

**INSULIN RESISTANCE IN TYPE 1 DIABETES:  
DETERMINANTS AND CLINICAL CONSEQUENCES**

by

Christina M. Shay

B.A., John Carroll University, 2001

M.A., Kent State University, 2004

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This dissertation was presented

by

Christina M. Shay

It was defended on

May 18, 2009

and approved by

Committee Members:

Bret H. Goodpaster, Ph.D.

Assistant Professor of Medicine, Department of Medicine  
School of Medicine, University of Pittsburgh

Sheryl F. Kelsey, Ph.D.

Professor of Epidemiology, Department of Epidemiology  
Graduate School of Public Health, University of Pittsburgh

Elsa M. Strotmeyer, Ph.D., M.P.H.

Assistant Professor of Epidemiology, Department of Epidemiology  
Graduate School of Public Health, University of Pittsburgh

Dissertation Advisor:

Trevor J. Orchard, M.D., M.Med.Sci.

Professor of Epidemiology, Department of Epidemiology  
Graduate School of Public Health, University of Pittsburgh

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Christina M. Shay, Ph.D.

University of Pittsburgh, 2009

Insulin resistance (IR) is well documented in type 1 diabetes (T1D) and is theorized to relate to diabetes complications, including renal and coronary artery disease (CAD). The hyperinsulinemic-euglycemic clamp technique provides accurate assessment of IR, yet the laborious, costly, and invasive nature of this technique is often inappropriate for large investigations. Increasing use of the Estimated Glucose Disposal (eGDR) equation in T1D makes further examination of this equation desirable as it may be improved with additional assessments. Leg adiposity has been favorably associated with IR and cardiovascular risk, but whether this protective tendency is similar in T1D populations is unknown. This dissertation examines whether diabetes complications or additional clinical factors (i.e. regional adiposity distribution) contributes to the estimation of IR in T1D. Differences in regional adiposity, and the extent to which these differences influence IR, were examined in T1D and individuals without diabetes. Associations between CAD risk factors and regional adiposity were also investigated in individuals with T1D.

No differences in IR were observed between T1D individuals with CAD or renal disease. All adiposity measures were detrimentally associated with IR, however, general obesity most strongly predicted IR in this population. Despite lower levels of adiposity, more severe IR was observed in individuals with T1D compared to non-diabetic individuals. Leg adiposity was

favorably associated with presence of CAD, even after controlling for general obesity, but this association was only observed in non-diabetes and in T1D individuals who were obese. Trunk and leg fat displayed equal yet opposite associations with CAD risk factors and increasing leg adiposity was associated with decreased risk for the presence of CAD in females with T1D.

This dissertation thus yields significant Public Health findings by providing evidence that IR is a prominent feature in T1D, is largely driven by adiposity, and can be estimated using clinical measures. Furthermore, the finding that leg adiposity was favorably associated with presence of CAD in individuals with T1D provides impetus to further study and underscores the complex association of adiposity with morbidity in T1D.

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## **PREFACE**

This dissertation is based upon a collection of studies conducted prior to and during my time at the University of Pittsburgh between August 2004 and June 2009 at the Department of Epidemiology, Graduate School of Public Health, Pittsburgh, Pennsylvania.

I would like to express my sincere gratitude to my dissertation advisor Trevor J. Orchard. Without his advice and unique support, this dissertation would never have become a reality. I am truly grateful to have been given the opportunity to work with such a renowned epidemiologist and I am truly honored to have been part of the EDC study. I will carry these experiences with me through my career. I would also like to thank my previous supervisor and dissertation committee member Bret H. Goodpaster for giving me the opportunity to gain experience with insulin clamp techniques and to work with the wonderful group of investigators at the Obesity and Nutrition Research Center. I would also like to give great thanks to Elsa M. Strotmeyer for allowing me to explore the EDC DEXA data as part of my dissertation project. I would also like to thank my final dissertation committee member, Sheryl F. Kelsey, for her great co-operation and effort in finalizing these manuscripts. To the faculty and staff at the Department of Epidemiology at the University of Pittsburgh I am extremely grateful for your assistance through my graduate coursework and for their positive attitude through this arduous dissertation process. I will miss you all.

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## 1.0 INTRODUCTION

Insulin resistance (IR), which has been well documented in type 1 diabetes (T1D), is theorized to be related to a number of diabetes complications [1-7]. The hyperinsulinemic-euglycemic clamp technique [8] provides the most accurate assessment of IR, although the labor intensive, costly, and relatively invasive nature of this technique is often inappropriate for use in large-scale population studies. Alternative techniques developed to assess IR in T1D commonly rely on the measurement of fasting plasma insulin levels. Since the insulin concentrations in T1D needed for the computation of these methods more likely reflect timing and dosing of replacement therapy, rather than resistance, investigations in T1D make limited use of alternative methods. The Estimated Glucose Disposal (eGDR) equation [9], validated by hyperinsulinemic-euglycemic clamp studies, is a noted method of estimating IR in T1D in many epidemiologic T1D investigations [10-13]. The increasing use of this method makes desirable a further examination of this equation as it may be improved with additional clinical factors (e.g. more detailed adiposity measures).

A large amount of evidence links obese states with IR in both diabetic and non-diabetic states [14-18]. Adipose tissue in the abdomen is more strongly associated with IR than other depots due to its potential for influencing lipolysis through release of free-fatty acids into the portal circulation [19]. This association between trunk fat mass and insulin sensitivity seems clear; however considerable variability in the results regarding the relationship between insulin

sensitivity and regional adiposity in humans exists [20]. Besides abdominal fat, another adipose depot that has gained attention in regards to its potential influence on IR and associated disease states (e.g. coronary artery disease (CAD)), is the lower extremity and/or hips. Recent investigations in T2D and non-diabetic populations have suggested that leg fat and the propensity to store fat in the legs is favorably associated with measures of insulin sensitivity [21-24]. Increasing amounts of absolute leg fat has similarly been associated with cardiovascular risk [25, 26] and the propensity to store fat in the lower body has been associated with favorable cardiovascular profiles [27]. This notion that some degree of cardio-metabolic protection may be afforded by the tendency to deposit fat in gluteal-femoral depots is appealing, but whether this protective metabolic tendency is similar in T1D populations has yet to be evaluated.

Current definitions distinguish between T1D, characterized by autoimmune beta cell destruction, and the broader T2D, with varying ranges of IR and insulin deficiency. Despite more than three decades of research, the cause of T1D remains unknown although it is clearly an autoimmune disease in many cases (sub-classified as Type 1 A). Its incidence has seen a dramatic increase and its clinical features have become increasingly difficult to distinguish from T2D and emerging experimental evidence highlights a marked overlap between these two diabetic conditions [28-30]. While the literature has focused on body composition as a risk factor for IR in T2D [16, 31-34], it lacks data examining these associations in T1D. Moreover, further investigation into the manifestation of CAD and IR as they may be associated with regional adiposity in T1D may better distinguish the risk factors specific to worsening disease states in T1D and help develop new public health control measures. For these reasons, a research dissertation based on the following investigations is proposed:

- A. A reexamination of the eGDR equation in the assessment of IR in T1D in order to evaluate the contribution of DEXA-assessed regional body composition measures as they may increase the predictability of the eGDR equation. An additional goal of this investigation is the examination of whether IR differs across strata of CAD and renal disease in T1D.
- B. An examination of IR and DEXA-assessed regional adiposity distribution between non-diabetics and individuals with T1D. A main goal of this investigation is the examination of whether IR or regional adiposity differs between individuals with and without T1D and whether any differences in IR can be attributed to regional adiposity.
- C. An examination of the associations between CAD and CAD risk factors, and both total and regional adiposity in individuals with T1D. An emphasis of this investigation is the examination of associations between the different regional adiposity depots and CAD risk factors and to determine if regional adiposity assessments are associated with the presence of CAD in T1D.

## **2.0 BACKGROUND**

### **2.1 DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS**

The National Diabetes Data Group in the United States and the World Health Organization (WHO) released reports addressing diabetes diagnostic criteria in 1979 and 1980 [35, 36]. These diagnostic guidelines for the diagnosis of diabetes consisted of a fasting plasma glucose measurement of  $\geq 140$  mg/dL and/or a two-hour oral glucose tolerance test (OGTT) plasma glucose measurement of  $\geq 200$  mg/dL after a 75-g glucose load. A two-hour OGTT measurement of 140-199 mg/dL was also used for the diagnosis of impaired glucose tolerance (IGT), a group considered at increased risk for developing diabetes. Prior to the adoption of such guidelines, criteria were often inconsistent and clinicians frequently relied on their clinical experience rather than standardized criteria in diagnosing diabetes cases.

Since the acceptance of these standards, two more recent reports on diabetes diagnostic criteria were published in 1997 and 1998. These reports, released from the World Health Organization [37] and the American Diabetes Association (ADA) Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [38], were highly influenced by the considerable increases in knowledge regarding the etiology of diabetes and more information on the predictive value of different blood glucose values. The updated WHO report included lowering the fasting plasma glucose level to  $\geq 126$  mg/dL for the diagnosis of diabetes. The range for IGT diagnosis was also

changed to allow for the new WHO fasting level. The new category of Impaired Fasting Glucose (IFG) category, was proposed by the WHO to encompass values which are above normal but below the diagnostic cut-off for diabetes (plasma  $\geq 110$  to  $< 126$  mg/dL; whole blood  $\geq 101$  to  $< 110$  mg/dL). IFG, similar to IGT, was considered to be both abnormal and a risk factor for the development of diabetes (although not yet diagnostic of diabetes).

The ADA Expert Committee on the Diagnosis and Classification of Diabetes Mellitus was convened to address the desire to eliminate the need for the OGTT for the diagnosis of diabetes. These conferences resulted in the lowering of the fasting plasma glucose diagnostic criteria from  $\geq 140$  mg/dL to  $\geq 126$  mg/dL to best identify those with a two-hour value  $\geq 200$  mg/dL. In addition, the IFG classification replaced the IGT categorization (which would no longer be identified) and was defined as 110–125 mg/dL. The Expert Committee published a follow-up report in 2003 which retained the previous fasting, two-hour diagnostic criteria, and the IGT criteria. However, the this report resulted in a change of the IFG criteria to 100–125 mg/dL, a lowering from the previous lower threshold of 110 mg/dL [39]. As of today, these criteria are the current diagnostic criteria for the diagnosis of diabetes mellitus.

## **2.2 PREVALENCE AND INCIDENCE OF DIABETE MELLITUS**

Diabetes mellitus is the most common endocrine disorder, affecting almost 6% of the world's population and more than 97% of these patients are diagnosed with T2D [40]. Prevalence of diabetes mellitus, particularly T2D, is dramatically increasing and has reached epidemic proportions in many areas across the globe. It is calculated that more than 150 million people have been diagnosed with diabetes worldwide, and this number is estimated to rise above

300 million by 2025 [41]. Reports on the annual incidence of T1D range from 1.9-7.0 per 100,000 in Africa, 0.13-10 per 100,000 in Asia, approximately 4.4 per 100,000 in Australasia, 3.4-36 per 100,000 in Europe, 2.62-20.18 per 100,000 in the Middle East, 7.61-25.7 per 100,000 in North America, and 1.27-18 per 100,000 in South America. Similarly, population prevalence of T2D is reported to range from 0.3-17.9% in Africa, 1.2-4.6% in Asia, 0.7-11.6% in Europe, 4.6-40% in the Middle East, 6.69-28.2% in North America, and 2.01-17.4% in South America [40].

According to the American Diabetes Association (ADA), 20.8 million children and adults in the United States (7.0% of the population) have diabetes [42]. Of those cases, 14.6 million people actually been diagnosed while an estimated 6.2 million go undiagnosed. The ADA also reports that 54 million individuals in the United States have the condition known as impaired glucose tolerance (IGT) which places them at increased risk for developing diabetes. Statistics for diabetes prevalence by age group in the United States are startling. As of 2005, 1.5 million new cases of diabetes were diagnosed in people aged 20 years or older and the numbers are increasing. In individuals younger than 20 years, 0.22% (176,500 individuals) have diabetes. Similar reports from the ADA suggest that about 1 in every 400 to 600 children and adolescents has been diagnosed with diabetes, and two million adolescents (1 in 6 overweight/obese adolescents) aged 12-19 are estimated to be pre-diabetic (IGT). Although T2D occurs in children, nationally representative information needed to monitor diabetes incidence and prevalence in children in the United States is not yet available. Clinical reports and regional studies suggest that T2D is being diagnosed more frequently in children and adolescents, particularly in American Indians, African Americans, and Hispanic/Latino Americans [40, 43]. In individuals older than 20 years, 9.6% (20.6 million) have diabetes while in those 60 years and

older, 20.9% (10.3 million) of all people in this age group have diabetes. With regards to gender, the prevalence of diabetes has a near equal distribution. In individuals aged 20 years or older, 10.5% (10.9 million) men and 8.8% (9.7 million) women.

The prevalence of diabetes in the United States also varies dramatically by race/ethnicity. Among non-Hispanic whites, 18.7% (3.1 million) aged 20 years or older have diabetes. In non-Hispanic blacks, 13.3% (3.2 million) aged 20 years or older have diabetes. After adjusting for population age differences, the ADA reports that non-Hispanic blacks are 1.8 times as likely to have diabetes as non-Hispanic whites. Mexican Americans, the largest Hispanic/Latino subgroup, are 1.7 times as likely to have diabetes as non-Hispanic whites. In American Indians and Alaska Natives aged 20 years or older, 12.8% (99,500 individuals) have diabetes. Taking into account population age differences, American Indians and Alaska Natives are 2.2 times as likely to have diabetes as non-Hispanic whites., In Hawaii, Asians, Native Hawaiians, and other Pacific Islanders aged 20 years or older are more than 2 times as likely as whites to have diagnosed diabetes after adjusting for population age differences. Asians living in California were 1.5 times as likely to have diagnosed diabetes as non-Hispanic whites [44].

When examining the specific types of diabetes, T1D is more common in Caucasians than in individuals of Latino, African-American, or other non-Caucasian backgrounds. There are also major ethnic differences in susceptibility to T2D, which are suspected to be largely genetically determined. People of African-American, American Indian, Asian American, Latino, and Pacific Islander background have a higher risk of developing T2D [45, 46]. Despite the strong evidence suggesting genetic contribution to both obesity and T2D, the increasing rates in these conditions, in both developed and developing countries, appears to be due to a changing balance between energy intake and energy expenditure through physical activity [41]. There is also a tendency

for an increased prevalence of T2D to be concentrated in lower socioeconomic groups in developed countries and higher socioeconomic groups in developing countries [47]. This occurrence most likely reflects the adoption of a “healthier” lifestyle by individuals with higher levels of education in developed countries, while it is generally the affluent in developing countries that enjoy a high calorie intake and low level of physical activity.

Overall, the profiles of risk factors for the development of T1 or T2D are diverse. Studies report the following to be some of the most common risk factors for developing T1D: being ill in early infancy, early foods (early exposure to cow's milk in infancy and not being breast fed), having a parent with T1D, advanced maternal age, having a mother with preeclampsia during pregnancy, and obesity. With regards to T2D, the National Institutes of Health report significant major risk factors being age 45 or older, family history of diabetes, ethnicity (African American, Hispanic/Latin American, American Indian and Alaska Native, Asian American, or Pacific Islander), a history of gestational diabetes or having given birth to at least one baby weighing more than 9 pounds, inactive lifestyle, high blood pressure (140/90 mm/Hg or higher), HDL cholesterol < 35 mg/dL or triglyceride level  $\geq$  250 mg/dL or higher, and/or a diabetes test history of IFG or IGT, obesity [48]. Above all other risk factors, obesity is considered to be the number one risk factor for the development of T2D. Obesity in children has long been linked to a higher risk for T2D, the common risk factor potentially being an obesity related increase in insulin secretion. Obese states in childhood are theorized to overstress the beta cells increasing their susceptibility to damage by overactive immune factors and eventually destruction in children genetically vulnerable to T1D. Excess adiposity appears to play a strong role in IR in T2D, and potentially in T1D, but adipose distribution is also theorized to be a significant risk factor in both conditions. Exploring the influence of adipose distribution on IR,

and the potential distinction of this association in T1 and T2D, is imperative to the understanding and treatment of IR as a clinical consequence of the two conditions. Further evaluation of the relationship between adiposity and IR may also offer valuable information related to weight control evaluation and treatment practices in T1 and T2D.

### **3.0 INSULIN PATHOPHYSIOLOGY**

#### **3.1 INSULIN SECRETION AND ACTION**

Insulin, an anabolic hormone responsible for maintaining glucose homeostasis, plays a major role in the regulation of carbohydrate, lipid, and protein metabolism [49]. Insulin has two basic functions: 1) it facilitates the rate of transport of glucose across the cell membrane in adipose tissue and muscle, and 2) it increases the rate of glycolysis and it stimulates the rate of glycogen synthesis in a number of tissues, including adipose tissue, muscle, and liver. Insulin is also involved in decreasing the rate of glycogen breakdown in muscle and liver and the inhibition of rates of glycogenolysis and gluconeogenesis in the liver. In addition to its role in regulating glucose metabolism, insulin promotes lipid synthesis, suppresses lipid degradation, and increases amino acid transport into cells [50, 51]. Insulin stimulates growth, DNA synthesis, and cell replication and it also modulates transcription, altering the cell content of numerous mRNAs [52].

Insulin is synthesized as a prohormone in the beta cells of the islets of Langerhans. Once its signal peptide is removed in the cisternae of the endoplasmic reticulum, generating proinsulin, it is packaged into secretory vesicles in the Golgi. The molecule is then folded into a unique structure and locked in this conformation by the formation of two disulfide bonds. Protease activity cleaves the center third of the molecule, which detaches as C-peptide, leaving

the amino terminal B peptide disulfide bonded to the carboxy terminal A peptide [53]. The body produces both insulin and C-peptide at the same rate as part of the activation and division of proinsulin in the pancreas. As a result, both may be measured to evaluate how much insulin in the blood is due to endogenous production and how much is from exogenous sources. While insulin tests will reflect the total, C-peptide describes the endogenous insulin.

Plasma glucose levels are primarily regulated by insulin secretion from beta cells. An increased uptake of glucose by pancreatic beta cells leads to a simultaneous increase in cellular metabolism. The increase in metabolism yields an elevation in the adenosine triphosphate (ATP)/adenosine diphosphate (ADP) ratio. This in turn results in an inhibition of an ATP-sensitive  $K^+$  channel. The net result is a depolarization of the cell leading to  $Ca^{2+}$  influx and insulin secretion. [54]. Once secreted insulin is directly infused, via the portal vein, to the liver where it exerts its metabolic effects. The effects are the response of the activation of the insulin receptor, which belongs to the class of cell surface receptors that exhibit intrinsic tyrosine kinase activity [55]. With respect to hepatic glucose homeostasis, the effects of insulin receptor activation are specific events that lead to an increase in the storage of glucose with a concomitant decrease in hepatic glucose release.

## **3.2 INSULIN AND SUBSTRATE METABOLISM**

### **3.2.1 Carbohydrate Metabolism**

Insulin mediates the entry of glucose into muscle, adipose, and several other tissues in the body. The only mechanism by which glucose can be transported into the cell is by facilitated diffusion

through the use of hexose transporters. In many tissues, specifically skeletal muscle, the major transporter used for uptake of glucose (called GLUT4) is made available in the cell through the action of insulin. Without the presence of insulin, GLUT4 transporters are useless for transporting glucose. The binding of insulin to cell receptors leads to the rapid attachment of those vesicles to the plasma membrane and insertion of the glucose transporters. This occurrence allows the cell the ability to efficiently break down glucose for energy. As blood levels of insulin decrease and insulin receptors are no longer occupied, the GLUT4 transporters are recycled back into the cell [56].

Insulin is also responsible for the stimulation of the liver for the storage of glucose in the form of glycogen. Much of the glucose absorbed in the small intestine is immediately taken up by cells in the liver and stored as glycogen. Insulin has additional effects on the liver, including the stimulation of glycogen synthesis. Insulin is used in this way to activate the enzyme hexokinase, which phosphorylates glucose, trapping it within the cell. Additionally, insulin can also act to inhibit the activity of glucose-6-phosphatase. Insulin is involved in the activation several of the enzymes (e.g. phosphofructokinase and glycogen synthase) that are directly involved in glycogen synthesis. The overall hepatic effect of insulin is when glucose quantities are abundant, insulin stimulates excess glycogen storage in the liver for later use.

In relation to the effect of insulin on blood glucose stabilization, insulin secretion ceases when blood glucose concentrations are low. Without insulin, hepatic glycogen synthesis ceases and enzymes responsible for breakdown of glycogen become active. Glycogen breakdown is stimulated not only by the absence of insulin, but by the presence of glucagon, which is secreted when blood glucose levels fall below the normal range. Many of the cells in the body are unable to utilize glucose without insulin and they begin to use alternate fuels for energy. Neurons,

however, require a constant supply of glucose, which in the short term, is provided from glycogen reserves.

