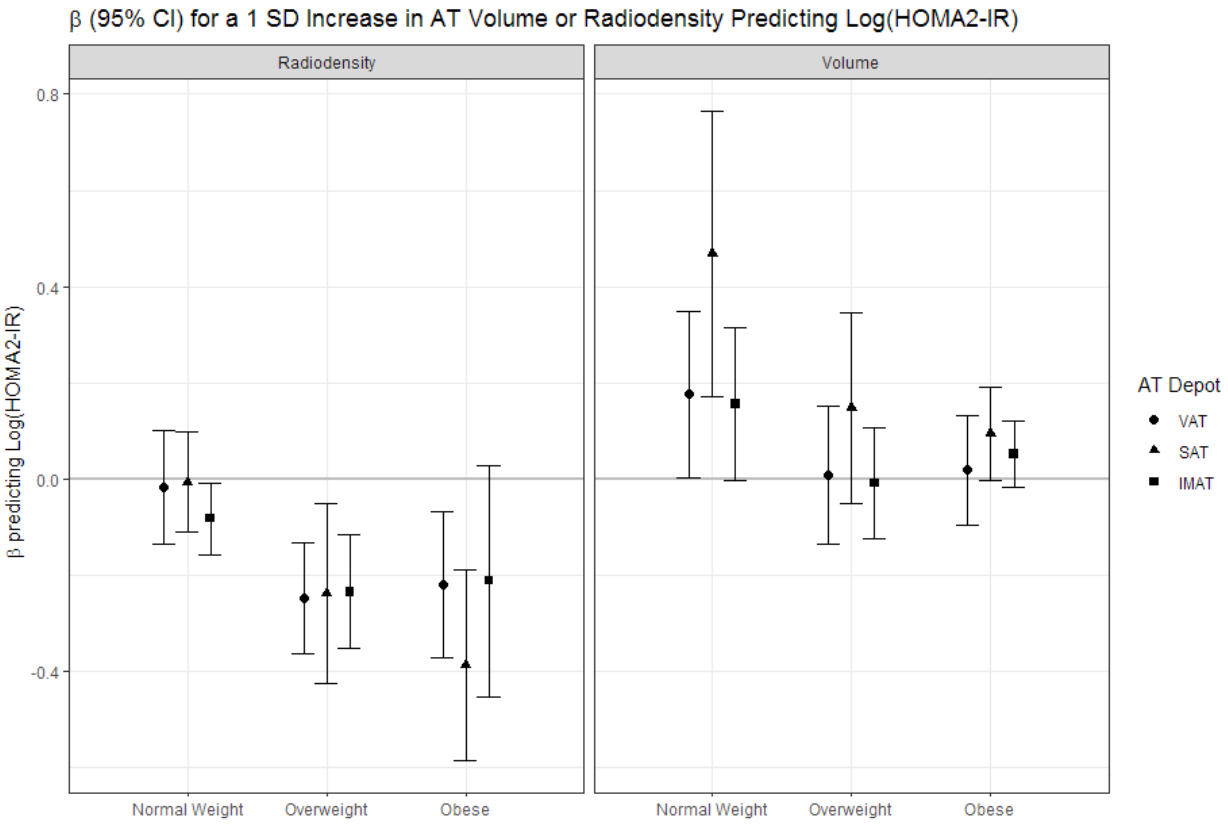
Abbreviations: AT= Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue, CI = Confidence Interval



Appendix B Figure 2 Multiple linear regression coefficients (95% confidence intervals) for AT depot radiodensity and volume predicting log-insulin levels, stratified by obesity status

Models adjusted for age, alcohol intake, smoking, walking, TV watching, AND the simultaneous adjustment of a specific adipose tissue depot's volume and radiodensity. Models did not adjust for multiple AT depots. CI's above or below the 0.0 line are statistically significant.

Abbreviations: AT= Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue, CI = Confidence Interval



Appendix B Figure 3 Multiple linear regression coefficients (95% confidence intervals) for AT depot radiodensity and volume predicting log-HOMA2-IR levels, stratified by obesity status

Models adjusted for age, alcohol intake, smoking, walking, TV watching, AND the simultaneous adjustment of a specific adipose tissue depot's volume and radiodensity. Models did not adjust for multiple AT depots. CI's above or below the 0.0 line are statistically significant.