### **3.2.2 Lipid Metabolism**

The pathways for metabolism of lipids and carbohydrates elaborately connected. In relation to the involvement of insulin with carbohydrate metabolism, the effects of insulin on lipid metabolism are also significant. From an overall perspective, insulin suppresses the amount of fat which is being used for energy. Not only does it promote the preferential oxidization of carbohydrates over lipids, insulin is also involved in the stimulation of lipid accumulation in adipose tissue [57].

A main role of insulin in lipid metabolism is the promotion of fatty acid synthesis in the liver. Despite insulin-stimulated hepatic glycogen synthesis in the liver, synthesis becomes strongly suppressed as glycogen accumulates to high levels (roughly 5% of liver mass). When the liver is saturated with glycogen, any additional glucose taken up by hepatocytes is shunted into pathways leading to synthesis of fatty acids, which are expelled from the liver as lipoproteins. The lipoproteins are broken down in the circulation, providing free fatty acids for use in other tissues, including adipocytes, where they are used to synthesize triglycerides. Insulin is further involved in the accumulation of triglycerides in fat cells since it also influences the inhibition of lipolysis. Insulin does so by inhibiting intracellular lipase which is responsible for the release of fatty acids. In adipocytes, insulin facilitates the entry of glucose where it can be used to synthesize glycerol. Adipocytes can then utilize glycerol, and fatty acids from the liver, to synthesize triglycerides [58].

#### **4.0 TYPE 1 AND TYPE 2 DIABETES INSULIN RESISTANCE**

Diabetes mellitus is a metabolic disease characterized by hyperglycemia. T1D is a multifaceted condition distinguished, in most cases, by the autoimmune-mediated destruction of pancreatic beta cells that ultimately develops into an insulin deficient state [59, 60]. T1D is most commonly diagnosed in children and adolescents, though can occur at any age, and is frequently identified by symptomatic hyperglycemia, requiring a need for exogenous insulin replacement. In contrast to T1D, the etiology of T2D is a condition characterized by low levels of response to insulin from primary target tissues (i.e. adipose, muscle and liver cells) [61, 62], a condition more commonly referred to as IR. It is hypothesized that at some point, the pancreatic beta cells are unable to compensate for the IR by increasing insulin secretion, and it is at this point that T2D then appears [63]. This decreased ability of the body to use insulin to lower blood glucose concentrations [64] is posited to be a cascading condition correlated with severity of disease in T2D; the mitigating factors hypothesized to be both genetic and metabolic in nature [62, 65-70].

IR is an area of increasing investigation in both T1D and T2D and may be associated with the progression of complications related to diabetes [4, 7, 13, 64, 66, 69, 71-78] as well as with diabetes etiology. It is well documented that individuals with diabetes are at increased risk for the development of many conditions, including cardiovascular disease (CVD) [13, 66, 77, 79-83], kidney disease [81, 84, 85], retinopathy [86-89], and neuropathies [79, 83, 90, 91]. Even though individuals with T1D and T2D are known to be particularly at high risk for

cardiovascular disease (CVD), as well as other microvascular complications, these risks have not been fully accounted for by conventional risk factors. In attempts to explore diabetes related complications, IR has been intensely investigated as a potential risk factor for many CVD and nephropathy related conditions [13, 69, 72, 78, 92, 93]. The conclusions from the majority of these investigations have suggested insulin sensitivity as a strong inverse correlate of CVD [73, 78, 94, 95] and nephropathy related complications [3, 6, 96], yet some investigations have reported conflicting results [71, 97].

IR has been suggested to mediate atherosclerosis largely by its effect on endothelial function [98-101]. Coronary artery calcification (CAC) strongly predicts endothelial dysfunction in patients with suspected CAD [102]. Additionally, many of the manifestations of IR, including hypertension, elevated insulin, elevated blood glucose, dyslipidemia, high triglycerides, and low HDL cholesterol predispose an individual to the development of CAD as well as the development of IR. Despite the information available concerning the association between IR and CVD in diabetes, the majority of these investigations examine these relationships individually in T1 or T2 with no comparisons between the two conditions. Since CAC is a strong independent predictor of endothelial dysfunction and the development of CVD [102], a direct comparison of IR in both T1 and T2 as it may relate to level of CAC may have significant implications for the conception of diabetes related CVD at various clinical stages.

#### **4.1 BODY COMPOSITION, INSULIN RESISTANCE, AND TYPE OF DIABETES**

Research shows a strong relationship between obese states and the incidence and prevalence of T2D [103-106]. Many investigations examine the relationship between body composition and

T2D [16, 107-115], but few investigations examine body composition in T1D [116-121]. Reports on body composition in T1D have suggested that individuals with T1D have similar body composition to the respective non-diabetic controls [118, 122, 123]. The major body composition focus of many of these investigations has been bone mineral density [118, 124, 125] in adolescent T1D populations [116, 119, 120, 123, 126-128] with very little emphasis on adiposity. With the limited availability of data in the area of body composition and adiposity in T1D, even fewer investigations compare adiposity between T1 and T2D [114, 129]. One such investigation examining body composition and fat distribution in women concluded that total body fat and abdominal fat were reported to be higher for those with T2D than for those with T1D, yet lean tissue mass was similar across both groups [114]. Besides being overweight or obese compared to the T1D participants, the T2D subjects exhibited a higher degree of abdominal fat distribution; this supports previous findings of lower WHR in T1D when compared to T2D populations [130]. Such findings indicate that individuals with T1 belong to different anthropometric body types than individuals with T2D, which may be reflective of the different pathologies associated with adiposity across the two diabetes states.

In the last few decades, diabetes research has intensely focused on IR as it may be related to body composition and adiposity. More recently, studies have re-examined the notion that obesity is not uniform throughout the body in terms of function and effect. These investigations show that the particular anatomical compartment in which adipose tissue is stored (regional distribution) is important in understanding the relationship between obesity and disturbances in glucose and lipid metabolism. Despite the relationship between generalized obesity and an increased risk for IR and its metabolic complications, whether regional adiposity confers an excess risk of IR is not fully understood. Current review articles have addressed this debate at

both a clinical and molecular level [105, 131-133], yet their conclusions remain controversial. The literature examining adiposity in non-diabetic and T2D populations, which largely suggests that regional adiposity is a more important determinant of IR than body size alone [104, 107-109, 112, 134, 135], indicates the need for more comprehensive comparisons of this relationship between T1 and T2D.

#### **4.1.1 Abdominal and Lower Extremity Adiposity and Cardio-Metabolic Risk**

It is widely accepted that generalized obesity is a risk factor for IR and T2D, and a significant risk factor for CVD, yet it has been shown that not every obese patient is insulin resistant or at high risk of diabetes and CVD [31]. Literature has emerged over the last few decades suggesting the accumulation of adipose tissue in a particular anatomical compartment (regional adiposity) confers an excess risk of IR and its complications.

Since men carry approximately 15–18% of their total body fat within the abdominal cavity and women carry approximately 7–8% in that area [136], it is not surprising that the metabolic influence of this adipose depot has been examined. The adipose tissue in the abdominal area can be subdivided into intraperitoneal (IP) and retroperitoneal (RP) tissue compartments. From an anatomical standpoint, this fat depot is theorized to be metabolically influential as a result of the unique venous drainage of the IP adipose tissue; it drains directly into the liver through the portal vein compared with the RP adipose tissue, which drains into the systemic circulation. Since it is theorized that free fatty acids, glycerol and other cytokines released from the IP adipose tissue have specific effects on hepatic metabolism of glucose, triglycerides, insulin, and other substrates and hormones, excess accumulation of visceral

adiposity resulting in excess release of these products may ultimately lead to various forms of metabolic dysregulation.

Results from several large cross-sectional and longitudinal studies provide evidence that simple measures of abdominal adiposity (i.e. waist circumference) remain a significant predictor of CVD and T2D after control for the disease risk predicted by indices of body mass index [48, 137, 138]. These findings are further supported by a large amount of literature implicating abdominal obesity, as measured by various imaging techniques (e.g. CT, MRI, DEXA), in the development of numerous metabolic risk factors across various populations [139-141]. Despite some controversy regarding the impact of regional adiposity on IR, for any given amount of total body fat, the subgroup of individuals with a selective excess of intra-abdominal, or visceral, adipose tissue are generally accepted to be at substantially higher risk of being characterized by IR and by the features of metabolic syndrome [32, 142].

Another adipose depot that is hypothesized to exert metabolic influence is the areas located in the lower extremities and in the hips. Although both the central and peripheral subcutaneous adipose tissue depots drain into the systemic circulation, accumulation of fat in these depots may confer different susceptibilities to developing IR or cardiovascular disease. There is a potential physiological rationale for why lower-extremity adiposity decreases the risk for metabolic dysfunction as it is related to the heterogeneity of regional adipose tissue metabolism. There is data demonstrating that adipocytes from visceral abdominal regions are more sensitive to stimuli related to the breakdown of fat. It is further suggested that these same cells are more resistant to suppression of fat breakdown by insulin than are adipocytes from gluteal-femoral subcutaneous regions [143, 144]. On the basis of these regional differences in the regulation of lipolysis, it is hypothesized that the relative daily systemic flux of free fatty

acids would be higher in individuals with a high accumulation of abdominal fat than in those with lower body fat localization, due both to a heightened sensitivity to the stimulation of lipolysis and to an impaired suppression of lipolysis in abdominal adipocytes.

Although adipose tissue content of the thigh and legs has generally not been considered a correlate of cardio-metabolic dysfunction, new evidence is emerging that may suggest a metabolically protective effect regarding this regional area of adiposity. Along with the extensive prospective evidence identifying waist-hip ratio as an independent CVD risk factor [145], improvements in technology have allowed more precise measurements of regional fat distribution that permit differentiation of various fat depots, including trunk fat, arm fat, and leg fat. Recent investigations employing such measures have suggested that leg fat mass is favorably associated with measures of insulin sensitivity [21-23, 141, 146] and several studies have also shown that the association between waist-hip ratio or waist-thigh ratio and glucose metabolism of in T2D was not only associated with a larger waist circumference but also positively correlated with hip or thigh circumference [147, 148]. Along with IR, evidence also exists indicating that this fat depot may have comparable positive effects on the risk for cardiovascular disease and its associated risk factors. Different measures of leg and thigh fat have been favorably associated with a number of CVD risk factors such as fasting serum measures of glucose, total cholesterol, high density lipoprotein cholesterol, triglycerides, metabolic syndrome and peripheral and central arterial stiffness [21, 24, 26, 141, 146, 149-157]. Additionally, these associations have been identified in a variety of populations including healthy individuals [23, 150], the overweight and obese [25, 146], the elderly [21, 26, 153], Japanese [155, 156], and African-Americans [157]. These findings support the notion that some degree of metabolic protection may be afforded by the propensity to deposit fat in gluteal-femoral depots, yet the examination of

adiposity variables assessed by DEXA (arm fat, leg fat, and trunk fat) in T1D populations is noticeably missing from the literature.

## **4.2 ASSESSMENT OF INSULIN RESISTANCE IN TYPE 1 AND TYPE 2 DIABETES**

Considerable variety exists in the techniques used to measure IR in non-diabetics and in individuals with T2D. Direct measurements of IR are preferred when examining all populations, yet the costly and labor intensive nature of such methods can be limiting. Plasma insulin concentrations measured in individuals with T1D will more likely reflect (at time of sampling) dose, timing, and absorption of recent insulin injections than IR. Because fasting plasma insulin levels cannot be measured in T1D, this assessment cannot be used to estimate IR this population. Consequently, the use of any methodology relying on assessment of fasting plasma insulin in estimating IR is not appropriate for use in T1D.

### **4.2.1 Hyperinsulinemic-Euglycemic Clamp and Infusion Techniques**

Due to the limitations of an IR assessment technique that relies on estimations of fasting plasma insulin in T1D, researchers have generally used a “direct” assessment as the standard technique. A well-established technique for assessment of insulin sensitivity in the majority of populations, including T1D, is the hyperinsulinemic-euglycemic clamp. This technique administers exogenous insulin intravenously at rates designed to maintain a pre-set hyperinsulinemic plateau. Simultaneously, the plasma glucose concentration is “clamped” at the normal fasting or any pre-

existing (euglycemic) level by means of an exogenous intravenous (IV) infusion of glucose. By doing this, insulin action is measured “directly” under comparable conditions of stimulus (fasting plasma insulin concentration) and substrate (plasma glucose concentration). When a steady state is attained, the exogenous glucose infusion rate equals the amount of glucose disposed of by all the tissues in the body, providing a quantification of whole-body insulin sensitivity [8].

Despite the "gold standard" validity of the hyperinsulinemic-euglycemic clamp, its time cost requirements have led to simplified approaches in quantification of IR. In the last 20 years, various indices of IR have been proposed using data from proxy measures. Although current literature frequently reports these estimation methods, it is important to understand the methodology employed when using such techniques since their validity and reliability in assessing IR can vary greatly.

A commonly utilized approach for the estimation of IR is the minimal model proposed by Bergman *et al* .[67, 158, 159]. This technique employs a frequently sampled IV glucose tolerance test (FSIVGTT) which measures the level of first phase insulin response and insulin secretion from the body. From the data obtained by the FSIVGTT, a computer-assisted model (minimal model) is used to calculate an insulin sensitivity index along with a measure of the acute endogenous response of insulin to glucose. The minimal model, which accounts for both insulin and glucose concentrations during the FSIVGTT, uses a simplified mathematical representation of the glucose—insulin relationships. By entering the measured insulin concentration into a linear model, insulin sensitivity and glucose effectiveness are estimated by least-squares fitting of the FSIVGTT glucose concentration profile.

The insulin sensitivity test (IST) is a more simplistic assessment which involves IV infusion of a defined glucose load and a fixed-rate infusion of insulin over approximately three hours. Somatostatin may be infused simultaneously to prevent insulin secretion, inhibit hepatic gluconeogenesis, and delay secretion of counter-regulatory hormones such as glucagon, growth hormone, cortisol, and catecholamines [160]. The mean plasma glucose concentration over the last 30 minutes of the test reflects IR. Although lengthy, IST is less labor intensive than clamp techniques; the FSIVGTT and IST also require fewer blood samples compared to clamp techniques.

Over a decade ago, researchers proposed the Insulin Tolerance Test (ITT) as a simple and inexpensive alternative to more sophisticated techniques [161]. The ITT primarily measures the insulin-stimulated uptake of glucose into skeletal muscle. A less complicated version of IST, the ITT measures the decline in serum glucose after the administration of an IV bolus of regular insulin (0.1–0.5 U/kg). Several insulin and glucose levels are sampled over a 15 minute period (depending on the protocol used). Because of the brevity of this test, the risk of counter-regulatory hormones interfering with its results is minimal, making the additional of pharmaceutical agents with this test not necessary. Although considered a safe test of insulin sensitivity, this test is infrequently used due to patient tolerance issues that arise as due to the hypoglycemia experienced during the test (sweating, drowsiness, shakiness, hunger, difficulty with concentration, etc.).

#### **4.2.2 Fasting Methods for Assessing Insulin Resistance**

Various adaptations of insulin-glucose comparisons have been applied examining fasting blood samples. These protocols have been widely adapted into varying methods of IR estimation.

Regardless of the protocol, the majority of these methodologies are based on the general concept that a high insulin level implies the presence of IR when theoretically measured at normal blood glucose level.

Fasting serum insulin is an inexpensive assay that does not require any mathematical calculations. Although assessment of fasting serum insulin is less variable than is the case for other fasting procedures in non-diabetic patients, interpretations of the results can vary depending on the patient population. Therefore, caution is advised when interpreting the results. A fasting level of 30  $\mu\text{U}/\text{mL}$  indicates greater IR in a diabetic individual than in a non-diabetic person. This is because a similar basal insulin level would not proportionately suppress glucose in a T2D patient as well as in the non-diabetic individual [160]. Conclusions from this assessment of IR are based solely on the assumption insulin levels reflect only the ability of insulin to reduce serum glucose. However, with decreasing beta cell function, a diminished production of circulating insulin occurs and thus the results may not accurately reflect insulin sensitivity. Consequently, the exclusive use of insulin levels for the estimation of IR is contraindicated in IGT or T2D individuals [162].

The Glucose/Insulin (G:I) ratio, introduced in 1998, was first reported as an accurate index of IR in women with polycystic ovary syndrome (PCOS) [163, 164]. The ratio of glucose to insulin is easily calculated from blood samples, with lower values depicting higher degrees of IR. A G:I ratio of less than 4.5 has been shown to be sensitive (95%) and specific (84%) for IR in a group of women with PCOS, when compared to a control group [163]. Recently, a number of studies have suggested that the G:I ratio represents a useful method for assessing IR in other populations [160, 165, 166].

Similar to fasting insulin levels, the G:I ratio appropriately reflected the physiology underlying the determinants of IR both in normal fasting or steady state fasting conditions [167-169]. In fasting non-diabetic individuals, mechanisms used to maintain normal blood sugar involve the regulation of both glucose production in the liver and insulin secretion by the pancreas. Under steady state fasting conditions, high insulin levels measured when glucose is in the normal range corresponds to a state of IR. Accordingly, the reciprocal value of insulin (1/insulin) is a well known proxy for insulin sensitivity that decreases as fasting insulin levels rise denoting an increase in IR [168-170]. In non-diabetic subjects, G:I is functionally equivalent to 1/insulin since all subjects have similar fasting glucose levels.

Various methods to assess IR that rely on more advanced computations of the insulin and glucose relationship are also available. Clinical researchers have widely employed the HOMA-IR to assess IR [165]. Rather than using fasting insulin or a G:I ratio, the product of the fasting values of glucose (GO) (expressed as mg/dL) and insulin (IO) (expressed as  $\mu\text{U/mL}$ ) is divided by a constant:  $(\text{IO} \times \text{GO})/405$ . The constant 405 is replaced by 22.5 if glucose is expressed in S.I. units. Since both HOMA-IR and fasting insulin values increase in insulin-resistant individuals while the G:I ratio decreases, the HOMA-IR values correlate well with hyperinsulinemic-euglycemic clamp assessments [166]; and HOMA-IR has been frequently used to assess changes in IR after treatments [171-174].

The request for simpler tests to assess IR has yielded a revised concept for the estimation of IR based on a single sample of fasting insulin [175]. Researches then explored the hyperbolic relationship between fasting insulin and IR proposed by Hermans *et al.* [176] which described the model of insulin sensitivity  $S_1 = \alpha/\text{Insulin (I)}$ , with the coefficient  $\alpha$  as a constant. Data from 70 normal and overweight participants who previously FSIVGTTs for the calculation of IR via

the minimal model [159] were examined. Statistical analysis revealed  $S_I \times I = \sim 40$  best describes the relationship with IR via the minimal model. A second sample of 49 normal and overweight subjects where HOMA-IR index [166] was previously calculated was subsequently examined. The results showed that the 40/I index gave a better prediction of minimal model-derived  $S_I$  ( $r = 0.882$ ,  $p < 0.0001$ ) than either the HOMA-IR ( $r = 0.546$ ,  $p < 0.01$ ) or fasting plasma insulin ( $r = 0.589$ ,  $p < 0.01$ ). Conclusions from this investigation suggest that 40/I, with the specific methods utilized, would be a more precise marker of IR than fasting insulin values alone.

#### **4.2.3 Post-Glucose Load Assessments of Insulin Resistance**

The OGTT, a foundation in the diagnosis diabetes in the past, and recently of IGT and T2D in pregnant and non-pregnant women, also permits estimation of IR. Because no IV catheterization or insulin infusion is needed, the OGTT is better suited for assessment of large populations than the other clinical techniques assessing IR. Many formulas for the assessment of IR have been derived based on the OGTT values. In 1990, Cederholm and Wibell examined the validity of a formula for  $S_I$ , derived from an OGTT. Their results suggested estimating IR based on four timed samples of insulin and glucose (0, 30, 60, and 120 min) values. A comparison of this formula to the hyperinsulinemic-euglycemic clamp demonstrated fairly good agreement [177]. Along with  $S_I$ , many other attempts have been made at exploring possible calculations and validating simple measures of IR using OGTT values. The  $S_I$  formula has thus been modified to use only the 0 and 120 min post-glucose challenge insulin and glucose concentrations [178]. The individual protocols also examine a variety of correlates of IR, including hypertension and sodium sensitivity. The resulting index,  $ISI_{0,120}$ , has been reported to correlate well with hyperinsulinemic-euglycemic clamp measures ( $r = 0.63$ ,  $P < 0.001$ ) [178].

Another algorithm for the estimation of SI and Acute Insulin Response (AIR), a measure of IR and beta-cell function, is available using data from OGTTs. The BIGTT test, derived from multiple simple and inexpensive physiological measurements [179] uses samples from OGTTs and a tolbutamide-modified FSIVGTTs. Model development and internal validation of the BIGTT, using Bergman's minimal model to calculate SI, was performed. Multiple linear regression was then applied to develop predictive equations of log SI and log AIR using information collected on gender, BMI, plasma glucose, and serum insulin levels obtained during the OGTT. The results suggest a high correlation between the obtained estimates of SI (BIGTT-SI) and AIR (BIGTT-AIR) with FSIVGTT-derived values of SI ( $R^2 = 0.77$ ) and AIR ( $R^2 = 0.54$ ). The two validation investigations performed in this study displayed similar results.

Hyperinsulinemic-euglycemic clamp and insulin-modified FSIVGTT tests [180] are also the basis for the development of the Quantitative Insulin Sensitivity Check Index (QUICKI), a commonly used simplistic index of IR [181]. Researchers, who subjected the data from the standard methods to various transformations, ultimately defined the proxy equation as  $QUICKI = 1/[\log(IO) + \log(GO)]$ , where IO is the fasting insulin level, and GO is the fasting glucose level assessed from a fasting blood sample. Clinical researchers favor this measure due to its high correlation with the hyperinsulinemic-euglycemic clamp ( $r = 0.69$ ;  $p < 0.04$ ). This method is limited, however, since it only focuses on fasting blood samples and does not take into consideration non-fasting glucose—insulin relationships. This technique neither considers the limitations assessing fasting plasma insulin in T1D nor does it have value in severe T2D populations who are unable to discontinue their medication regime, thereby affecting the assessment of insulin and glucose.

With the various techniques available to assess IR in humans, researchers should account for many diverse factors when comparing diabetic populations. Most likely due to the time and financial issues associated with the hyperinsulinemic-euglycemic clamp technique, the majority of IR literature use estimation measures (previously described) as proxy measures of IR. Although acceptable valid instruments for use in populations where fasting plasma insulin can be measured, these techniques are not appropriate for use in T1D populations. Investigators are currently proposing these simplistic estimation formulae as useful indices of IR, particularly in epidemiologic studies [182-193]. Yet, the utility for the formulae in assessing IR in T1D is minimal due to the use of fasting plasma insulin assessment being central to the methodologies.

#### **4.2.4 Estimation Equations for Insulin Resistance in Type 1 Diabetes**

Estimation equations based on patient characteristics and on clinical parameters of T1D are available for use in this population [9, 194]. The most common technique for estimating IR in T1D is the Estimated Glucose Disposal (eGDR) equation [9]. Hyperinsulinemic-euglycemic clamp studies (n = 24) were performed to directly assess IR, and the relationship between IR and the following patient characteristics and clinical parameters of the disease was examined: hypertension, waist-hip ratio (WHR), triglyceride and HDL cholesterol levels, family history of type 2 diabetes, and glycemic control (HbA1). Using multiple linear regression, the combination of risk factors that yielded the highest adjusted  $r^2$  value with the clamp values were WHR, hypertension status, and HbA1 ( $r = 0.57$ ,  $P < 0.001$ ). The study yielded the estimation equation:  $eGDR = 24.31 - 12.22(WHR) - 3.29(HTN) - 0.57(HbA1)$ . This estimation equation has been applied to the entire population from the Pittsburgh Epidemiology of Diabetes Complications study in order to determine the effect of IR on diabetes complications [13, 78, 195-197].

Recently, a similar investigation in children and adolescents with T1D investigated a simplified estimation of IR based on patient characteristics and clinical parameters of the disease course [194]. The hyperinsulinemic-euglycemic clamp technique was performed in 142 children and adolescents with T1D (79 boys, 63 girls), ages 7.7 to 20.3. Researchers examined plasma cholesterol, HDLc, triglycerides, HbA1c, height, weight, waist circumference, blood pressure, body mass index, and daily dose of insulin. They found correlations between index M (a measure of glucose disposal) and insulin dose ( $r=-0.34$ ,  $p<0.05$ ) and HbA1c ( $r=-0.17$ ;  $p=0.04$ ). Significant relationships between index M and waist circumference, lipids and blood pressure were also discovered. After performing multiple linear regression analysis examining all significant clinical parameters, the model with the strongest correlation with index M yielded the equation:  $M \text{ index} = 17.065 + 1.547(\text{gender: boys}=1, \text{ girls}=0) - 0.183(\text{age}) - 0.117(\text{Waist circumference}) - 2.019(\text{Daily insulin dose}) - 0.016(\text{LDLc}) + 0.041(\text{DBP})$ . Even though this equation seems promising for use in adolescent populations, its use in other T1D age groups or T1D individuals with diabetes related complications has not yet been validated.

In light of investigations exploring the validity of these IR equations for use in T1D, their usefulness in assessing IR in T2D populations has not been examined. Consequently, the only acceptable “direct” assessment of IR that can be jointly evaluated in T1 and T2D is the hyperinsulinemic-euglycemic clamp technique; however, few reports are available comparing in vivo IR across these two populations [198-202]. Although these equations are based on relationships between hyperinsulinemic-euglycemic clamp assessments and certain adiposity measures (i.e. WHR and waist circumference), no current studies have examined the relationship between IR and adiposity as assessed by more specific adiposity measures such as dual x-ray absorptiometry or computed tomography. The addition of such adiposity measures to the

estimation equations may prove useful in increasing the validity and reliability of these measures of IR, yet further examination is warranted.

## 5.0 TECHNIQUES FOR THE ASSESSMENT OF BODY COMPOSITION

Due to the metabolic influences of adipose tissue, researchers have explored various techniques assessing body composition when exploring the physiologic effects of the tissue. In population studies, the two most common indices of fatness or obesity include 1) body mass index (BMI = body mass/stature<sup>2</sup>) where body mass is recorded in kilograms (kg) and stature in meters (m); and 2) regional adiposity, commonly estimated by either WHR or summing measurements from raised skin folds. Despite their convenience and popularity, the above measures are still crude assessments of adiposity.

BMI, best used as an index of nutritional status [203] and risk for disease outcome [204-206], can be categorized into underweight, normal weight, and overweight/obesity. In adults, BMI predicts clinical outcomes such as cardiovascular disease and T2D; however, its predictive value for children and adolescents is less clear [207, 208]. Despite being considered the most common anthropometric index used for the classification of general obesity [156], the usefulness of BMI in relation to body composition remains controversial. While studies show significant correlations between BMI and percent body fat [207-209], BMI is also reported to have up to a two-fold range of variation in fatness for a given BMI value in adults and children [209, 210].

Waist circumference (WC) provides a simple measure of central or trunk fatness and is considered a better predictor of adverse outcomes such as lipid profile or IR than total body fat

[207]. In adults, WC has been independently associated with morbidity after adjustment for a relative weight or BMI [211, 212]. Similar findings are now being reported in children [212]. Studies investigating the relation of WC with measures of visceral adiposity obtained from magnetic resonance imaging (MRI) have consistently shown correlations in the range of 0.4 to 0.8, although its associations with total abdominal fat tend to be higher than those with visceral fat [213-216]. In contrast, studies reporting the association between WHR and visceral fat are more inconsistent than the association with WC, yet some investigations have found no significant relationships [213, 214].

Since their introduction in the 1970's, *skinfold thickness* measurements have been used to rank individuals in terms of relative "fatness" or to assess the size of specific subcutaneous fat depots [217]. Skinfold thickness data is best used as raw values, where they act as reliable indices of regional fatness. The measurements can then be converted into standardized scores which are useful for longitudinal evaluations. The advantages for skinfold measurements include being quick and easy to obtain in most age groups, yet the limitations for their use lie in the low repeatability of the measurements. Intra-observer and inter-observer errors are low compared to between-subject variability, but accuracy and precision have been poor in obese individuals, including adolescents [218, 219].

Two-component methods divide the body into fat mass (FM) and fat-free mass (FFM). These methods address components of weight, avoiding the need to predict total masses from regional or superficial proxies. However, one limitation is that they remain dependent on theoretical assumptions, such as constancy of the composition of FFM. A two-compartment technique that has garnered great attention in recent literature is dual energy x-ray absorptiometry (DEXA). DEXA, originally developed to measure bone mineral density, has

shown to perform valid quantification of various other tissues. The measurements from a DEXA scan are based on a three-compartment model that separates the body into total body mineral, FFM, and FM. These assessments of body composition are determined by the differential absorption of *x*-rays of two different energies and subsequent calculations are performed in order to obtain the desired values. DEXA results have been reported to vary according to body shape and outcome. Due to the high concentration of visceral organs in the trunk region, composition assessment in this area involves substantial prediction rather than measurement [207, 220, 221]. As a result, soft tissue estimation in the trunk area is less accurate than in the limbs. DEXA may provide useful information on relative fat and lean masses as a single measurement in an individual, particularly with respect to limb lean mass. However, such assessments need the development of normal reference data in order to accurately assess disease populations.

Magnetic Resonance Imaging (MRI), another two-compartment technique, is used to estimate the volume of adipose tissue rather than the assessment of tissue mass. This technique produces images based on spatial variations in the phase and frequency of the energy absorbed and emitted by the target tissues. The imaging, which analyzes the absorption and emission of energy in the radio frequency range of the electromagnetic spectrum, primarily addresses hydrogen nuclei located either in water or fat. It uses these data to discern tissue types in "imaging slices" which can then be summed to calculate regional tissue volumes [222].

Despite MRI providing high quality imaging data, this technique has relatively high cost and limited availability. Certain difficulties also arise when attempting to compare results from MRI analysis with those obtained by other techniques. MRI results make assumptions concerning the fat content of adipose tissue and the density of fat. While the density of fat does not vary significantly, large variation can exist in the fat content of adipose tissue. Additionally, when

MRI estimates fat mass, it bases the assessment on the fat mass present in the tissue being scanned [223]. This measure greatly differs from the outcomes of other methods, including densitometry which assesses total fat mass. As a result, many researchers deem the capacity of MRI to estimate regional body composition as one of the few accurate and viable approaches for the estimation of visceral adipose tissue.

Despite the increasing reports of MRI as a preferred method of assessing regional adiposity, emerging evidence also supports the use of computed tomography (CT) as a direct assessment of regional adiposity [224, 225]. CT assessments, which assume that fat has a lower attenuation than other tissues, produce cross-sectional radiographs that are suggested to be very useful in the assessment of visceral adiposity [225]. Since CT is much more sensitive to slight differences in attenuation when compared to standard radiography, this technique offers great clarity for assessment of adipose tissue [226]. Its ability to obtain clear images of soft tissue gives CT similar advantages to those of MRI images in relation to its capacity for estimation of regional body composition. CT images of the abdomen allow computerized measurement of total fat area, as well as the differentiation of subcutaneous fat from visceral fat. Although CT has been widely used to assess regional adiposity in a variety of populations [111, 223, 224, 226-230], certain limitations arise. Similar to MRI, this methodology is costly and limited in availability. CT, whose assessment is based on the fat mass present in the target tissue, also presents challenges when comparing it to other methods such as densitometry or hydrometry. Additionally, the use of this technology requires radiation exposure to participants which increases in relation to the length of the scanning protocol used.

In attempts to decrease the cost of CT scanning protocols and limit radiation exposure to participants, researchers have examined the validity of few or single-slice CT assessments of

visceral adiposity. An investigation of whether a single CT scan of the abdomen provides an accurate indication of overall abdominal adiposity shows that rankings of total abdominal area, total fat area, and subcutaneous and visceral fat areas are relatively consistent no matter which abdominal level is chosen [225]. This study reports correlations of 0.89 to 0.99 between single scans and the average values for all scans, suggesting that a single CT image contains the same information on adiposity as a series of scans. These results support the use of single scans at different anatomical sites to limit the radiation exposure in future CT studies of body composition. If only a single scan at one site can be obtained, the level of the umbilicus may be the most useful, because it contains the largest percentage of fat in the body and best allows differentiation of visceral from subcutaneous fat.

## 6.0 SUMMARY AND CONCLUSIONS

Obesity and IR are major metabolic abnormalities in the natural history of T2D, and current investigations are suggesting similar incidences in T1D. These abnormalities can often be identified many years before the appearance of any impairment in glucose homeostasis, yet the true relationship between these abnormalities and how they may vary in T1D and T2D is still unclear. Adiposity (i.e. visceral fat) is suggested to play a major role in affecting insulin action and consequently the development of CVD, yet these associations have also not been fully examined in T1 and T2D. Elucidation of these associations may have implications for the identification of key targets in the prevention and/or treatment of diabetic conditions. Accordingly, the following investigations are proposed for a research dissertation under the theme, “IR in Type 1 Diabetes: Determinants and Clinical Consequences”:

1. *Does DEXA-determined regional adiposity add to the prediction of insulin resistance in type 1 diabetes?: A re-examination of the eGDR equation*

The purpose of this investigation is to determine if regional adiposity assessments (e.g. arm fat, leg fat, trunk fat) improve the estimation of IR by standard risk factors and to examine whether IR differs across strata of CAD and renal disease in T1D.

2. *Exploring the relationship between regional adiposity and insulin resistance in type 1 diabetes*

The purpose of this investigation is to compare levels of IR and DEXA-assessed regional adiposity distribution (i.e. leg, arm, and trunk fat) between non-diabetic and T1D individuals and to examine whether the associations between regional adiposity and IR differ between non-diabetics and individuals with T1D.

3. *Regional adiposity and risk for coronary artery disease in type 1 diabetes: Does lower body adiposity reduce the risk?*

The purpose of this investigation is to examine the associations between prevalent CAD and CAD risk factors, IR, and DEXA-assessed regional adiposity in individuals with T1D and to determine if regional adiposity assessments assist in the characterization of CAD in T1D.

**7.0 ARTICLE 1: DOES DEXA-DETERMINED REGIONAL ADIPOSITY ADD TO  
THE PREDICTION OF INSULIN RESISTANCE IN TYPE 1 DIABETES? A RE-  
EXAMINATION OF THE EGDR EQUATION**

To be submitted for publication

Christina M. Shay<sup>1</sup>, Bret H. Goodpaster<sup>2</sup>, Frederico G.S. Toledo<sup>2</sup>, Sheryl F. Kelsey<sup>1</sup>, Elsa  
Strotmeyer<sup>1</sup> and Trevor Orchard<sup>1</sup>

<sup>1</sup>Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh,  
Pittsburgh, Pennsylvania

<sup>2</sup>Department of Medicine, University of Pittsburgh School of Medicine, University of Pittsburgh,  
Pittsburgh, Pennsylvania

## 7.1 ABSTRACT

**BACKGROUND:** Insulin resistance (IR) occurs in type 1 diabetes (T1D) and is related to a number of diabetes complications, including cardiovascular and renal disease. The insulin clamp technique is the most accurate assessment of IR in T1D, but the resource intensive nature of this technique is often inappropriate for use in large epidemiologic studies. The Estimated Glucose Disposal (eGDR) equation has been increasingly used in T1D studies making further examination of this technique desirable. Lower body adiposity has been favorably associated with IR, but whether this measure of body composition contributes to the prediction of IR in T1D has yet to be examined. Thus, we set out to determine if IR differs across strata of CAD and renal disease in T1D and to examine whether regional adiposity assessments improve the estimation of IR by standard risk factors.

**METHODS:** Participants were recruited from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, a 20-yr prospective study of childhood onset T1D. Data was analyzed from the 18-year examination and ancillary insulin clamp studies (n=30). Insulin sensitivity was assessed by hyperinsulinemic-euglycemic clamp technique and adiposity distribution was assessed by dual x-ray absorptiometry (DEXA). Other diabetes factors were also assessed.

**RESULTS:** Multivariate linear regression revealed that leg, arm, trunk and total % FM were all negatively associated with IR after adjusting for eGDR variables. Diabetes duration, WC, daily insulin dose, and presence of overt nephropathy were the risk factors most strongly associated

with IR in this sample. After controlling for these risk factors and gender, all regional adiposity measures remained negatively associated with IR, however % FM was the DEXA adiposity measure that most strongly contributed to the eGDR estimation of IR.

CONCLUSIONS: All measures of regional adiposity were negatively associated with IR in T1D thus these data do not support the hypothesis that lower body adiposity have a protective effect on IR in T1D, but rather that general obesity and abdominal obesity are similarly detrimental to IR in this population. Whether excess lower body adiposity has a metabolically protective effect in T1D, or whether it is merely reflective overall or central adiposity, which is likely the case in this T1D sample, requires further examination.

## 7.2 INTRODUCTION

Although the prognosis in patients with type 1 diabetes (T1D) has improved considerably over the last 50 years, the morbidity and mortality rates still greatly exceed that of the general population. Coronary artery disease (CAD) in T1D is the leading cause of death and, as in the general population, is likely to result from an amalgam of different factors. Substantial evidence exists suggests that much, but not all, of the higher CAD mortality in T1D is related to the development of renal disease [231-233]. However, even in the absence of concurrent renal disease there is a considerable increase in CAD risk in T1D subjects [234]. It has been increasingly recognized that insulin resistance (IR), usually associated with type 2 diabetes, also occurs in T1D and has been related to a number of diabetes complications, including cardiovascular and renal disease [3, 13, 66, 69, 78]. One hypothesis is that an insulin resistant subgroup of T1D individuals may be predisposed for both CAD and renal disease and the temporal relationship may be partly dependent

on other cardiovascular risk factors (e.g. IR) [235]. Therefore, as an underlying component of multiple complications in T1D, the measurement of IR is of particular interest.

The hyperinsulinemic-euglycemic clamp technique [8] provides the most accurate assessment of IR, although its labor intensive, costly, and invasive nature makes it inappropriate for use in large-scale population studies. Alternative techniques developed to assess IR in non-diabetic and type 2 diabetes (T2D) populations commonly rely on the measurement of fasting plasma insulin levels [158, 159, 178, 179, 236]. Since plasma insulin concentrations in T1D more likely reflect timing and dosing of replacement therapy, rather than resistance, these methods are not appropriate for use in T1D populations. The Estimated Glucose Disposal (eGDR) equation, validated by hyperinsulinemic-euglycemic clamp studies, is a method of estimating IR in T1D [9] and has been used in a number of epidemiologic T1D investigations [10-13]. This equation which employs readily obtainable clinical assessments (waist-hip ratio, HbA<sub>1c</sub>, and hypertension) has been shown to identify patients with T1D who are likely to have IR ( $R^2=0.63$ ) and is comparable to the insulin based formula in the non-diabetic population. The increasing use of this method makes desirable a further examination of this equation to see if it is improved with additional clinical factors.

The purpose of this study was thus two-fold: 1) to determine if regional adiposity assessments (DEXA) improve the estimation of IR by standard risk factors; and 2) to examine whether IR differs across strata of CAD and renal disease in T1D in participants from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, a 20-yr prospective study of childhood onset T1D.

## 7.3 METHODS

### 7.3.1 Study Population and Subject Recruitment

Subjects were participants from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC), a 20-year prospective follow-up study of childhood-onset type 1 diabetes mellitus which has been previously described in detail [237]. Briefly, participants were diagnosed (or seen within 1 year of diagnosis) between 1950 and 1980, before age 17, at the Children's Hospital of Pittsburgh. This population has been shown to be representative of the T1D population in Allegheny County, Pennsylvania [238]. Participants for the current investigation were recruited after subjects had attended the 18-year EDC exam (November 2004 – November 2006). Based on the results from the 18-year EDC study visit, patients were determined eligible for the glucose clamp study and a subgroup of participants with specific diabetes-related complications status were studied. Participants were ineligible if they were anemic or if their hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) was  $\geq 11.0\%$ . The cutoff level of  $\geq 11.0\%$  for HbA<sub>1c</sub> was chosen to minimize the likelihood of excessive hepatic production due to poor glycemic control [239]. Subjects were also ineligible if they used any steroid medications which affect insulin sensitivity within 6 weeks of testing.

### 7.3.2 Clinical Evaluation and Procedures

Details regarding the clinical and metabolic evaluation for the EDC study have been previously reported [240]. As part of the EDC exam, total cholesterol (TC) and triglycerides were measured enzymatically [241, 242] and high-density lipoprotein cholesterol (HDL-C) levels

were determined by a precipitation technique (heparin and manganese chloride) with modification of the Lipid Research Clinics method [243]. Non-HDL-C levels were calculated by subtracting HDL-C from total cholesterol. Blood samples were analyzed for HbA<sub>1c</sub> using the DCA 2000 analyzer (Bayer Diagnostics, Tarrytown, NY). Results from at least two of three timed urine collections (24-hour, overnight, and random timed post-clinic visit urine) were used to determine albumin excretion rates (AER). Both a standardized medical history and clinical examination were performed by a trained internist to classify participants according to CAD and renal status. Upon hospital admission for insulin sensitivity testing, height and weight were measured using calibrated scale and stadiometer. Sitting blood pressures were measured in the right arm after a 5-min rest period using a Dynamap electronic blood pressure monitor (GE Healthcare, Fairfield, CT).

### **7.3.3 Coronary Artery Disease and Renal Disease Classification**

Since one of the goals of this investigation was to examine in patients with T1D the interrelationships between renal disease, CAD, and IR, participants were recruited based on the presence or absence of CAD and/or renal disease yielding 4 groups with a maximum of 10 participants in each group. CAD cases (CAD+) comprised of a positive clinical history (EDC clinic physician diagnosed angina and/or ischemic ECG (Minnesota codes 1.3, 4.1-4.3, 5.1-5.3, 7.1) at the time of examination with coronary artery calcification (CAC)  $\geq 100$ , myocardial infarction (either pathological Q waves (Minnesota codes 1.1, 1.2) or findings on review of previous hospital records), hospital record or validated angiographic evidence of  $\geq 50\%$  or more stenosis with or without revascularization, CAC  $\geq 400$  without presence of clinical disease was also included in the definition for CAD cases. Overt nephropathy (ON+) was defined as albumin

excretion rate (AER) of greater than or equal to 200 micrograms per minute or an albumin/creatinine (A/C) ratio of greater than or equal to 105 micrograms per minute.