Abbreviations: AT= Adipose Tissue, VAT = Visceral Adipose Tissue, SAT = Subcutaneous Adipose Tissue, IMAT = Intermuscular Adipose Tissue, CI = Confidence Interval

Appendix C Paper 2 Supplementary Tables

Appendix C Table 1 Additional Abdominal and Thigh Characteristics, Overall and by BMI Category

Variable	Mean (SD), Median (IQR), or N(%)				
	Overall (N=610)	Normal (N=177)	Overweight (N=266)	Obese (N=167)	P-value
Body Composition Tissue Measures					
Abdominal IMAT Volume (cm^3) *	27.6 (21.5, 37.0)	21.7 (9.1)	28.4 (23.0, 36.0)	37.0 (28.5, 49.9)	<.0001
Abdominal Muscle Volume (cm^3) *	165.7 (148.4, 183.6)	153.8 (23.1)	167.7 (24.6)	182.3 (28.2)	<.0001
Other Abdominal Volume (cm^3) *	115.5 (25.3)	114.0 (26.4)	108.2 (93.7, 127.2)	123.4 (23.8)	0.0007
Thigh Muscle Attenuation (HU)	43.6 (40.5, 45.6)	43.2 (3.8)	40.1 (37.7, 43.7)	43.1 (39.9, 45.3)	0.1491

* Abdominal measures in 608 men (264 Overweight)

Appendix C Table 2 Multivariable-Adjusted Regressions for Abdominal Composition Tissues Only, with Continuous Risk Factors (Top) and Ordinal Risk Factors (Bottom), (N=610)

Risk Factor	VAT	ASAT
Log Glucose *	0.02 (-0.02, 0.05)	0.01 (-0.02, 0.05)
Log Insulin *	**0.10 (0.04, 0.17)**	**0.09 (0.02, 0.17)**
Log HOMA-IR *	**0.12 (0.05, 0.19)**	**0.11 (0.03, 0.19)**
Type 2 Diabetes Categories	1.10 (0.83, 1.47)	1.13 (0.79, 1.62)

* Additionally adjusted for antidiabetic medication use

Appendix C Table 3 Multivariable-Adjusted Regressions for Thigh Composition Tissues Only, with Continuous Risk Factors (Top) and Ordinal Risk Factors (Bottom), (N=610)

Risk Factor	TSAT	Thigh IMAT	Thigh Muscle
Log Glucose *	0.02 (-0.03, 0.06)	0.02 (-0.04, 0.07)	**-0.15 (-0.25, -0.04)**
Log Insulin *	**0.15 (0.07, 0.24)**	-0.001 (-0.10, 0.10)	0.08 (-0.13, 0.28)
Log HOMA-IR *	**0.17 (0.07, 0.27)**	0.02 (-0.10, 0.13)	-0.07 (-0.33, 0.17)
Type 2 Diabetes Categories	1.01 (0.68, 1.50)	1.26 (0.79, 1.98)	**0.25 (0.10, 0.63)**

* Additionally adjusted for antidiabetic medication use

Appendix C Table 4 Multivariable-Adjusted Regressions for Body Composition Tissues with Continuous Risk Factors (Top) and Ordinal Risk Factors (Bottom), Including Abdominal IMAT and Muscle Estimates (N=608)

Risk Factor	VAT	ASAT	Abdominal IMAT	Abdominal Muscle	TSAT	Thigh IMAT	Thigh Muscle
Log Glucose *	-0.003 (-0.04, 0.03)	**0.07 (0.02, 0.12)**	0.03 (-0.02, 0.08)	**-0.11 (-0.20, -0.02)**	-0.03 (-0.08, 0.01)	-0.01 (-0.06, 0.03)	-0.05 (-0.14, 0.04)
Log Insulin *	**0.11 (0.04, 0.18)**	0.05 (-0.06, 0.17)	-0.06 (-0.17, 0.04)	0.16 (-0.01, 0.34)	0.08 (-0.02, 0.19)	-0.06 (-0.17, 0.05)	0.10 (-0.11, 0.31)
Log HOMA-IR *	**0.10 (0.02, 0.19)**	**0.13 (0.01, 0.24)**	-0.03 (-0.15, 0.08)	0.05 (-0.17, 0.28)	0.05 (-0.07, 0.17)	-0.08 (-0.19, 0.04)	0.05 (-0.22, 0.32)
Type 2 Diabetes Categories	0.94 (0.69, 1.30)	**2.05 (1.17, 3.60)**	1.10 (0.66, 1.82)	**0.37 (0.16, 0.85)**	0.66 (0.40, 1.10)	1.02 (0.61, 1.70)	0.41 (0.15, 1.14)