#### **7.3.4 Adiposity Assessment**

During the EDC exam, waist circumference (WC) was assessed as a measure of visceral adiposity as part of the regular EDC exam. WC was measured horizontally at midpoint between the highest point of the iliac crest and the lowest part of the costal margin in the mid-axillary line. Distribution of adiposity was measured by dual x-ray absorptiometry (DEXA) using a Hologic QDR4500A scanner (Hologic QDR system software 12.3) to determine whole body fat mass (FM) lean body mass (LBM), total body FM (FM %), arm FM, leg FM, and trunk FM. FM in the arms and legs was calculated as the sum of both corresponding appendages. The separation between trunk and leg regions was made by two oblique lines passing through the femoral necks and the separation between trunk and arm regions was made by two oblique lines passing through the humeral heads. Trunk FM included both subcutaneous and visceral FM of this anatomical region and the measures of arm and leg FM was a total of both corresponding appendages.

#### **7.3.5 Assessment of Insulin Sensitivity**

All participants were admitted to the University of Pittsburgh Clinical Translational Research Center on the evening before insulin sensitivity testing. All participants fasted overnight after receiving a standard dinner (7 kcal/kg) and snack and any long-acting injection of insulin was withheld at least 24 hours prior to insulin sensitivity testing. Short-acting insulin was used to

control post-prandial hyperglycemia the evening before the study. 14 of the 30 participants used an insulin pump and for these participants basal rates and prandial boluses of insulin were continued on the day preceding insulin sensitivity testing. At 10 pm, an intravenous (IV) catheter was placed in a forearm vein for blood sampling and a second catheter was placed in the antecubital region of the opposite arm for all IV infusions. An intravenous infusion of insulin was then started to control blood glucose; those using subcutaneous insulin pumps discontinued the infusion at this time. The overnight target range for blood glucose was 90-150 mg/dl and venous glucose was monitored hourly (or more frequently as needed) with adjustments to the insulin infusion made based on a pre-determined dosing algorithm. At the initiation of the insulin clamp procedure, the insulin infusion was increased to 40 mU/m<sup>2</sup>/min, and blood glucose was measured at 5 min intervals to achieve and maintain euglycemic values (85-95 mg/dl), with an adjustable infusion of 20% dextrose using the “glucose clamp” method [8]. The insulin infusion was continued for a minimum of 2 and a maximum of 4 hours until steady-state metabolic conditions had been attained. Blood was collected for the determination of steady-state plasma insulin during the clamp procedure. Insulin was measured using an immunoassay on a DPC 2000 (Human Insulin Eliza, Linco Laboratories, Billerica, MA). Glucose disposal rate (GDR) was calculated based on glucose infusion rates adjustment for body weight (mg·min<sup>-1</sup>·kg<sup>-1</sup>) and M-values were calculated after adjustment for lean body mass (mg·min<sup>-1</sup>·kgLBM<sup>-1</sup>). The Insulin Sensitivity Index (ISI) was also computed to potentially account for decreased insulin clearance in individuals with renal disease [8] which has been theorized to alter insulin clamp assessments in individuals with renal disease [71]. The ISI is a measure of tissue sensitivity to insulin adjusted for circulating insulin levels was calculated by dividing the M-value by the mean plasma insulin concentrations and multiplied by 100 (ISI= 100 x M/plasma insulin). All

measures of insulin sensitivity were calculated based on based glucose infusion rates during a 20 minute period of euglycemia after beyond the 2 hour study time point.

### 7.3.6 Statistical Analyses

Univariate differences between complication groups were evaluated using Analysis of Variance (ANOVA). Pearson correlation coefficients were used to examine associations between continuous variables. Non-normally distributed variables (e.g. albumin excretion rate [AER]) were transformed by natural log prior to testing.  $P < 0.05$  was considered statistically significant. Multivariate linear regression was used to examine the influence of the regional adiposity measures on the eGDR equation, the components of the eGDR formula were forced into a linear regression model and each adiposity variable was individually added to the model with sex. examine variables most strongly associated with insulin sensitivity. To reexamine a new set of predictors for the estimation of IR, multivariate linear regression was used and all variables with a univariate association ( $P < 0.25$ ) with insulin sensitivity were made available for modeling, and a significance of  $P > 0.05$  was applied for exclusion from the model. Because of colinearity with duration ( $r = 0.86$ ) and a stronger association between duration and insulin sensitivity (M-value), age was not used in multivariate analyses. Similarly, since a strong correlation was also observed between WC and BMI ( $r = .83$ ,  $p < .001$ ), and WC was most strongly associated with insulin sensitivity, BMI was not used in multivariate analyses for the full sample. For adiposity measures that were significantly correlated with WC or WHR (i.e. trunk FM), separate models were fit investigating the influence of each relative adiposity measure on the prediction of the baseline insulin sensitivity model excluding these variables. The majority of the regional adiposity variables were significantly correlated with WC, so each DEXA assessment was

regressed onto WC and the standardized residuals were retained. In order to address the combined contribution of WC and regional adiposity to the prediction of IR, additional models were fit with the best prediction variables (including WC) and the residual values for each adiposity variable along with sex were added to the model. Since total FM and FM % were also highly correlated in this sample ( $r=.97$ ,  $p<.01$ ), and FM % is a measure of relative adiposity, FM % was used in the regression modeling. Models were compared using  $R^2$  values and the model having the highest such value was designated the final (best) model. No formal correction was performed for multiple comparisons. Analysis was performed using SPSS for Windows software version 16.0 (SPSS, Chicago, IL).

Twenty nine out of thirty participants included in the analysis were fully examined according to protocol. DEXA body composition assessments were not performed on one individual due to mechanical issues. Since similar results were obtained when analyses for the baseline model were performed with and without this subject, data for the baseline model is presented including this individual (N=30). LBM assessments for the calculation of the M-value for this individual were taken from a bioelectrical impedance assessment as part of the regular EDC exam.

## **7.4 RESULTS**

### **7.4.1 Participant Characteristics**

The overall sample of 30 participants had a diabetes duration of  $38.8 \pm 7.0$  years (mean  $\pm$  SD) and were  $47.7 \pm 7.9$  years of age. These findings are similar to the distribution of participants

with CAD or ON in the original EDC glucose clamp investigation where following number of participants had diabetes complications: 16 CAD-/ON-, 6 CAD+, 1 ON+, and 1 CAD+/ON+. Insulin sensitivity adjusted for LBM (M-values) ranged from 2.2-11.4  $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg LBM}^{-1}$  and insulin sensitivity additionally adjusted for plasma insulin (ISI) ranged from 3.1-25.9  $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}\cdot\text{uUmL}^{-1}$  and these assessments of insulin sensitivity were strongly correlated ( $r = .73$ ,  $p < .01$ ). No gender differences in insulin sensitivity or clinical characteristics were apparent except that men in this sample exhibited higher blood pressures, especially DBP when compared to females (75.8 vs. 62.6 mmHg,  $p = .003$ ) (Table 1). The 0.9% higher HbA<sub>1c</sub> was not significant ( $p = .07$ ). BMI, WC, FM, trunk FM, and arm FM were not significantly different between the sexes, but males had higher body weight ( $p = .02$ ), and LBM ( $p < .001$ ), and lower FM % ( $p = .005$ ) and leg FM ( $p = .001$ ), compared to females.

Correlations between insulin sensitivity (M-value) and clinical participant characteristics overall and by gender are presented in Table 2. In all participants, moderately strong associations were observed between M-value and triglycerides, daily insulin dose, BMI, WC, FM %, trunk FM, leg FM, and arm FM. A significant positive association was also observed between insulin sensitivity and both serum creatinine and AER in this sample. When stratified by gender, arm FM, trunk FM, FM %, and BMI were strongly associated with M-values in the females only, respectively, although WC was equally correlated in both genders.

Upon stratification by disease classification (Table 3), CAD+ individuals had significantly longer duration of diabetes compared to CAD- individuals and ON+ participants had significantly higher AER compared to ON- individuals. All other demographic or diabetes characteristics were similar between the complication groups except that the four CAD+/ON+ individuals had significantly higher BMI, WC, and serum creatinine when compared to

individuals without either diabetes complication. No significant difference in M-value or ISI was observed between CAD+, ON+, or CAD+/ON+ individuals when compared to those without diabetes complications.

#### **7.4.2 Optimization of the eGDR Equation**

Subsequent analyses were performed to refine the eGDR equation and determine if a particular DEXA adiposity measure more optimally predicts the eGDR equation (Table 4). The original eGDR equation was based on GDR adjusted for body weight, opposed to the new M-value adjusted for LBM, and did not predict GDR adjusted for LBM as well. In addition to the original eGDR variables (HbA<sub>1c</sub>, hypertension and WHR) and sex, FM % (p<.001) offered the optimal prediction of GDR corrected for body weight ( $R^2=.62$ , p<.001) similar to eGDR prediction in the earlier study group [9]. Height was also added to these models to account for the influence of this variable on relative adiposity distribution and doing so did not significantly change the results (data not shown).

#### **7.4.3 Re-estimation of Glucose Disposal Rate**

As similar associations were seen for both the ISI and M-values measures of insulin sensitivity, and measures of insulin sensitivity adjustment for lean body mass (LBM) are now preferred for glucose clamp studies, M-values adjusted for LBM are used as the measure of insulin sensitivity in subsequent analyses.

The results from a forward selection multivariate linear regression of the dependant variable insulin sensitivity (M-value) revealed that duration of diabetes, WC, presence of overt

nephropathy, and daily insulin dose provided the combined best estimation (Table 5). CAD status was not offered to the model since it was not univariately associated with M-values. Even though it was not selected for the final model, gender was forced into the final model but did not significantly after the final baseline model ( $p=.93$ ) and was subsequently removed. The regression equation that best describes insulin sensitivity (M-value) =  $9.916 + .018*(\text{diabetes duration}) - 3.792*(\text{daily insulin dose}) + 2.263*(\text{Overt Nephropathy}) - .064*(\text{WC})$ .

Separate models individually forcing leg, arm, trunk, and FM % into the baseline model were examined to assess their contribution to the estimation of M-value after additional adjustment for gender. Since WC was highly correlated with all adiposity variables in this sample, the measure was removed from this first set of models including these variables. The addition of the adiposity variables to the baseline model revealed that trunk FM ( $p=.03$ ), leg FM ( $p=.04$ ), and FM % ( $p=.02$ ) were independently associated with insulin sensitivity (M-value). No interactions were observed between gender and the adiposity measures and the regional adiposity measures did not improve the  $R^2$  beyond that seen for WC or FM % (Table 5). Next, the combined influence of WC and the regional adiposity measures was subsequently examined. The residuals from each regression were then placed into the full model with WC and the contribution of each regional adiposity measure was examined (Table 6). The DEXA adiposity measures that contributed to the estimation beyond the association with WC were trunk FM ( $p=.02$ ) and FM % ( $p=.03$ ), whereas Leg FM ( $p=.12$ ) and Arm FM ( $p=.14$ ) did not independently contribute to the estimation of insulin sensitivity (M-value) after accounting for WC.

Since stronger associations between regional adiposity and insulin sensitivity (M-value) were observed in females, separate models examining the influence of regional adiposity on M-value each gender were fit (Table 7). The baseline variables from the best prediction equation

derived from the overall sample were included and the regional adiposity variables were again individually added to examine the contribution of these variables to prediction of insulin sensitivity. The best female equation included diabetes duration, BMI, overt nephropathy, and daily insulin dose ( $R^2 = .82$ ), while in men the best equation included diabetes duration, WC, overt nephropathy, and daily insulin dose ( $R^2 = .72$ ). The DEXA measures improved prediction of insulin sensitivity over WC in men ( $R^2$  increased from .72 to .85), but BMI remained a stronger adiposity-related predictor of insulin sensitivity over all DEXA adiposity measures in women.

## 7.5 DISCUSSION

Although over the past three decades IR has been well documented in T1D, its assessment in T1D remains a challenge which is often inappropriate for use in large scale investigations. Many risk factors associated with IR, including dyslipidemia, obesity, and hypertension, similarly cluster with CAD in T1D [3, 13, 69, 244], leading to IR accelerated atherosclerosis, as in non-diabetic populations [245]. Similarly, many investigations suggest that IR, and its correlates, are also independently associated with chronic kidney disease [246, 247]. The current data suggests that severity of IR (assessed by the hyperinsulinemic-euglycemic clamp method) may not differ cross-sectionally between individuals with T1D who have developed overt nephropathy or CAD when compared to individuals who have not developed such complications, however, this contrasts with our earlier data demonstrating that our eGDR equation predicted the subsequent incidence of CAD or ON. Additionally, all DEXA-assessed regional adiposity measures were found to have an independent negative contribute to the prediction of IR when used in

conjunction with gender and the eGDR variables or the newly derived regression equation. Leg FM was found to have a negative association, rather than a protective association, with insulin sensitivity in T1D which contrasts the results of previous investigations which have observed a protective effect of femoral-gleuteal fat storage on insulin sensitivity in diabetic and non-diabetic populations [21, 24, 141, 248].

Substantial evidence exists regarding CAD risk and IR in T1D [31, 66, 73, 82] yet conflicting results have emerged regarding the relationship between insulin sensitivity and renal disease in individuals with T1D [3, 6]. The current findings suggest no association between direct assessment of insulin sensitivity and the presence of either CAD or ON in individuals with T1D, even after adjustment for circulating insulin levels during the insulin clamp procedure (ISI). These findings contrast results from our earlier reports [78, 195, 235, 249] that IR is associated with the development of renal and cardiovascular diseases in T1D. Many factors associated with the estimation of insulin resistance in T1D have changed since the development of the eGDR equation nearly a decade ago. The more frequent use of angiotensin converting enzyme inhibitor (ACE) and/or angiotensin II receptor blockers (ARB) for both blood pressure control and/or renal protection is an influential factor in the historically held association between hypertension, insulin resistance, and renal disease in diabetic populations [43]. More recent examination of calcium antagonist and ACE inhibitor therapy have associated their use with better metabolic profiles in diabetic populations and similar reports have also reported that ACE use may improve insulin resistance [250]. The majority of participants in the current study were taking ACE/ARB medication at the time of insulin sensitivity testing (n=21), but despite previous suggestions that these medication may alter insulin sensitivity [251], withdrawing participants from these medications for the purpose of this study would have caused undue health

risk. No differences in insulin sensitivity (M-value) were observed between individuals taking and not taking ACE/ARB medication ( $p=.62$ ) and ACE/ARB medication use as a categorical variable was also offered for variable selection in models examining insulin sensitivity (M-value) but was not selected as an independent predictor. Accounting for the use of these medications by forcing the variable into the final models did not significantly alter the current findings (data not shown), but the influence of these medications on the association between IR, CAD, and renal disease requires further investigation. Additionally, though no differences in severity of IR were observed between the different diabetes complication groups, the cross-sectional nature of the current findings does not allow more detailed prospective examination of IR in the development of renal and cardiovascular disease and the potential protective role of ACE/ARB medication. Further investigation of the temporal relationship between IR and the development of micro- and macrovascular diabetes complications is warranted.

Since the development of the original eGDR equation, more recent improvements have been made to the insulin clamp procedure (i.e. adjustment for LBM via DEXA assessment), thus adjusted data from this new sample of T1D participants was used to re-examine the estimation of IR. In this investigation, duration of diabetes, WC, daily insulin dose (u/kg), and overt nephropathy were most strongly associated with IR in T1D. Duration of diabetes (or age), daily insulin [252, 253] dose, and WC have all been previously associated with IR in T1D [194, 252, 253] and previous studies have shown WC to be a strong predictor of IR, as well as cardiovascular and kidney disease risk factors [254-258], supporting this observed relationship with IR in this T1D sample with long standing disease and complications. The strong positive association observed between presence of overt nephropathy and insulin sensitivity was contrary to our initial hypotheses. Previous investigations have suggested that presence of albuminuria

may be associated with increased IR in T1D [259] but the evidence is equivocal [71]. In our study, steady-state plasma insulin concentrations during the insulin clamp procedure were higher in individuals with ON compared to participants without (48.5 vs. 60.7  $\mu\text{U}/\text{mU}$ ,  $p=.11$ ) but these differences did not reach statistical significance (most likely due to small sample sizes). Increased circulating insulin would promote glucose uptake, therefore increasing glucose disposal in these participants, but subsequent analyses adjusting for circulating plasma insulin levels (ISI) did not alter the overall findings. The observation of increased M-values in participants with nephropathy in this investigation is not necessarily suggestive of increased insulin sensitivity in individuals with ON, but more likely evidence that circulating insulin levels in individuals with nephropathy is an important factor to account for when assessing IR with insulin infusion techniques.

When used in addition to the components of the original eGDR equation (i.e. HbA<sub>1c</sub>, WHR, and hypertension), DEXA adiposity measures (regional and FM %) can be used to more precisely predict insulin in T1D resistance after accounting for gender. In our analyses, total body adiposity (FM %) most improved the estimation of insulin sensitivity. These findings, combined with the negative associations between all regional fat measures (including WC) with insulin sensitivity, further supports the established relationship between general obesity and IR in T1D. These findings also add reinforcement to the need for therapies designed to reduce overall adiposity in T1D, such as weight loss and exercise intervention. Such lifestyle interventions have been shown to improve insulin sensitivity in T2D and non-diabetic populations [260-263] and may hold additional benefit for reductions in IR and obesity-related diabetic complications in T1D.

Since all regional DEXA examined were positively associated with WC (Table 25), more complex analyses of the residual effects of the regional adiposity after controlling for the influence of WC was required. These analyses revealed interesting results, firstly is that the independent negative effect of leg and arm FM on IR was removed after controlling for WC. This finding likely reflects the known association between IR and abdominal obesity and these associations overpower, in this population, the influence of leg and arm adiposity distribution on insulin resistance. Additionally, FM % had an independent negative association after taking WC into account which further supports the known association between IR and general obesity. Despite these findings, whether excess lower body adiposity has a metabolically protective effect in other populations (e.g. T2D or obese), or whether it is merely reflective of overall adiposity, which is likely the case in this T1D sample, requires further examination.

Since the DEXA assessment of regional adiposity distribution has been shown to provide useful information regarding various components of IR [141, 264-266], the appeal in examining more comprehensive assessments of adiposity distribution as it related to IR in T1D is evident. Previous investigations have found opposite contributions of trunk and leg fat mass to IR, with increased lower body adiposity offering metabolic protection in non-diabetic and T2D populations [24, 141, 248, 267]. Our current findings do not support this leg fat association in T1D, but suggest that increasing leg adiposity is directly reflective of increasing general adiposity, and therefore the associations are metabolically detrimental in nature. In particular note in these data, however, is the different association of IR and adiposity, per se, and its distribution in women compared with men. Differences in adiposity distribution between the genders has been well described using DEXA and other anthropomorphic techniques, the majority of investigations providing evidence that women in the general population have less

relative lean mass and more total and peripheral fat storage, particularly in the hips and legs [268-271]. If women also have higher FM % compared to men, which is likely to be reflected in regional adiposity stores in T1D, these differences may explain the stronger association between regional and total adiposity with IR in females in this population. Since the majority of investigations examining gender differences in adiposity and the association with disease risk focus solely on abdominal adiposity distribution, it is unclear whether the patterns of peripheral fat storage in females may further explain the increased risk for other cardio-metabolic conditions in women.

Further investigation is also needed to explore the metabolic feasibility of IR-related associations with regional adiposity across genders, particularly in T1D. Strong correlations between all DEXA-adiposity measures (both absolute and relative) introduce difficulty with the examination of the combined effect of regional adiposity in the prediction of IR. WC was also highly correlated with all DEXA adiposity measures, particularly with trunk FM, and the two abdominal adiposity measures have similar independent associations with IR in this sample. The similar association between WC and trunk FM with IR may suggest that the more simplistic and clinically available measurement of WC is able to be easily measured and interpreted in both genders, and may therefore be a more clinically relevant measure in identifying IR in long standing T1D.

A few strengths and limitations of the current investigation should be noted. The gold-standard technique used to assess IR in this investigation (hyperinsulinemic-euglycemic clamp) adds strength to the investigational results. However, due to the labor intensive, costly, and relatively invasive nature of the clamp procedure, the sample size for this investigation was limited. Therefore the results from the regression analysis should be carefully interpreted.

Alternative assessments of insulin sensitivity (e.g. the frequently sampled intravenous glucose tolerance test) are also available for use in individuals with T1D, however, the testing is similarly time intensive and invasive which would also influence the number of participants recruited. The ability to examine the clinical presence of disease in this T1D population also affords valuable information towards the relationship between IR and diabetes complications. Investigations into the relationship between CAD and renal status as they may be influenced by IR are an important route into potentially preventing diabetes complications through the therapeutic management of IR.

Another factor that is strongly associated with IR that was not directly assessed in this investigation is physical activity level. Since participants were recruited based on the presence of clinical CAD, strenuous exercise testing would have placed undue health risk on the participants. As part of the EDC exam, questionnaires assessing both daily caloric expenditure and sports participation were administered to individuals in this investigation and the results were not associated with IR either in the sample as a whole or when stratified by disease group. More accurate assessment of physical activity levels may have added further clarification to factors associated with the estimation of IR in T1D.

In conclusion, in our subjects with T1D, no associations were observed between presence of CAD or ON and IR. Measures of DEXA-assessed regional and total adiposity were negatively associated with insulin sensitivity in T1D and these measures improved the prediction of the eGDR equation for the identification of T1D individuals at risk for the IR. Despite previous findings in non-diabetic and T2D populations suggesting opposite metabolic influences of trunk and leg FM on glucose metabolism [141, 146, 267], these findings do not support this claim and further support the negative association between general obesity, rather than a

protective effect with gluteal-femoral FM stores, and insulin sensitivity in T1D. The cross-sectional nature of this investigation only allows the examination of associations between IR, presence of diabetes complications, and DEXA-assessed adiposity. Further longitudinal investigations are required to elucidate the causal/temporal relationship between increasing adiposity and the development of IR, CAD, and ON in T1D populations.

## 7.6 TABLES

Table 1. Characteristics of participants with type 1 diabetes by gender (n=30)

Characteristics	Overall	Females (n = 14)	Males (n = 16)
Age (years)	47.7 ± 7.9	47.2 ± 8.4	48.2 ± 7.7
Diabetes duration (years)	38.8 ± 7.0	38.6 ± 7.4	35.2 ± 3.0
Ever smoked, n (%)	11, 36.7	4, 28.6	7, 43.8
AER, µg/min (med(IQR))	12.0 (5.8-102.6)	10.5 (4.3-95.6)	14.9 (6.3-177.2)
Serum Creatinine (mg/dl)	1.10 ± .40	1.10 ± .48	1.09 ± .34
HbA <sub>1c</sub> (%)	7.7 ± 1.2	8.1 ± 1.2	7.3 ± 1.2
Pulse (bpm)	69.2 ± 11.7	67.8 ± 12.4	70.4 ± 11.3
Hypertension, n (%)	9, 30.0	4, 44.4	5, 55.6
SBP (mmHg)	130.6 ± 20.5	126.2 ± 23.7	134.5 ± 17.0
DBP (mmHg)	69.6 ± 9.9	62.6 ± 7.0	75.8 ± 7.7*
WBC (10 <sup>3</sup> /mm <sup>2</sup> )	6.6 ± 1.7	6.5 ± 1.4	6.7 ± 1.9
ACE/ARB Inhibitors, n (%)	21, 70.0	11, 78.6	10, 62.5
Total Cholesterol (mg/dl)	165.3 ± 27.7	163.6 ± 28.5	167.0 ± 27.8
LDLc (mg/dl)	90.5 ± 27.2	80.2 ± 25.6	101.7 ± 25.3
HDLc (mg/dl)	55.7 ± 15.1	59.9 ± 16.2	51.8 ± 13.4
Non-HDLc (mg/dl)	109.7 ± 27.6	103.7 ± 26.9	115.2 ± 27.9
Triglycerides (mg/dl)	88.3 ± 43.8	88.1 ± 52.1	88.5 ± 37.0
Daily Insulin Dose (u/kg)	0.58 ± 0.25	0.56 ± 0.22	0.59 ± 0.28
LDL Medication, n (%)	17, 56.7	8, 57.1	9, 56.2
Weight (kg)	76.7 ± 14.3	70.3 ± 13.7	82.3 ± 12.7*
BMI (kg/m <sup>2</sup> )	27.1 ± 4.5	27.1 ± 4.6	27.1 ± 4.5
Waist Circumference (cm)	90.8 ± 12.6	87.9 ± 13.3	93.4 ± 11.7
Waist-Hip Ratio	0.90 ± 0.08	0.87 ± 0.09	0.92 ± 0.06
LBM (kg)	53.5 ± 10.5	44.1 ± 5.9	61.1 ± 6.1*
FM (kg)	20.8 ± 8.5	23.1 ± 8.4	19.0 ± 8.4
FM (%)	27.4 ± 9.1	33.3 ± 7.3	22.6 ± 7.8*
Trunk FM (kg)	9.9 ± 5.1	10.3 ± 5.4	9.5 ± 5.0
Leg FM (kg)	7.2 ± 2.9	8.8 ± 2.7	5.9 ± 2.3*
Arm FM (kg)	2.7 ± 1.3	3.1 ± 1.2	2.4 ± 1.2
Insulin Sensitivity (M-value) (mg·min <sup>-1</sup> ·kg LBM <sup>-1</sup> )	5.8 ± 2.3	6.0 ± 2.1	5.7 ± 2.6
Insulin Sensitivity Index (mg·min <sup>-1</sup> ·kgLBM <sup>-1</sup> ·uUmL <sup>-1</sup> )	12.8 ± 6.6	13.6 ± 6.5	12.0 ± 6.8

Data are shown as mean (SD) unless otherwise noted

\* Significantly different than Females, p<0.05

**Table 2. Pearson correlation of insulin resistance factors and insulin sensitivity (M-value) in type 1 diabetes**

(n=30)

Variable	Pearson correlation with M-value (mg·min·kgLBM)					
	Overall		Females		Males	
	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Age (yrs)	.08	.67	.18	.55	.01	.96
Duration (yrs)	.28	.13	.33	.26	.24	.36
SBP (mmHg)	.09	.64	.10	.73	.11	.69
DBP (mmHg)	-.05	.79	-.19	.53	.09	.74
HbA <sub>1c</sub> (%)	.20	.30	-.01	.98	.34	.20
Total Cholesterol (mg/dl)	-.01	.95	-.02	.95	-.01	.99
HDL (mg/dl)	.21	.25	.37	.19	.03	.91
Non-HDL (mg/dl)	-.13	.49	-.24	.44	-.01	.96
LDL (mg/dl)	.04	.85	.01	.99	.30	.38
Triglycerides (mg/dl)	-.41	.04	-.39	.19	-.40	.14
Daily Insulin Dose (u/kg)	-.67	<.01	-.68	<.01	-.66	<.01
Serum Creatinine (mg/dl)	.49	<.01	.58	.03	.43	.10
AER (µg/min)§	.40	.03	.24	.41	.56	.03
Leg FM (kg)	-.27	.16	-.45	.13	-.24	.40
Arm FM (kg)	-.29	.12	-.58	<.01	-.15	.60
Trunk FM (kg)	-.36	.06	-.53	.06	-.24	.36
FM (%)	-.22	.26	-.52	.07	-.17	.52
BMI (kg/m <sup>2</sup> )	-.39	.03	-.53	.05	-.29	.28
WHR	-.27	.16	-.18	.54	-.34	.21
Waist Circumference (cm)	-.48	<.01	-.45	.11	-.50	.05

§Log transformed before statistical testing.

Table 3. Characteristics of participants with type 1 diabetes by complication group (N= 30)

Characteristics	CAD- ON- (5 F/5 M)	CAD+ (5 F/5 M)	ON+ (3 F/3 M)	ON+ CAD+ (1 F/3 M)
Age (years)	43.1 ± 5.7	52.4 ± 7.1*	43.3 ± 6.4	54.3 ± 6.7*
Diabetes duration (years)	35.0 ± 6.0	42.2 ± 7.7	35.2 ± 3.0	45.3 ± 2.2*
BMI, kg/m <sup>2</sup>	24.3 ± 3.7	28.0 ± 4.0	26.7 ± 3.3	32.4 ± 4.9*
Waist Circumference	85.2 ± 13.3	91.7 ± 9.6	89.6 ± 7.6	108.4 ± 15.5*
Waist-Hip Ratio	0.87 ± 0.08	0.90 ± 0.08	0.89 ± 0.05	0.98 ± 0.09
Ever smoked (n,%)	1, 10.0	5, 50.0	3, 50.0	2, 50
AER (µg/min) (med(IQR))	5.1 (2.8-9.8)	9.7 (6.4-17.6)	447.1 (283.0-629.8)*	88.7 (55.8-116.5)*
Serum Creatinine (mg/dl)	.88 ± .14	.97 ± .21	1.31 ± .49	1.61 ± .57*
HbA <sub>1c</sub> (%)	7.6 ± 1.3	7.6 ± 1.0	8.3 ± 1.7	6.9 ± 0.8
Pulse (bpm)	67.6 ± 13.0	68.5 ± 9.1	75.0 ± 13.4	66.3 ± 13.8
Hypertension (n,%)	1, 10.0	2, 20.0	3, 50.0	3, 75.0
SBP (mmHg)	116.2 ± 17.0	137.0 ± 23.7	133.2 ± 11.2	147.0 ± 10.1*
DBP (mmHg)	69.0 ± 10.5	71.0 ± 9.1	73.2 ± 5.8	62.5 ± 14.6
WBC (10 <sup>3</sup> /mm <sup>2</sup> )	6.5 ± 1.9	6.8 ± 1.3	7.2 ± 2.0	5.5 ± 1.6
ACE/ARB Inhibitors (n, %)	4, 40.0	8, 80.0	5, 83.3	4, 100.0
Total Cholesterol (mg/dl)	160.7 ± 32.9	163.3 ± 27.7	173.3 ± 27.1	171.7 ± 13.6
LDLc (mg/dl)	89.6 ± 29.0	89.6 ± 26.8	99.0 ± 46.2	87.3 ± 7.5
HDLc (mg/dl)	55.1 ± 14.5	55.6 ± 16.8	56.0 ± 12.0	57.3 ± 24.8
Non-HDLc (mg/dl)	105.6 ± 34.6	107.7 ± 27.6	117.3 ± 30.8	114.3 ± 1.7
Triglycerides (mg/dl)	75.9 ± 34.6	90.0 ± 37.0	97.8 ± 36.9	136.7 ± 86.9
Daily Insulin Dose (u/kg)	0.59 ± 0.29	0.59 ± 0.20	0.48 ± 0.18	0.72 ± 0.38
LDL Medication (n, %)	5, 50.0	8, 80.0	2, 33.3	2, 50.0
Insulin Sensitivity (M-value) (mg·min <sup>-1</sup> ·kg LBM <sup>-1</sup> )	4.95 ± 1.97	5.39 ± 1.68	7.49 ± 1.57	6.71 ± 4.30
Insulin Sensitivity Index (mg·min <sup>-1</sup> ·kgLBM <sup>-1</sup> ·uUmL <sup>-1</sup> )	12.11 ± 5.57	11.95 ± 6.74	16.78 ± 5.98	10.33 ± 9.46

\* Significantly different than CAD-, ON- Group, p<0.05.

**Table 4. Multiple linear regression model for dependent variable insulin sensitivity (GDR adjusted for body weight ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ )) in type 1 diabetes - eGDR variables**

	<b>Model 1</b>	<b>Model 2</b>	<b>Model 3</b>	<b>Model 4</b>	<b>Model 5</b>
<b>HbA<sub>1c</sub> (%)</b>	0.4 ± 0.3	0.3 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.3
<b>Waist : Hip Ratio</b>	-5.9 ± 4.0	-7.3 ± 3.4*	-4.6 ± 3.6		-3.9 ± 3.6
<b>Hypertension</b>	0.2 ± 0.7	1.2 ± 0.6	0.9 ± 0.6	1.2 ± 0.6	1.1 ± 0.5
<b>Sex§</b>		-0.3 ± 0.7	0.1 ± 0.7	0.5 ± 0.5	-1.2 ± 0.9
<b>Leg FM (kg)</b>		-0.4 ± 0.1**			
<b>Arm FM (kg)</b>		-0.9 ± 0.3**			
<b>Trunk FM (kg)</b>		-0.2 ± 0.1**			
<b>FM %</b>		-0.2 ± 0.1**			
<b>R<sup>2</sup></b>	0.18	0.57	0.59	0.47	0.62

\*  $P < 0.05$ , \*\* $P < 0.01$ , § $P < 0.01$ .

Sex: Negative value indicates lower GDR in men

**Table 5. Multiple linear regression model for dependent variable insulin sensitivity (M-value) ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}$ ) in type 1 diabetes; regression coefficients ( $\pm\text{SE}$ )**

	<b>Model 1 (FW selection)</b>	<b>Model 2</b>	<b>Model 3</b>	<b>Model 4</b>	<b>Model 5</b>
<b>Diabetes Duration</b>	$0.1 \pm 0.0^*$	$0.1 \pm 0.0^*$	$0.1 \pm 0.1^*$	$0.1 \pm 0.0^*$	$0.1 \pm 0.0^*$
<b>Waist Circumference</b>	$-0.7 \pm 0.0^{**}$				
<b>Overt Nephropathy</b>	$2.4 \pm 0.6^{**}$	$2.4 \pm 0.6^{**}$	$2.2 \pm 0.6^{**}$	$2.5 \pm 0.6^{**}$	$2.3 \pm 0.6^{**}$
<b>Insulin Dose (u/kg)</b>	$-3.5 \pm 1.4^*$	$-4.6 \pm 1.1^{**}$	$-4.9 \pm 1.2^{**}$	$-4.0 \pm 1.3^{**}$	$-4.8 \pm 1.3^{**}$
<b>Sex§</b>		$-0.8 \pm 0.6$	$-0.6 \pm 0.6$	$-0.5 \pm 0.6$	$-1.3 \pm 0.7$
<b>Leg FM (kg)</b>		$-0.2 \pm 0.2^*$			
<b>Arm FM (kg)</b>			$-0.5 \pm 0.3$		
<b>Trunk FM (kg)</b>				$-0.2 \pm 0.1^*$	
<b>FM %</b>					$-0.1 \pm 0.4^*$
<b><math>R^2</math></b>	0.72	0.73	0.70	0.71	0.72

Variables offered to model 1: Duration, waist circumference, overt nephropathy, serum creatinine, LDL-C medication use, WBC, ACE/ARB medication use

\* $P < 0.05$ , \*\* $P < 0.01$ .

Sex: Negative value indicates lower GDR in men

**Table 6. Multiple linear regression model for dependent variable insulin sensitivity (M-value) ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}$ ) examining the combined effect of waist circumference and regional adiposity in type 1 diabetes; regression coefficients ( $\pm\text{SE}$ )**

	<b>Model 2</b>	<b>Model 2</b>	<b>Model 3</b>	<b>Model 4</b>
<b>Diabetes Duration</b>	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0*	0.1 $\pm$ 0.0*
<b>Waist Circumference</b>	-0.2 $\pm$ 0.1*	-0.1 $\pm$ 0.0*	-0.1 $\pm$ 0.0**	-0.3 $\pm$ .1**
<b>Overt Nephropathy</b>	2.4 $\pm$ 0.7**	2.1 $\pm$ 0.7**	2.4 $\pm$ 0.6**	2.1 $\pm$ 0.6**
<b>Insulin Dose (u/kg)</b>	-3.6 $\pm$ 1.4*	-3.9 $\pm$ 1.4*	-3.0 $\pm$ 1.4*	-3.8 $\pm$ 1.3*
<b>Sex§</b>	-0.8 $\pm$ 0.8	-0.8 $\pm$ 0.8	-1.4 $\pm$ 0.8	-2.0 $\pm$ 1.0
<b>Residuals of Leg FM (kg)</b>	1.9 $\pm$ 1.2			
<b>Residuals of Arm FM (kg)</b>		1.0 $\pm$ 0.7		
<b>Residuals of Trunk FM (kg)</b>			1.1 $\pm$ 0.5*	
<b>Residuals of FM (%)</b>				-0.1 $\pm$ 0.4*
<b>R<sup>2</sup></b>	0.73	0.71	0.71	0.72

\* $P < 0.05$ , \*\* $P < 0.01$ .

Sex: Negative value indicates lower GDR in men

Table 7. Multiple linear regression model for dependent variable insulin sensitivity (M-value) ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg LBM}^{-1}$ ) by gender in participants with type 1 diabetes; regression coefficients ( $\pm\text{SE}$ )

FEMALES			MALES		
	$\beta\pm\text{SE}$	<i>p</i>		$\beta\pm\text{SE}$	<i>p</i>
<b>Model 1</b>			<b>Model 1</b>		
<b>Duration</b>	0.09 ± 0.05	.08	<b>Duration</b>	0.12 ± 0.07	.10
<b>BMI (<math>\text{kg}/\text{m}^2</math>)</b>	-0.32 ± 0.10	.01	<b>BMI (<math>\text{kg}/\text{m}^2</math>)</b>	-0.07 ± 0.05	.18
<b>Overt Nephropathy</b>	2.71 ± 0.73	.005	<b>Overt Nephropathy</b>	2.29 ± 0.89	.03
<b>Insulin Dose (u/kg)</b>	-3.37 ± 1.85	.10	<b>Insulin Dose (u/kg)</b>	-3.74 ± 1.94	.08
<b>R<sup>2</sup></b>	.82		<b>R<sup>2</sup></b>	.72	
<b>Model 2</b>			<b>Model 2</b>		
<b>Diabetes Duration</b>	0.01 ± 0.05	.82	<b>Diabetes Duration</b>	0.16 ± 0.05	<.01
<b>Overt Nephropathy</b>	1.98 ± 0.93	.07	<b>Overt Nephropathy</b>	3.27 ± .72	<.01
<b>Insulin Dose (u/kg)</b>	-6.69 ± 1.84	.007	<b>Insulin Dose (u/kg)</b>	-2.96 ± 1.35	.05
<b>Leg FM (kg)</b>	-0.31 ± 0.15	.07	<b>Leg FM (kg)</b>	-0.22 ± 0.14	.15
<b>R<sup>2</sup></b>	.75		<b>R<sup>2</sup></b>	.85	
<b>Model 3</b>			<b>Model 3</b>		
<b>Diabetes Duration</b>	0.03 ± 0.05	.60	<b>Diabetes Duration</b>	0.14 ± 0.07	.06
<b>Overt Nephropathy</b>	2.23 ± 0.92	.44	<b>Overt Nephropathy</b>	2.19 ± 0.86	.03
<b>Insulin Dose (u/kg)</b>	-5.26 ± 1.97	.03	<b>Insulin Dose (u/kg)</b>	-5.08 ± 1.59	<.01
<b>Arm FM (kg)</b>	-0.79 ± 0.35	.06	<b>Arm FM (kg)</b>	-0.34 ± 0.38	.40
<b>R<sup>2</sup></b>	.76		<b>R<sup>2</sup></b>	.72	
<b>Model 4</b>			<b>Model 4</b>		
<b>Diabetes Duration</b>	0.05 ± 0.06	.39	<b>Diabetes Duration</b>	0.15 ± 0.06	.05
<b>Overt Nephropathy</b>	2.72 ± 1.04	.54	<b>Overt Nephropathy</b>	2.46 ± 0.87	.02
<b>Insulin Dose (u/kg)</b>	-4.01 ± 2.41	.14	<b>Insulin Dose (u/kg)</b>	-4.33 ± 1.71	.03
<b>Trunk FM (kg)</b>	-0.22 ± 0.11	.08	<b>Trunk FM (kg)</b>	-.13 ± .10	.21
<b>R<sup>2</sup></b>	.74		<b>R<sup>2</sup></b>	.74	
<b>Model 5</b>			<b>Model 5</b>		
<b>Diabetes Duration</b>	0.04 ± 0.05	.42	<b>Diabetes Duration</b>	0.14 ± 0.07	.06
<b>Overt Nephropathy</b>	2.29 ± 0.86	.03	<b>Overt Nephropathy</b>	2.30 ± 0.86	.02
<b>Insulin Dose (u/kg)</b>	-5.63 ± 1.76	.01	<b>Insulin Dose (u/kg)</b>	-4.86 ± 1.62	.01
<b>FM %</b>	-0.14 ± 0.05	.03	<b>FM %</b>	-0.06 ± .06	.32
<b>R<sup>2</sup></b>	.79		<b>R<sup>2</sup></b>	.73	

**8.0 ARTICLE 2: EXPLORING THE RELATIONSHIP BETWEEN REGIONAL  
ADIPOSIY AND INSULIN RESISTANCE IN TYPE 1 DIABETES**

To be submitted for publication

Christina M. Shay<sup>1</sup>, Bret H. Goodpaster<sup>2</sup>, Frederico G.S. Toledo<sup>2</sup>, Sheryl F. Kelsey<sup>1</sup>, Elsa  
M. Strotmeyer<sup>1</sup> and Trevor J. Orchard<sup>1</sup>

<sup>1</sup>Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh,  
Pittsburgh, Pennsylvania

<sup>2</sup>Department of Medicine, University of Pittsburgh School of Medicine, University of Pittsburgh,  
Pittsburgh, Pennsylvania

## 8.1 ABSTRACT

**BACKGROUND:** The anatomical compartment in which fat tissue is stored is known to influence glucose metabolism, particularly abdominal adiposity. Recent studies have also shown that a propensity to store fat in the lower body has a protective effect on insulin resistance, but this association has not been explored in type 1 diabetes (T1D). The purpose of this study was to compare regional adiposity distribution between non-diabetic and T1D individuals of similar age and BMI and to examine whether the associations between regional adiposity and IR differ between these populations.

**METHODS:** Twenty-nine participants recruited from the Epidemiology of Diabetes Complication Study (EDC), a 20-yr prospective study of childhood onset diabetes and fifty-six non-diabetic individuals underwent insulin sensitivity assessment by hyperinsulinemic-euglycemic clamp (40 mU/m<sup>2</sup>/min) and regional and total adiposity quantification by dual X-ray absorptiometry (DEXA).