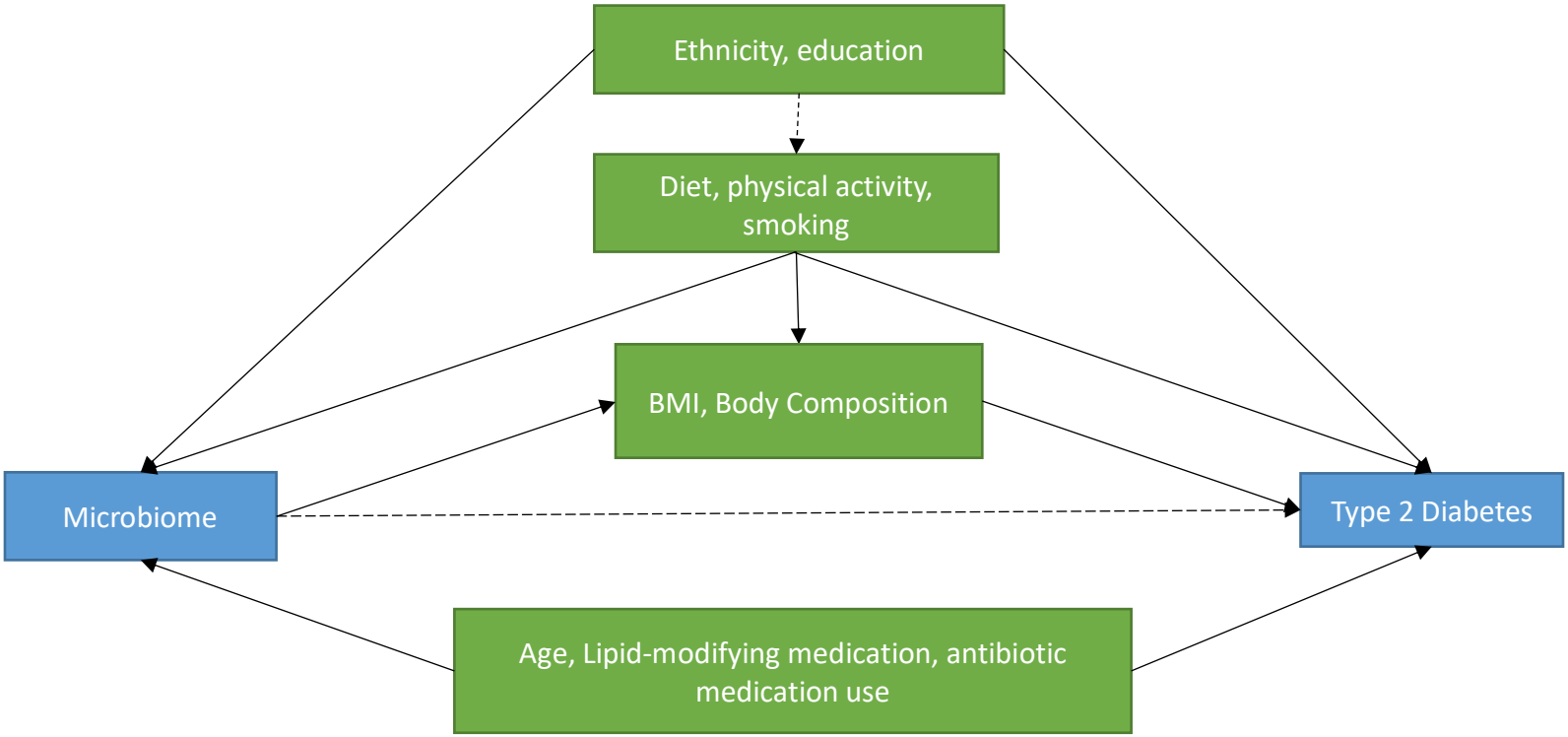
* Additionally adjusted for antidiabetic medication use

Appendix C Table 5 Multivariable-Adjusted Regressions for Body Composition Tissues with Continuous Risk Factors (Top) and Ordinal Risk Factors (Bottom), Including Thigh Muscle Density (N=610)

Risk Factor	VAT	ASAT	TSAT	Thigh IMAT	Thigh Muscle Volume	Thigh Muscle Density (per SD increase)
Log Glucose *	0.001 (-0.03, 0.03)	0.06 (-0.01, 0.11)	-0.03 (-0.07, 0.02)	0.005 (-0.05, 0.06)	-0.08 (-0.17, 0.02)	0.01 (-0.02, 0.04)
Log Insulin *	**0.10 (0.03, 0.17)**	0.06 (-0.05, 0.16)	0.03 (-0.07, 0.14)	0.03 (-0.09, 0.14)	0.01 (-0.21, 0.23)	**0.10 (0.04, 0.15)**
Log HOMA-IR *	**0.10 (0.02, 0.18)**	**0.11 (0.002, 0.23)**	0.01 (-0.11, 0.12)	0.03 (-0.10, 0.16)	-0.06 (-0.33, 0.21)	**0.10 (0.04, 0.16)**
Type 2 Diabetes Categories	0.95 (0.70, 1.28)	**1.77 (1.03, 3.04)**	0.70 (0.42, 1.16)	1.16 (0.67, 2.00)	**0.33 (0.11, 0.97)**	1.08 (0.84, 1.38)

* Additionally adjusted for antidiabetic medication use

Appendix D Paper 3 Supplementary Figure



Appendix D Figure 1 Conceptual model for association of the microbiome with type 2 diabetes

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