**RESULTS:** Overall, T1D was associated with decreased insulin sensitivity compared to the non-diabetic individuals, respectively (5.8 vs. 8.2 mg·min<sup>-1</sup>·kgLBM<sup>-1</sup>, p<.01). T1D was also associated with increased total FM (p<.001) but similar proportion (%) of fat mass (FM) stored in the trunk (p=.82) and lower % FM in the legs (p=.03) after adjustments for age, sex, and height. Multivariate linear regression demonstrated that % FM in legs was independently positively associated with insulin sensitivity associated with insulin resistance (p<.01) and higher % FM in the trunk was independently and negatively associated with insulin sensitivity (p<.01) after adjustment for diabetes status, age, sex, height, and total FM. Stratification by diabetes

group revealed that the only adiposity assessment that was independently associated with insulin sensitivity was total FM ( $p=.03$ ).

CONCLUSIONS: These findings suggest that lower adiposity may play a protective role in glucose metabolism for individuals without diabetes, but this association may not be evident in T1D. These findings also suggest that insulin resistance is a prominent feature of T1D and that general obesity may overpower the potential influence of regional adiposity storage in this population. The biological plausibility for a protective effect of increased leg adiposity against IR remains uncertain, and requires further exploration, particularly in individuals with T1D.

## 8.2 INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia. Type 1 diabetes (T1D) is a multifaceted condition distinguished, in most cases, by the autoimmune-mediated destruction of pancreatic beta cells that ultimately develops into an insulin deficient state [59, 60]. T1D is most commonly diagnosed in children and adolescents, though can occur at any age, and is frequently identified by symptomatic hyperglycemia, requiring a need for exogenous insulin replacement. In contrast to T1D, the etiology of type 2 diabetes (T2D) is a condition characterized by low levels of response to insulin from primary target tissues (i.e. adipose, muscle, and liver cells) [61, 62], a condition more commonly referred to as insulin resistance (IR). It is hypothesized that at some point, the pancreatic  $\beta$ -cells are unable to compensate for the IR by increasing insulin secretion, and it is at this point that T2D then appears [63].

In the last few decades, diabetes research has intensely focused on IR as it may be related to body composition and adiposity. Examining the notion that adiposity is not uniform

throughout the body in terms of metabolic influence, recent studies have demonstrated that the particular anatomical compartment in which fat tissue is stored (regional adiposity) is important in understanding the relationship between obesity and disturbances in glucose metabolism[20, 32]. Abdominal, or visceral fat, is theorized to be metabolically influential given that excess adipose tissue accumulation in this region is associated with excess release of metabolic byproducts that lead to forms of metabolic dysregulation[20]. With increasing utilization of imaging techniques for the assessment of regional adiposity (e.g. dual x-ray absorptiometry (DEXA)) another adipose depot that has been more recently hypothesized to exert metabolic influence is the areas located in the lower extremities and in the hips. Recent investigations in T2D and non-diabetic populations have suggested that leg fat is favorably associated with measures of insulin sensitivity [21-24] and that some degree of metabolic protection may be afforded by the propensity to deposit fat in gluteal-femoral depots but whether this protective metabolic tendency is similar in T1 populations has yet to be evaluated.

Despite the extensive literature exploring IR and adiposity in non-diabetic and T2D populations which largely suggests that regional adiposity is a more important determinant of IR than body size alone [104, 107-109, 112, 134, 135], few investigations have explored these associations in T1D and even fewer investigations explore differences in these associations when compared to non-diabetic populations. The most likely cause of the deficiency of investigations into the association between body composition and IR in T1D is the difficulties associated with the direct assessment of IR in this population. Many indirect assessments of IR are available, but these techniques rely on the measurement of fasting plasma insulin levels. Since the insulin concentrations in T1D needed for the computation of these methods more likely reflect timing and dosing of replacement therapy, these techniques are often inappropriate. The

hyperinsulinemic-euglycemic clamp technique [8] provides the most accurate assessment of IR and is therefore the most suitable techniques for the assessment of IR in T1D, although the labor intensive, costly, and relatively invasive nature of this technique is often inappropriate for use in large-scale population studies.

Utilizing a sample of non-diabetic individuals and participants from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, a 20-yr prospective study of childhood onset diabetes and a group of non-diabetic individuals, the purpose of this study was two-fold: 1) to compare DEXA-assessed regional adiposity distribution (i.e. leg, arm, and trunk fat) between non-diabetic and T1D individuals from the EDC study; and 2) to examine whether the associations between regional adiposity and IR differ between non-diabetics and individuals with T1D.

## **8.3 PARTICIPANTS AND METHODS**

### **8.3.1 Participants and Recruitment**

Volunteers for the current investigation consisted of 29 participants with T1D (13 females, 16 males) and 56 non-diabetic individuals (28 females, 28 males). The present study was performed as an ancillary study to the Pittsburgh Epidemiology of Diabetes Complications Study (EDC), a 20-year prospective follow-up study of childhood-onset type 1 diabetes mellitus which has been previously described in detail [237]. Briefly, T1D participants were diagnosed (or seen within 1 year of diagnosis) between 1950 and 1980, before age 17, at the Children's Hospital of Pittsburgh. This population has been shown to be representative of the T1D population in

Allegheny County, Pennsylvania [238]. The participants with T1D were recruited after subjects had attended the 18-year EDC exam (November 2004 – November 2006). All participants had a screening medical history, physical examination, and screening laboratory tests which included fasting blood lipids. Non-diabetic individuals underwent a 75-g oral glucose tolerance test after an overnight fasting to confirm normal glucose tolerance (2-hour blood glucose < 140 mg/dL). Participants were excluded if they were anemic or if HbA<sub>1c</sub> ≥11.0%, a cut point which was chosen to minimize the likelihood of excessive hepatic production due to poor glycemic control in the T1D participants [239]. Subjects were also ineligible if they used insulin-sensitizing agents within 3 months or any medications which affect insulin sensitivity within 6 weeks of metabolic testing.

### **8.3.2 Regional and Total Body Composition**

Waist circumference (WC) was assessed as a measure of visceral adiposity and body mass index (BMI) was computed based on measurements of height and weight on the day of insulin sensitivity testing (kg/m<sup>2</sup>). Distribution of adiposity was measured by dual x-ray absorptiometry (DEXA) (Hologic QDR 4500A; Hologic, Bedford, MA; DPX-L; Lunar Corp, Madison, WI) to determine fat mass (FM), fat free mass (FFM), leg FM, arm FM, and trunk FM. The separation between trunk FM and leg FM was made by two oblique lines passing through the femoral necks and the separation between trunk FM and arm FM was made by two oblique lines passing through the humeral heads. Total body adiposity was assessed as total body fat in kg and percent body fat (%FM). Trunk FM included both subcutaneous and visceral fat of this anatomical region. Leg and arm FM was the total fat in both corresponding limbs and was calculated in total kg. A measure of regional adiposity relative to total body FM was calculated as a

percentage of total FM in the legs (% FM in legs), arms (% FM in arms), and trunk (% FM in Trunk).

### **8.3.3 Assessment of Insulin Resistance**

Insulin sensitivity was determined using the hyperinsulinemic-euglycemic clamp method as described previously[8]. Subjects were instructed to avoid alcohol consumption and strenuous activity for 48 hours preceding these studies. On the evening before measurement of insulin sensitivity, subjects were admitted to either the General Clinical Research Center or the Clinical Translational Research Center and all participants received a standard dinner (7 kcal/kg) with 50% of energy from carbohydrate, 30% from fat, and 20% from protein.

For T1D participants, an intravenous (IV) catheter was placed in a forearm vein for blood sampling and a second catheter was subsequently placed in the antecubital region of the opposite arm for all IV infusions on the evening before the clamp procedure (at approximately 2200 hours). An IV infusion of insulin was then started to control blood glucose; those using subcutaneous insulin pumps discontinued the infusion at this time. The overnight target range for blood glucose was 90-150 mg/dl and venous glucose was monitored hourly (or more frequently as needed) with adjustments to the insulin infusion made based on a pre-determined dosing algorithm. Any long-acting injection of insulin was withheld at least 24 hours prior to insulin sensitivity testing. Short-acting insulin was used to control post-prandial hyperglycemia the evening before the study. For non-diabetic controls, IV catheters were placed in the forearm and opposite antecubital vein at approximately 0700 hours, immediately preceding insulin sensitivity testing. The insulin dose for all clamp procedures was  $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ , where  $\text{m}^2$  represents body surface area (Humulin; Eli Lilly, Indianapolis, IN) to achieve and maintain euglycemic

values (85-95 mg/dl), with an adjustable infusion of 20% dextrose. Plasma glucose concentrations were measured by using an automated glucose oxidase reaction (YSI 2300 glucose analyzer; Yellow Springs Instrument Co, Yellow Springs, OH) and glucose concentrations were determined at 5-min intervals during the clamp procedure. The insulin infusion was continued for a minimum of 2 and a maximum of 4 hours until steady-state metabolic conditions had been attained. Insulin sensitivity (M-value) was quantified for 30 minutes of the clamp procedure during steady-state and adjusted for lean body mass ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}$ ).

### **8.3.1 Statistical Analyses**

Since all variables examined were normally distributed, univariate group differences were evaluated using Student's t-test. General linear models were used to examine differences between T1D and non-diabetes groups adjusting for measures known to influence adiposity distribution, included age, sex, and/ or height as appropriate. Multivariate linear regression was used to examine the association between regional fat measures (arm FM, leg FM, trunk FM) and diabetes status (0 = non-diabetic, 1 = T1D) after adjustment for age, sex, and height. Multivariate linear regression was also used to examine the association between insulin sensitivity (M-value), regional fat measures and diabetes status after adjustment for covariates. Since FM was highly correlated with all absolute measures of regional adiposity, FM and the regional measures could not be combined in the same models. Thus, relative regional adiposity measures (% FM in Legs, Arms, and Trunk) were used to examine influence of regional adiposity accounting for total body adiposity (FM). Two-way interactions between diabetes state and the regional fat measures as well as interactions between sex and regional adiposity measures were also tested for

significance.  $P < 0.05$  was considered statistically significant. No formal correction was performed for multiple comparisons. Analysis was performed using SPSS for Windows software version 16.0 (SPSS, Chicago, IL).

## **8.4 RESULTS**

### **8.4.1 Metabolic Characteristics**

The metabolic characteristics of the investigational groups are presented in Table 8. The mean age of the T1D participants in this sample was slightly higher than the non-diabetic group (48.0 vs. 44.2 years,  $p=.08$ ) and males and females were equally distributed between study groups. Additionally, participants with T1D had significantly higher SBP, but lower DBP, total cholesterol, LDL cholesterol and triglycerides compared to the non-diabetic group. The range of M-values was 3.01-15.71  $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg FFM}^{-1}$  in the non-diabetic group and 2.24-11.41  $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg FFM}^{-1}$  in the individuals with T1D. The average rate required to maintain euglycemia during the clamp procedure was approximately 30% higher in the T1D individuals which is indicative of elevated insulin resistance in this group (5.8 vs. 8.2  $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg FFM}^{-1}$ ,  $p=.002$ , respectively).

### **8.4.2 Participant Characteristics and Body Composition**

Unadjusted and adjusted values for the anthropomorphic characteristics of T1D and non-diabetic participants are presented in Table 9. Upon examination of unadjusted group measurements,

BMI was slightly lower in the T1D group ( $p=.06$ ) and body weight, WC, FFM, arm FM, % FM in legs, and % FM in trunk were not different between T1D and non-diabetics. Despite absolute and regional similarities between central adiposity, FM was 8.9 kg higher in non-diabetics and 8.4% more body weight was accounted for by fat ( $p<.001$ ) in this group ( $p=.01$ ). Unadjusted values of leg FM were also 3.7 kg lower ( $p<.001$ ) and trunk FM was 4.9 kg lower ( $p<.001$ ) in the T1D group. Despite higher overall leg FM in non-diabetics, there was no difference in unadjusted % FM in legs ( $p=.33$ ) between groups. Non-diabetic individuals also had significantly lower % FM in arms ( $p<.001$ ) despite similar levels of absolute arm FM between groups.

Since it is recognized that age, sex, and height influence adipose tissue distribution, the data was adjusted. After adjustment, no significant group differences in BMI, arm FM and % FM in trunk were observed, however, % FM in arms was higher and body weight, WC, FM, % FM, leg FM, trunk FM, and % FM in legs were statistically lower in T1D participants.

### **8.4.3 Correlates of Insulin Sensitivity**

Table 10 shows the Pearson correlation of the risk factors with insulin sensitivity (M-value). Because of prior reports indicating a consistent association between both age and sex and insulin sensitivity and adiposity [82, 248, 253], partial Pearson correlations of M-values adjusted for age, sex, and height are also shown in Table 3. BMI and WC were negatively associated with insulin sensitivity across both groups, particularly in non-diabetic individuals. After adjustment, the majority of adiposity measures were correlated with insulin sensitivity in non-diabetic individuals, and of those, % FM in trunk had the strongest correlation. Of particular note, is the strong positive correlation between % FM in legs and insulin sensitivity in the non-diabetic

group, a pattern that was not evident in T1D. In T1D, FM, % FM, and the absolute measures of regional fat (kg), but not the relative regional adiposity measures (%), were modestly negatively associated with insulin sensitivity. In non-diabetics, there was also a strong positive correlation of insulin sensitivity with HDL cholesterol, and negative associations with SBP and DBP. A similar negative association was observed between insulin sensitivity and triglycerides in both T1D and non-diabetic individuals.

#### **8.4.4 Leg Fat Mass**

A multivariate linear regression model was used to further examine the effect of diabetes status on leg FM (Table 11). After accounting for the influence of age, sex, height, and FM there was a borderline lower level of leg FM associated with T1D ( $-.85 \pm .48$ ,  $p=.08$ ). Height had independent negative effect on amount of leg FM ( $-.09 \pm .02$ ,  $p<.001$ ) and every kilogram increase in FM was associated with a .33 kilogram increase in leg FM ( $p<.001$ ).

#### **8.4.5 Arm Fat Mass**

After accounting for the influence of age, sex, height, and FM (Table 11), a significant group difference in arm FM (kg) was observed. T1D was associated with a  $1.06 \pm .16$  adjusted mean increase in arm FM ( $p<.001$ ). In this model, neither sex, age, nor height had an independent effect on arm fat, but every kilogram increase in FM was significantly associated with a  $.10 \pm .01$  increase in arm FM ( $p<.001$ ).

#### **8.4.6 Trunk Fat Mass**

After accounting for the influence of age, sex, height, and FM (Table 11), trunk FM was similar between the two groups ( $-.49 \pm .62$ ,  $p=.44$ ). In this model, neither sex nor age had an independent effect on trunk FM, but height was positively associated with trunk FM ( $p=.001$ ) and every kilogram increase in FM was significantly associated with a  $.55 \pm .03$  increase in trunk FM ( $p<.001$ ).

#### **8.4.7 Relationship of Body Composition to Insulin Resistance**

The hypothesis that differences in body composition may contribute to the greater IR seen in T1D was next examined. Multivariate linear regression models for the estimation of insulin sensitivity (M-values) are presented in Table 12. Model 1 examined the influence of age, sex, height, FM, and diabetes group on insulin sensitivity and a significant effect of diabetes group in this model further suggesting that individuals with T1D had lower insulin sensitivity compared to non-diabetic individuals ( $-3.04 \pm .70$ ,  $p<.001$ ). In model 2, % FM in legs was positively associated with insulin sensitivity ( $p=.001$ ), even after adjustment for total body FM, and the addition of this measure slightly decreased the association between T1D and IR, but the presence of T1D group remained highly significant. After adjustment for diabetes group, age, sex, height, and FM, no independent associations were observed between IR and % FM in arms ( $p=.32$ ) (Model 3) yet a strong independent negative association between insulin sensitivity and % FM in trunk ( $p<.001$ ) was observed (Model 4). When compared to the influence of total FM alone, the regional adiposity variable that explained the largest difference in IR between diabetes groups compared to the was % FM in legs ( $-3.04 \pm .70$  vs.  $-2.61 \pm .67$ ). Of particular note is that

across all models examined, T1D was strongly independently associated with decreased insulin sensitivity, even after adjustment for total and regional adiposity.

No two-way interactions for insulin sensitivity were observed between diabetes group x sex, or any of the regional adiposity measures x sex, but significant interactions were observed between diabetes group x % FM in legs ( $p=.002$ ) and diabetes group x % FM in trunk ( $p=.02$ ). Due to these interactions, separate forward selection models examining the influence of regional adiposity on insulin sensitivity were fit within each study group (Table 13). In T1D, FM ( $p=.001$ ) was the only adiposity measure that was independently associated with insulin resistance after adjustment for sex, age and height. In non-diabetics, % FM in legs and trunk were both independently associated with insulin sensitivity and the best model ( $R^2=.56$ ) exhibited a strong positive association between % FM in legs and insulin sensitivity ( $.24 \pm .05$ ,  $p<.001$ ).

## 8.5 DISCUSSION

IR is a prominent state that has been previously described in T1D [2, 272], yet it is typically less severe than in T2D where IR is largely driven by obesity. Since the particular anatomical compartment in which adipose tissue is stored (regional distribution) is important in understanding the relationship between obesity and disturbances in glucose metabolism, the current investigation was undertaken to compare adipose tissue distribution between T1D and non-diabetics individuals and to examine whether these differences are associated with excess risk for IR in T1D. A novel aspect of the present study is the use of advanced imaging techniques (DEXA) for the assessment of regional adiposity in both T1D and non-diabetic

individuals which allows a more comprehensive exploration of the effect of T1D on body composition. More importantly, insulin sensitivity was measured between these two groups using the complicated glucose clamp method which, when combined with the DEXA analyses, allowed examination of the influence of regional adiposity and IR.

In the current investigation, men and women with T1D exhibited approximately 30% more severe IR than non-diabetics which affirms the presence of IR in this population. These findings are consistent with previous investigations reporting similar disparities in IR associated with T1D [199, 201, 253, 273]. Several group differences existed in DEXA-assessed adipose tissue distribution which may be associated with the elevated IR in T1D. After adjustment for age, sex, and height, individuals with T1D in this sample had significantly higher FFM, higher % FM in the arms (%) ( $p < .001$ ), less total fat (FM), lower proportion of their total body weight as fat (FM %) and lower % FM in the legs ( $p = .03$ ) when compared to non-diabetic individuals. An intriguing finding is that participants with T1D exhibited borderline lower levels of % FM in legs ( $p = .03$ ) compared to non-diabetic individuals, and higher % FM in the legs was independently associated with insulin sensitivity (M-value) ( $p < .001$ ), regardless of diabetes status. These findings, which are similar to associations between IR and leg adiposity reported in T2D populations [248], imply that individuals with both T1 and T2D diabetes may have a propensity to store less fat in their legs at a given level of adiposity compared to non-diabetic individuals. If higher levels of leg fat are favorably associated with measures of insulin sensitivity, and diabetic individuals store less body fat in the femoral-gluteal region, consequently, lower levels of leg fat may be a contributing factor in diabetic states of IR. After adjustment for adiposity factors and diabetes, greater amounts % FM in the trunk was also independently associated with IR adding further support to the well-known relationship between upper-body adiposity and glycemic

dysregulation [32, 104, 274]. This finding, along with a strong inverse correlation between % FM in the legs and trunk in this sample ( $r=-.86$ ,  $p<.001$ ), provides additional evidence that increased amounts of fat stored in the abdomen relative to the lower body may be a risk factor for the development of IR.

Another interesting finding is that T1D was associated with a greater propensity to store fat in the arms and is, to our knowledge, the first time that this association has been reported and is worthy of further exploration. Arm circumference and triceps skin fold assessments are commonly used as a region of interest when using various anthropomorphic techniques [275, 276], and we hypothesize that this association is reflective of overall lower levels of abdominal adiposity regardless of the alternate region of fat storage. From our results, it could be suggested that the leg area would be more a metabolically protective fat storage region than in the arms (models 2 & 3, Table 12), yet insufficient evidence exists to determine if arm fat, per se, produces harmful consequences on glucose metabolism. This finding, however, does not explain the increased IR in T1D as % FM in the arms was not independently associated with IR ( $p=.66$ ).

Some of the most interesting findings in this investigation are the strong associations between regional adiposity and IR, even after adjustment for diabetes, but stratification by diabetes group revealed that these associations are only evident in the non-diabetic individuals. The association between % FM in legs and % FM in trunk remained strong in the non-diabetic individuals. Interestingly, there was no association between % FM in the trunk or legs after adjustment for total FM in individuals with T1D. Total FM was the only DEXA adiposity measure that was independently associated with IR in T1D suggesting that increasing general obesity, rather than regional adiposity, may more strongly associated with IR in this population. These findings add reinforcement to the need for therapies designed to reduce overall adiposity

in T1D populations, such as weight loss and exercise intervention. Such lifestyle interventions have been shown to improve insulin sensitivity in T2D and non-diabetic populations [260-263] and may hold additional benefit for reductions in IR and obesity-related diabetic complications in T1D.

Despite the increasing use of DEXA for the assessment of regional body composition and the glucose clamp technique to assess IR, this investigation is one of the first to use these method to explore adiposity trends in T1D as it is associated with IR in T1D. Nonetheless, some limitations of the present investigation should be noted. Despite accounting for demographic and anthropometric features that are associated with IR (age, sex, height, and FM), a clinical characteristic associated with IR that was not assessed in this investigation was level of physical activity. Previous investigations have demonstrated a relationship between exercise capacity and IR [277, 278], and the lack of control for this lifestyle factor limits the investigational results. The examination of physical activity levels as an influence adiposity and IR in T1D is also an area requiring further exploration. The sample size for the groups in this study was also limited based on epidemiologic standards yet the number of T1D participants is comparable to that of other similar insulin clamp investigations [4]. The laborious and expensive nature of the gold-standard technique of assessing IR in T1D often requires an exchange between sample size and data quality.

In conclusion, the present investigation provides further evidence of the presence of more severe IR in T1D when compared to non-diabetic individuals. More importantly, the independent inverse association between lower leg adiposity and IR further substantiates the notion that some degree of metabolic protection is afforded by the propensity to deposit fat in gluteal-femoral depots in non-diabetic individuals, but whether this association is evident in T1D requires further

exploration. The current investigation also reinforces the negative metabolic effects of excess accumulation of abdominal adipose tissue associated with IR; however, this association may be attenuated by the effects of increased general adiposity in individuals with T1D. Despite the current findings, the biological plausibility for a protective effect of increased leg adiposity against IR remains uncertain and requires further exploration in all populations, particularly in individuals with T1D.

## 8.6 TABLES

**Table 8. Clinical and metabolic parameters in non-diabetic and type 1 diabetic participants**

	Non-Diabetics (28F/28M)	Type 1 Diabetics (13F/16M)
<b>Clinical Assessments</b>		
Age (years)	44.2 ± 10.2	48.0 ± 7.9
Systolic Blood Pressure (mmHg)	120.8 ± 16.3	129.8 ± 20.4*
Diastolic Blood Pressure (mmHg)	75.7 ± 8.7	69.5 ± 10.1**
Total Cholesterol (mg/dL)	189.4 ± 30.2	164.4 ± 27.6**
LDL Cholesterol (mg/dL)	112.2 ± 28.4	90.5 ± 27.2**
HDL Cholesterol (mg/dL)	53.3 ± 13.8	55.4 ± 15.3
Triglycerides (mg/dL)	127.1 ± 70.8	91.8 ± 44.8*
Insulin Sensitivity (M-value) (mg·min <sup>-1</sup> ·kg FFM <sup>-1</sup> )	8.2 ± 3.5	5.8 ± 2.4**

Values are unadjusted means ± SD.

\*Significantly different than the non-diabetic group, p<.05

**Table 9. Body composition assessments between non-diabetic and type 1 diabetic individuals**

	<i>Non-Diabetics (32F/32M)</i>	<i>Type 1 Diabetics (13F/16M)</i>	<i>Unadjusted P-value</i>	<i>Adjusted P-value<sup>1</sup></i>
Weight (kg)	82.1 ± 17.0	76.4 ± 14.5	.13	<b>&lt;.001</b>
BMI (kg/m <sup>2</sup> )	28.9 ± 4.8	26.9 ± 4.5	.06	.08
WC (cm)	95.3 ± 13.8	91.0 ± 12.9	.18	<b>.008</b>
<b>DEXA Assessments</b>				
FFM (kg)	49.8 ± 12.0	53.5 ± 10.4	.26	<b>.01</b>
FM (kg)	29.0 ± 10.1	20.1 ± 8.5	<b>&lt;.001</b>	<b>&lt;.001</b>
% FM	35.9 ± 9.7	27.5 ± 9.2	<b>&lt;.001</b>	<b>&lt;.001</b>
Leg fat (kg)	11.0 ± 4.0	7.3 ± 2.8	<b>&lt;.001</b>	<b>&lt;.001</b>
Arm fat (kg)	2.5 ± 1.0	2.7 ± 1.3	.29	.61
Trunk fat (kg)	14.8 ± 6.0	9.9 ± 5.1	<b>&lt;.001</b>	<b>&lt;.001</b>
FM in Legs (%)	37.7 ± 7.4	36.0 ± 7.0	.33	<b>.03</b>
FM in Arms (%)	8.3 ± 1.8	12.8 ± 2.1	<b>&lt;.001</b>	<b>&lt;.001</b>
FM in Trunk (%)	49.6 ± 8.3	46.0 ± 7.1	.05	.82

Values are unadjusted means ± SD. DEXA, dual x-ray absorptiometry; FFM, fat free mass; FM, fat mass; WC, waist circumference

<sup>1</sup>Data was adjusted for effects of sex, age, and height, except for the data of weight, BMI, FM, FFM, and % FM which were adjusted for age and sex.

DEXA, dual x-ray absorptiometry; FFM, fat free mass; FM, fat mass.

**Table 10. Pearson correlations between clinical and metabolic parameters and insulin sensitivity (M-value) in non-diabetic and type 1 diabetic participants**

	<i>Non-Diabetics (28F/28M)</i>				<i>Type 1 Diabetics (14F/16M)</i>			
	Pearson correlation with insulin sensitivity		Age, sex, and height adjusted Pearson correlation with insulin sensitivity		Pearson correlation with insulin sensitivity		Age, sex, and height adjusted Pearson correlation with insulin sensitivity	
	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
<b>Clinical Assessments</b>								
Age (years)	.29	.03			.08	.68		
Systolic Blood Pressure (mmHg)	-.24	.11	-.48	.001	.09	.64	.08	.69
Diastolic Blood Pressure (mmHg)	-.47	.001	-.47	.002	-.05	.80	-.02	.92
Total Cholesterol (mg/dL)	-.03	.82	-.04	.76	-.02	.94	-.01	.96
LDL Cholesterol (mg/dL)	-.15	.27	-.14	.32	.04	.85	.08	.74
HDL Cholesterol (mg/dL)	.62	<.001	.55	<.001	.22	.26	.20	.33
Triglycerides (mg/dL)	-.31	.02	-.28	.05	-.42	.03	-.41	.05
BMI (kg/m <sup>2</sup> )	-.67	<.001	-.67	<.001	-.40	.03	-.45	.02
WC (cm)	-.73	<.001	-.69	<.001	-.53	.005	-.55	.004
<b>DEXA Assessments</b>								
FM (kg)	-.44	.001	-.48	.001	-.36	.06	-.43	.02
%FM, of body weight	-.09	.50	-.38	.005	-.22	.26	-.38	.05
Leg fat (kg)	-.14	.31	-.33	.01	-.29	.13	-.40	.04
Arm fat (kg)	-.26	.06	-.43	.001	-.29	.12	-.38	.05
Trunk fat (kg)	-.62	<.001	-.69	<.001	-.36	.06	-.41	.03
FM in Legs (%)	.60	<.001	.66	<.001	.10	.62	.09	.65
FM in Arms (%)	.24	.07	.16	.31	-.06	.74	-.09	.66
FM in Trunk (%)	-.58	<.001	-.73	<.001	-.18	.34	-.19	.33

Values are unadjusted means ± SD.

**Table 11. Multiple linear regression models for dependent variables leg fat (kg), arm fat (kg), and trunk fat (kg); regression coefficients ( $\pm$ SE)**

	<i>Leg Fat (kg)</i>	<i>Arm Fat (kg)</i>	<i>Trunk Fat (kg)</i>
Diabetes Group†	$-.85 \pm .48$	$1.06 \pm .16^{**}$	$-.49 \pm .62$
Age (years)	$.01 \pm .02$	$.01 \pm 0.01$	$.00 \pm .03$
Sex§	$.58 \pm .40$	$-.13 \pm .13$	$-.70 \pm .51$
Height (cm)	$-.09 \pm .02^{**}$	$-.01 \pm .01$	$.10 \pm .03^{**}$
FM (kg)	$.32 \pm .02^{**}$	$.10 \pm .01^{**}$	$.55 \pm .03^{**}$
$R^2$	.85	.77	.90

\*  $P < 0.05$ , \*\* $P < 0.01$

§Negative value indicates lower adipose tissue in men

† Negative value indicates lower adipose tissue in type 1 diabetes

**Table 12. Multiple linear regression model for dependent variable insulin sensitivity (M-value) ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg FFM}^{-1}$ ); regression coefficients ( $\pm\text{SE}$ )**

	<i>Model 1</i>	<i>Model 2</i>	<i>Model 3</i>	<i>Model 4</i>
Diabetes Group†	-3.04 ± .70**	-2.61 ± .67**	-3.79 ± 1.03**	-3.47 ± .62**
Age (years)	.07 ± .03*	.07 ± .03*	.06 ± .03*	.07 ± .03*
Sex§	.45 ± .58	.14 ± .55	.49 ± .59	-.06 ± .55
Height (cm)	-.05 ± .03	.00 ± .03	-.05 ± .03	.00 ± .03
FM (kg)	-.13 ± .03**	-.10 ± .03**	-.13 ± .03**	-.10 ± .03**
Fat in Legs (%)		.14 ± .04**		
Fat in Arms (%)			.17 ± .17	
Fat in Trunk (%)				-0.15 ± .04**
$R^2$	0.28	0.39	0.30	0.42

\* $P < 0.05$ , \*\* $P < 0.01$

§Negative value indicates lower insulin sensitivity (M-value) in men

† Negative value indicates lower insulin sensitivity (M-value) in type 1 diabetes

**Table 13. Multiple linear regression models for dependent variable insulin sensitivity (M-value) stratified by diabetes group; regression coefficients ( $\pm$ SE)**

<i>Non-Diabetics</i>				
	<i>Model 1</i>	<i>Model 2</i>	<i>Model 3</i>	<i>Model 4</i>
<b>Age (years)</b>	.08 $\pm$ .04	.06 $\pm$ .04	.07 $\pm$ .05	.07 $\pm$ .04
<b>Sex§</b>	.96 $\pm$ .94	-.58 $\pm$ .82	.90 $\pm$ .94	-.24 $\pm$ .80
<b>Height (cm)</b>	-.05 $\pm$ .05	.01 $\pm$ .04	-.05 $\pm$ .05	.00 $\pm$ .04
<b>FM (kg)</b>	-.14 $\pm$ .04**	-.09 $\pm$ .04*	-.14 $\pm$ .04**	-.11 $\pm$ .04*
<b>Fat in Legs (%)</b>		.24 $\pm$ .05**		
<b>Fat in Arms (%)</b>			.26 $\pm$ .27	
<b>Fat in Trunk (%)</b>				-0.26 $\pm$ .05**
<b>R<sup>2</sup></b>	0.28	0.56	0.31	0.61
<i>Type 1 Diabetics</i>				
	<i>Model 1</i>	<i>Model 2</i>	<i>Model 3</i>	<i>Model 4</i>
<b>Age (years)</b>	.07 $\pm$ .06	.07 $\pm$ .06	.07 $\pm$ .06	.07 $\pm$ .06
<b>Sex§</b>	-1.16 $\pm$ 1.40	-1.70 $\pm$ 1.57	1.13 $\pm$ 1.44	-1.57 $\pm$ 1.56
<b>Height (cm)</b>	.02 $\pm$ .07	.02 $\pm$ .07	.02 $\pm$ .07	.02 $\pm$ .07
<b>FM (kg)</b>	-.13 $\pm$ .06*	-.16 $\pm$ .07*	-.14 $\pm$ .06*	-.16 $\pm$ .07*
<b>Fat in Legs (%)</b>		-.07 $\pm$ .08		
<b>Fat in Arms (%)</b>			.04 $\pm$ .23	
<b>Fat in Trunk (%)</b>				.06 $\pm$ .09
<b>R<sup>2</sup></b>	0.20	0.22	0.20	0.21

\*  $P < 0.05$ , \*\* $P < 0.01$

§Negative value indicates lower insulin sensitivity (M-value) in men

† Negative value indicates lower insulin sensitivity (M-value) in type 1 diabetes

**9.0 ARTICLE 3: REGIONAL ADIPOSITY AND RISK FOR CORONARY ARTERY  
DISEASE IN TYPE 1 DIABETES: DOES LOWER BODY ADIPOSITY LOWER THE  
RISK?**

To be submitted for publication

Christina M. Shay<sup>1</sup>, Bret H. Goodpaster<sup>2</sup>, Frederico G.S. Toledo<sup>2</sup>, Sheryl F. Kelsey<sup>1</sup>, Elsa  
M. Strotmeyer<sup>1</sup> and Trevor J. Orchard<sup>1</sup>

<sup>1</sup>Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh,  
Pittsburgh, Pennsylvania

<sup>2</sup>Department of Medicine, University of Pittsburgh School of Medicine, University of Pittsburgh,  
Pittsburgh, Pennsylvania

## 9.1 ABSTRACT

**BACKGROUND:** Coronary artery disease (CAD) is associated with increased morbidity and mortality in type 1 diabetes (T1D) and its occurrence has been reported to occur decades earlier compared to other populations. Although adipose tissue content of the thighs and legs has generally not been considered a correlate of traditional CAD risk factors, new evidence is emerging that may suggest a protective cardiovascular effect through favorable associations with CAD risk factors, but this association has not been evaluated in T1D.

**METHODS:** The relationship between regional adiposity, cardiovascular risk factors, and presence of CAD was compared between participants with CAD ( $CAC \geq 400$ , Minnesota codes 1.1, 1.2, 1.3, 4.1–4.3, 5.1–5.3, and 7.1;  $n=115$ ), from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, and those without ( $n=48$ ) using data collected from the 18-year exam. The total and regional adiposity was assessed by dual x-ray absorptiometry (DEXA) and insulin resistance was estimated using the eGDR formula. Other diabetes related measures were also examined.

**RESULTS:** Compared to non-CAD cases, T1D individuals with CAD had higher levels of fat stored in the trunk and lower amounts stored in the legs after controlling for age, height, and sex. Multivariate logistic regression analyses revealed that in females, after adjusting for age, HDLc, smoking, male sex, height, insulin resistance, and general obesity, increasing proportion of fat stored in the legs was associated with decreased risk for CAD (OR=.89;  $p=.05$ ), and increasing proportions of fat in the trunk was independently associated with increased risk for the presence of CAD (OR=1.16;  $p=.03$ ), but this association was not observed in males.

CONCLUSIONS: This is the first evidence that DEXA-assessed lower body adiposity may be cardio-protective in T1D, but this association seems to only exist in females. These results also support the detrimental effects of excess abdominal adiposity in T1D. Leg and trunk adipose adiposity may have independent and opposing effects on CAD risk and these findings may assist in improving the characterization of cardiovascular risk in T1D, particularly in women. Further investigation into the biological plausibility of these anthropomorphic trends is needed as these CAD risk factors may be modified with lifestyle intervention.

## 9.2 INTRODUCTION

Coronary artery disease (CAD) is the most common cause of death in type 1 diabetes (T1D). The development of CAD has been reported to occur decades earlier and at a 10-fold magnitude compared to non-diabetic individuals [231, 279-281]. Although the risk factors associated with the increased CAD morbidity and mortality in this population have been well documented for the past thirty years [231, 282], pathogenesis is still not well understood.

Although it is widely accepted that general obesity is a significant CAD risk factor [283], recent investigations have examined the notion that obesity throughout the body is not uniform in terms of influence on CAD risk [284]. Results from large cross-sectional and longitudinal studies provide evidence that even simple measures of central adiposity (e.g. waist circumference) remain significant predictors of CAD after controlling for the disease risk contributed by other standard factors [285, 286]. More specific measurement of abdominal adiposity by various imaging techniques (e.g. CT, MRI, DEXA), have confirmed findings

identifying the detrimental association between centrally located adiposity and the development of numerous CAD risk factors across various populations [287-290].

Besides abdominal fat, another adipose depot that has gained attention in regards to its potential influence on CAD risk is the lower extremity and/or hips. Although adipose tissue content of the thigh and legs has generally not been considered a correlate of traditional CAD risk factors, new evidence is emerging that may suggest a protective cardiovascular effect through favorable associations with CAD risk factors, such as insulin sensitivity [21-23] and lipid profiles [21, 291, 292]. Not only has increasing amounts of absolute leg fat been associated with reduced cardiovascular risk [25, 26], but the propensity to store fat in the lower body has also been associated with favorable cardiovascular profiles [27]. This notion that some degree of cardiovascular protection may be afforded by the tendency to deposit fat in gluteal-femoral depots is appealing, but few investigations have taken general obesity into account when assessing these associations. Since general obesity is a well established independent cardiovascular risk factor, and additional evidence suggests the strength of the metabolic protection afforded by lower body fat may intensify with increasing levels of obesity [146, 151], overall level of total adiposity must be accounted for when evaluating the association between cardiovascular risk and regional adiposity.

The purpose of this study was two-fold: 1) to examine the associations between CAD risk factors and both total adiposity and regional adiposity in individuals with T1D and 2) to determine if regional adiposity assessments assist in the characterization of those with both CAD and T1D utilizing data collected from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, a 20-yr prospective study of childhood onset T1D.

## 9.3 METHODS

### 9.3.1 Study Population

Subjects were participants in the Pittsburgh Epidemiology of Diabetes Complications Study (EDC), a 20-year prospective follow-up study of childhood-onset type 1 diabetes mellitus which has been previously described in detail [237]. Briefly, participants were diagnosed (or seen within 1 year of diagnosis) between 1950 and 1980, before age 17, at the Children's Hospital of Pittsburgh. This population has been shown to be representative of the T1D population in Allegheny County, Pennsylvania [238]. Analyses for the current cross-sectional investigation included data collected during the 18-year exam cycle (2004-2007) and included all EDC participants for which definitive CAD states and body composition information were available (N=163).

### 9.3.2 Clinical Evaluation and Procedures

As part of the EDC exam, height was measured using a stadiometer and weight was measured on a Detecto physician scale. Standardized sitting blood pressures and heart rate were measured after a 5-min rest period [293]. Hypertension was defined as systolic blood pressure (SBP)  $\geq$  140 mmHg or diastolic blood pressure (DBP)  $\geq$  90 mmHg or the reported use of medications for the purpose of blood pressure control. Total cholesterol was measured enzymatically [242]. High-density lipoprotein cholesterol (HDL-c) levels were determined by a precipitation technique (heparin and manganese chloride) with modification of Lipid Research Clinics method [243]. Non-HDL-c levels were calculated by subtracting HDL-c from total cholesterol. Blood

samples were analyzed for hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) using the DCA 2000 analyzer (Bayer Diagnostics, Tarrytown, NY). Coronary artery calcification (CAC) was assessed by electron beam computed tomography (Imatron, San Francisco, CA).

Estimated glucose disposal rate (eGDR), a measure of insulin sensitivity, was calculated using a formula derived from hyperinsulinemic-euglycemic clamp studies in T1D (involving HbA<sub>1c</sub>, WHR, and hypertension status) [9]. Results from at least two of three timed urine collections (24-hour, overnight, and random timed post-clinic visit urine) were used to determine albumin excretion rates (AER).

### **9.3.3 Coronary Artery Disease Classification**

Both a standardized medical history and clinical examination were performed by a trained internist to classify participants according to CAD status. CAD cases comprised of a positive clinical history (EDC clinic physician diagnosed angina and/or ischemic ECG (Minnesota codes 1.3, 4.1-4.3, 5.1-5.3, 7.1) at the time of examination with CAC  $\geq 100$ , myocardial infarction (either pathological Q waves (Minnesota codes 1.1, 1.2) or findings on review of previous hospital records), hospital record or validated angiographic evidence of  $\geq 50\%$  or more stenosis with or without revascularization. CAC  $\geq 400$  without presence of clinical disease was also included in the definition for CAD cases. Medication use was assessed by participant responses on the EDC medical history questionnaire. All current medications, along with the medication dose and reason for taking medications were recorded. All participants taking HMG CoA reductase inhibitors (statins or statin combination drugs), ezetimibe, probucol, dextrothyroxine, nicotinic acid and derivatives, and bile acid sequestrants were classified as taking LDLc lowering medication and participants taking angiotensin converting enzyme inhibitor (ACE) and/or

angiotensin II receptor blockers (ARB) medications for treatment of hypertension or the promotion of renal health were classified as taking ACE/ARB medications.

#### **9.3.4 Adiposity Assessment**

During the EDC exam, waist circumference (WC) and hip circumference was assessed as a measure of visceral adiposity as part of the regular EDC exam and calculated as a waist-hip ratio (WHR). WC was measured horizontally at midpoint between the highest point of the iliac crest and the lowest part of the costal margin in the mid-axillary line and hip circumference was measured at the widest point of the glutei, usually at the level of the greater trochanter. Distribution of adiposity was measured by dual x-ray absorptiometry (DEXA) using a Hologic QDR4500A scanner (Hologic QDR system software 12.3.) to determine whole body fat mass (FM), lean body mass (LBM), total body FM (%FM), arm FM, leg FM, and trunk FM. FM in the arms and legs was calculated as the sum of both corresponding appendages. The separation between trunk and leg regions was made by two oblique lines passing through the femoral necks and the separation between trunk and arm regions was made by two oblique lines passing through the humeral heads. Trunk FM included both subcutaneous and visceral FM of this anatomical region and the measures of arm and leg FM were a total of both corresponding appendages. A measure of regional adiposity relative to total body FM was calculated as a percentage of total FM in the legs (% FM in legs), arms (% FM in arms), and trunk (% FM in Trunk). Since adiposity assessments were calculated based on proportion of fat stored in particular region relative to total body FM, participants were excluded from the present analyses if any region of the body was amputated (n=3), if any plastic artifacts were scanned that may influence the computation of adipose tissue (n=14), or if any area of the body was excluded from

the DEXA scan (n=5). The protocol was approved by the University of Pittsburgh Institutional Review Board.

### **9.3.5 Statistical Analyses**

Non-normally distributed variables (e.g. heart rate and triglycerides) were transformed by natural log prior to testing. Non-parametric variables that were not normalized after transformation (e.g. AER and serum creatinine) were analyzed with non-parametric techniques. Group differences were examined using Student's *t*-test and Mann-Whitney-U test, as appropriate.  $P < 0.05$  was considered statistically significant. General linear models were used to determine group differences after adjusting for factors known to influence adiposity (e.g. age, sex, and/or height). Multivariate logistic regression with forward selection was used to examine variables most strongly associated with presence of CAD. All variables with a univariate association ( $P < 0.25$ ) with presence of CAD were made available for modeling. A significance of  $P > 0.10$  was applied for entry into the model and a  $P > 0.05$  was applied for exclusion from the model. Variables also known to influence risk for CAD and/or adiposity distribution were forced into models if not selected. Since many adiposity measures were significantly inter-correlated, separate models were fit investigating the influence of each regional adiposity measure on association with presence of CAD excluding other adiposity measures except FM (kg). For determination of the best overall model, Akaike's Information Criteria (AIC) was computed for each model and the model with the lowest AIC was considered the model containing the adiposity measures most closely associated with the presence of CAD. No formal correction was performed for multiple comparisons. Analysis was performed using SPSS for Windows software version 16.0 (SPSS, Chicago, IL).

## 9.4 RESULTS

### 9.4.1 Subject Characteristics

Characteristics of the 163 participants with T1D are presented by CAD status in Table 14. Participants with CAD were older, had diabetes for a longer duration, had showed a trend toward lower levels of HDL cholesterol, and had a larger proportion of individuals taking LDL cholesterol lowering medications. AER was lower in CAD cases and the distribution of participants by smoking status, HbA1c, total cholesterol, LDL cholesterol, non-HDLc, triglycerides, SBP, and level of insulin sensitivity (eGDR) were not significantly different between the groups. When body composition was examined in relation to gender (Table 15), men and women had similar BMI and trunk fat (kg), however, men exhibited significantly higher body weight, LBM, WC, WHR, and %FM in the trunk. Women displayed significantly higher levels of FM (kg), FM (%), leg fat (kg), arm fat (kg), % FM in the legs, and % FM in the arms. When body composition was examined with regard to CAD status (Table 16), individuals with CAD had higher WC, WHR, and a higher proportion of FM stored in the trunk along and significantly lower proportion of FM stored in the legs. These differences, along with higher trunk FM and BMI remained elevated in CAD cases after adjusting for factors influencing adiposity distribution (i.e. age, sex, and height). Weight, LBM, total FM (kg), and absolute levels of leg fat (kg), arm fat (kg), trunk fat (kg), and proportion of fat stored in the arms, were similar between groups with a trend towards higher FM % and trunk fat (kg) in individuals with CAD.

#### **9.4.2 Coronary Artery Disease Risk Factors and Regional Adiposity**

When examining the relationship between individual CAD risk factors and lower body adiposity (Table 17), moderate positive correlations were observed between % FM in legs and HDL cholesterol and insulin sensitivity (eGDR). Conversely, significant inverse correlations between % FM in the trunk and HDL cholesterol and insulin sensitivity (eGDR) were observed.

Moderate negative correlations were observed between % FM in legs and CAC, AER, serum creatinine, SBP, DBP, LDLc, non-HDLc, triglycerides, and BMI. Stronger negative correlations were observed between this measure of lower body adiposity and both WC and WHR. Similar, yet opposite, associations were observed with these CAD risk factors and % FM in Trunk compared to the associations observed with FM % in legs. Small positive correlations were observed between FM % in arms and diabetes duration, BMI, and WC while similar negative correlations were observed between this regional adiposity measure and insulin sensitivity (eGDR) and DBP. When examining associations between the individual regional adiposity measures, FM in the legs displayed a strong inverse correlation with % FM in the trunk and %, and a moderate inverse association with % FM in the arms. Results also suggested a positive association between % FM in the arms and % FM in the trunk, but these correlations did not reach statistical significance.

#### **9.4.3 Presence of Coronary Artery Disease and Regional Adiposity**

The results from a forward selection multivariate logistic regression model revealed that age and HDL cholesterol were the factors most strongly associated with presence of CAD. Even though they were not selected in the final model, additional variables were forced into the model due to

their established associations with both CAD and regional adiposity (i.e. sex, smoking status, insulin sensitivity (eGDR), and FM (kg)) [248, 294, 295]. Separate regression models individually forcing % FM in legs, % FM in arms, % FM in trunk, and FM % into the baseline model including which included FM (kg) were subsequently examined to assess to the prediction of CAD states after adjustment for total adiposity. Since WC was a variable used in the computation of insulin sensitivity (eGDR) and was highly correlated with all adiposity variables in this sample, the measure was removed multivariate analyses.

Results from univariate logistic regression analyses suggested that neither absolute (kg) nor total (%) FM was associated with presence of CAD, yet individuals with CAD had significantly lower % FM in the legs ( $p=.006$ ) and significantly higher % FM in the trunk ( $p=.01$ ) (Table 18). After controlling for other CAD risk factors, total FM (kg) was not associated with presence of CAD (Table 19, Model 1), however, a trend toward lower proportions of FM stored in the legs being associated with presence of CAD after controlling for total adiposity and other CAD risk factors (Table 5, Model 2) was observed. Multivariate analyses suggested that for every 1% increase in total FM stored in legs was associated with an approximate 7% reduction in the probability for CAD (OR=.93, 95% CI .85-1.00). The model including FM % in legs also had the lowest AIC value suggesting that increasing amounts of body fat stored in the lower body offers a protective effects against CAD, and that it may be the main driver of the regional contribution to the presence of CAD in T1D. A positive association between % FM in trunk was observed (Table 5, Model 3) suggests a detrimental association between this variable and the presence of CAD (OR=1.08, 95% CI 1.001-1.165). % FM in arms were not associated with presence of CAD after controlling for CAD risk factors and no interactions were observed between gender and any of the regional adiposity measures.

Since sex differences in adiposity are well established [295], separate models were fit examining the influence of regional adiposity on risk for CAD (Table 20). The baseline variables from the best prediction model derived from the overall sample were included and the regional adiposity variables were again individually added with FM (kg) to examine the contribution of these variables to prediction of presence of CAD (i.e. age, HDL, smoking status, eGDR, height and FM (kg)). After controlling for significant CAD risk factors, % FM in the legs and % FM in trunk exhibited stronger independent associations with CAD in females compared to the overall sample, while no regional adiposity measure was independently associated with presence of CAD in males. In females, every 1% increase in total FM stored in legs was associated with an approximate 12% reduction in CAD risk (OR=.89, 95% CI .79-.99) and every 1% increase in FM stored in the trunk region was associated with an approximate 16% increase in the risk for CAD (OR=1.16, 95% CI 1.01-1.33).

## 9.5 DISCUSSION

In this cross-sectional study of 163 type 1 diabetic patients, the primary new finding is that a preference to store body fat in the lower limbs, as measured by DEXA, appears to be associated with a lower prevalence of CAD, even after controlling for general obesity and other traditional CAD risk factors. These findings also confirm previous observations in T1D that increasing fat stored in the abdomen is associated with increased CVD risk [296]. It is also particularly interesting to note that when stratified by gender, the protective CAD association with a tendency to store fat in the lower body and the detrimental effect of trunk adiposity with CAD

risk both strengthened in females, while these associations were diminished in males. These observations again raise the central question as to whether trunk or leg fat is the driving force for CVD risk as these two components are inversely related. We suggest that “both” is the likely explanation as discussed below.

There has been great interest in identifying the regions of the body in which it is metabolically “optimal” to store adipose tissue in efforts to identify the anthropomorphic body type that is at lowest risk for CAD. Although there is little debate over the notion that excess general adiposity increases CAD risk, a significant body of knowledge exists concluding that the android pattern of fat distribution, independent of general obesity is positively associated increased CAD risk, even in the absence of obese states [297]. Evidence from early epidemiologic studies employing simplistic measures of central adiposity (e.g. waist circumference, waist-hip ratio) became the basis for the concept that excess storage of abdominal fat poses more metabolic risk than general obesity and that the distribution of fat deposits may be a better predictor of cardiovascular morbidity and mortality than the overall degree of adiposity [298, 299]. Despite the excess CAD risk in T1D, it was not until decades later that similar associations with abdominal adiposity began to be revealed in this population [197, 300-303]. The advent of more sophisticated imaging techniques (e.g. DEXA, MRI, CT) allowed independent examination of trunk and leg fat which provided more evidence for the theory that abdominal adipose tissue has the most atherogenic influence through the potential to release metabolic by-products (e.g. free fatty acids) into portal circulation [304-306]. Recent evidence is also emerging suggesting that increased lower-body adiposity is a reflection of a superior ability to “spillover” excess adiposity into the lower body (i.e. away from the abdomen) where it is less metabolically active and thus may be protective against cardio-metabolic disease risk [24-26,

307] . Data reported from Van Pelt *et al.* supported the protective association between lower body adiposity and numerous CAD risk factors such as blood lipid profiles and measures of insulin resistance [21, 24]. Comparable results were also reported from large epidemiologic cohort studies after protective associations between gluteal-femoral adiposity and arterial stiffness [26], glucose tolerance [147], and blood lipid levels [146, 151] were observed. Such previous investigations have examined these associations in high risk populations (e.g. post-menopausal women, type 2 diabetes) but to our knowledge, this is the first investigation into use DEXA-assessed regional adiposity to examine associations between presence of CAD and CAD risk factors and in T1D.

One of the strengths of the current analyses is the examination of the proportion of total body fat stored in the legs, as opposed to total leg fat mass, and its association with CAD risk factors. Previous investigations have used measures of absolute leg fat mass to examine associations with metabolic risk [21, 24, 248, 267] and since fat mass in any region (e.g. legs, trunk, arms) is influenced by total FM (kg), the examination of the combined contribution of these assessments on cardiovascular risk is difficult. Since the proportion of total body fat stored in a given anatomical region was strongly associated with total body adiposity in this T1D sample, the use of these measures of adiposity allowed examination of the combined effect of proportional leg fat and general obesity without concern for issues of colinearity. Examining whether a propensity to store fat in legs and ship is associated with CAD risk also asks a different question than whether leg fat, per se, is associated with CAD outcomes. If the propensity to store more adipose tissue in a given region, regardless of overall adiposity, has a cardio-protective effect, the classification of individuals based on this phenotype may help better

characterize individuals at risk for the development of CAD, particularly in high risk populations (e.g. T1D).

Besides the benefit of controlling for general obesity, the use of a proportional measure of regional adiposity allowed a more direct comparison of the protective effect of regional adiposity on CAD risk between individuals of different body sizes and genders. Although total FM (kg) was not associated with presence of CAD in this sample after adjustment for age, sex, and height, the influence of total adiposity and its potential attenuating effects on the protective association between lower body adiposity and CAD risk can not be ignored. There is significant evidence from longitudinal cohort investigations that increasing obesity is associated with increased cardiovascular mortality [308] yet little information is available on the role of obesity as it may attenuate the protective nature of lower body adiposity on CAD risk in T1D. Results from the current investigation suggest that the protective nature of gluteal-femoral fat storage may be independent of the detrimental effects of overall obesity on CAD risk in females with T1D and these findings support the need for further investigation into the metabolic plausibility of these associations.

The finding that lower body adiposity displayed a protective association with the presence of CAD in females and not men with T1D, remains intriguing. It is known women generally have a greater percentage of their total body weight stores as adipose tissue[309]. Additionally, men typically exhibit android obesity, characterized by accumulation of fat in the abdominal region, whereas women often display gynoid obesity, characterized with a greater proportion of their body fat in the gluteal-femoral region [310]. Since it has been established that there are sex differences in adipose tissue storage and metabolism [269, 311, 312], and that CAD risk is associated with body fat distribution, it is not surprising to consider that differences

may exist regarding the association between regional adiposity and CAD risk between the genders. Results from an investigation by Aasen et. al. suggest that a greater propensity to store fat in the lower body is protective against cardiovascular disease in obese women, but the association lessened in overweight women [146]. Women in the current investigation were found to have significantly higher levels of adiposity, including overall adiposity and a greater proportion of fat stored in the legs when compared to men. In light of these previous findings, we hypothesize that the enhanced cardio-protective effect of gluteal-femoral adiposity is most strongly exhibited in females due to the increased levels of adiposity in this gender. We also conclude that since previous findings on body composition in T1D have suggested that individuals with T1D have lower levels of overall adiposity compared to their respective non-diabetic controls [118, 313], it may be reasonable to conclude that the protective effect of lower body adiposity and CAD risk may not be limited to females, but may also be present in men with T1D but the association may be expressed more robustly in all individuals with T1D who have greater levels of adiposity, however, further exploration is warranted.

Amount of FM stored in the trunk was also detrimentally associated with CAD risk factors and these associations were nearly identical, but the inverse of, the associations observed with FM stored in the legs (Table 17). Previous investigations have similarly reported these inverse associations between trunk and leg adiposity in non-diabetic populations with CAD risk factors [21, 306] and postulations could be made that storage of adiposity in the legs is simply indicative of a propensity to store FM away from the trunk region. Results from the current investigation support this hypothesis in T1D, however, since the cardio-protective association of gluteal-femoral fat storage was independent of general obesity, we propose that additional metabolic mechanisms may be involved which require further investigation.

It is hypothesized that insulin sensitivity contributes to the development of CAD via increased adiposity in the visceral compartment which results in excess release of free-fatty acids into the portal circulation [19]. However, adjustment for insulin sensitivity (eGDR) did not substantially affect the associations between presence of CAD and either peripheral or central adiposity. Despite these findings, we cannot exclude the role of insulin sensitivity in the development of CAD since our assessment of insulin sensitivity was an indirect method and the current associations were examined using a cross-sectional approach. Insulin resistance, which has been well documented in type 1 diabetes (T1D), has been demonstrated as a risk factor for CAD [13, 66], but the labor intensive, costly, and relatively invasive nature of the direct assessment techniques is often inappropriate for use in large-scale population studies. Since more specific assessments of visceral adiposity or insulin resistance were unavailable, we were not able to explore this association any further in the current investigation. The connection between insulin resistance, per se, and CAD in T1D appears to be pertinent, but is less well defined in T1D than in other populations, likely due to difficulties in assessing this measure in insulin-dependant populations. The mechanisms and methodological issues regarding the inter-relationships between individual CAD risk factors and insulin resistance remain subjects of interest, particularly in T1D.

Since the DEXA assessment of body composition has been shown to provide useful information regarding various adiposity regions associated with CAD risk [306, 314] the appeal in examining more comprehensive assessments of these associations in T1D is evident. Although DEXA-assessment of composition is generally considered to be a reliable and valid assessment of abdominal and peripheral adipose tissue content [315, 316], a limitation of this assessment is that the distinction between visceral, subcutaneous, or intramuscular fat depots can

not be examined. In order to explore the specific biological plausibility of the current findings, additional studies are needed in T1D to explore the specific metabolic influence of abdominal and leg fat stores on CAD risk and outcomes.

In summary, a propensity to store adipose tissue in the lower body was favorably associated with CAD risk factors and negatively associated with presence of CAD in women with T1D, but this protective effect was not observed in the males. The lack of an association between lower body adiposity and presence of CAD in men with T1D may be due to the lower levels of adiposity in men and proportionally less adipose tissue stored in the lower body compared to women. Our findings that leg and trunk adipose tissue storage may have independent and opposing effects on CAD risk may reflect the metabolically protective ability to store body fat away from the abdomen, however, further investigation into the biological plausibility influencing these anthropomorphic trends is needed. Regardless of how excess fat is stored in the body, the public health message should remain aimed at the reduction of prevalent obesity and the prevention of weight gain due to excess adiposity, even in T1D.

## 9.6 TABLES

Table 14. Characteristics of type 1 diabetes participants by coronary artery disease status at 18-year exam -  
The Pittsburgh Epidemiology of Diabetes Complications Study

<i>Characteristics</i>	<i>CAD Negative</i>	<i>CAD Positive</i>	<i>P-value</i>
<b>N (% male)</b>	115 (51.30)	48 (56.25)	.56
<b>Age (years)</b>	44.01 (6.78)	49.40 (6.79)	< .01
<b>DIABETES duration (years)</b>	35.44 (6.03)	40.33 (7.13)	< .01
<b>Ever smoked, <i>n</i> (%)</b>	39 (34.5)	20 (41.7)	.58
<b>AER (<math>\mu\text{g}/\text{min}</math>)*</b>	5.98 (4.02-26.09)	5.27 (5.27-59.26)	.03
<b>Serum Creatinine (mg/dL)*</b>	1.00 (.80-1.10)	1.00 (.90-1.28)	.13
<b>HbA<sub>1c</sub> (%)</b>	7.5 (1.45)	7.15 (1.40)	.14
<b>Resting Heart Rate (bpm) ‡</b>	73.95 (10.77)	73.19 (11.31)	.69
<b>eGDR (mg/kg/min)</b>	7.66 (2.29)	7.05 (2.04)	.12
<b>Hypertension, <i>n</i> (%)</b>	30 (27.0)	18 (37.5)	.19
<b>Systolic blood pressure (mmHg)</b>	114.14 (15.34)	117.98 (15.88)	.16
<b>Diastolic blood pressure (mmHg)</b>	66.43 (10.97)	62.81 (10.80)	.06
<b>Taking ACE/ARB Inhibitors, <i>n</i> (%)</b>	61 (53.0)	27 (56.3)	.71
<b>Total Cholesterol (mg/dl)</b>	172.41 (30.57)	165.62 (31.61)	.21
<b>LDLc (mg/dl)</b>	97.93 (27.68)	94.50 (27.70)	.51
<b>HDLc (mg/dl)</b>	60.81 (17.16)	55.06 (15.36)	.05
<b>Non-HDLc (mg/dl)</b>	111.60 (28.61)	110.56 (29.85)	.84
<b>Triglycerides (mg/dl) ‡</b>	78.49 (36.53)	91.40 (50.32)	.09
<b>Taking LDL Medications, <i>n</i> (%)</b>	41 (36.3)	26 (54.2)	.04

All values are  $\bar{x}$  (SD) unless otherwise noted.

\*Data presented as median (interquartile range)

‡Log-transformed before statistical testing.

**Table 15. Body composition assessments of type 1 diabetes participants by gender at 18-year exam - The Pittsburgh Epidemiology of Diabetes Complications Study (N= 163)**

	<b>Females (n=75)</b>	<b>Males (n=86)</b>	<b><i>P-value</i></b>
<b>Weight (kg)</b>	68.61 (11.30)	80.65 (12.55)	<.001
<b>BMI (kg/m<sup>2</sup>)</b>	25.92 (4.33)	26.48 (3.84)	.39
<b>WC (cm)</b>	84.48 (10.62)	93.37 (11.02)	<.001
<b>Waist-Hip Ratio</b>	0.83 (0.08)	0.93 (0.07)	<.001
<b>LBM (kg)</b>	44.63 (5.11)	60.90 (7.27)	<.001
<b>FM (kg)</b>	22.84 (7.07)	18.01 (6.79)	<.001
<b>FM %</b>	33.17 (6.18)	22.34 (6.06)	<.001
<b>Leg fat (kg)</b>	8.76 (2.54)	5.73 (1.98)	<.001
<b>Arm fat (kg)</b>	3.04 (1.14)	2.28 (0.94)	<.001
<b>Trunk fat (kg)</b>	10.20 (4.27)	9.07 (4.20)	.08
<b>% FM in Legs</b>	39.50 (7.70)	32.67 (5.18)	<.001
<b>% FM in Arms</b>	13.15 (2.15)	12.51 (1.78)	.04
<b>% FM in Trunk</b>	43.38 (6.94)	48.58 (6.38)	<.001

Values are unadjusted means (SD). FM, fat mass; LBM, lean body mass; WC, waist circumference.

**Table 16. Body composition assessments of T1D participants by CAD status at 18-year exam - The Pittsburgh  
Epidemiology of Diabetes Complications Study (N= 163)**

	<b>CAD Negative</b>	<b>CAD Positive</b>	<i>Unadjusted P-value</i>	<i>Adjusted P-value<sup>1</sup></i>
<b>Weight (kg)</b>	75.09 (13.36)	74.95 (13.58)	.95	.20
<b>BMI (kg/m<sup>2</sup>)</b>	25.93 (3.91)	26.92 (4.39)	.16	.05
<b>WC (cm)</b>	88.09 (11.49)	91.85 (11.81)	.06	.05
<b>Waist-Hip Ratio</b>	0.87 (0.09)	0.91 (0.09)	.01	.03
<b>LBM (kg)</b>	53.54 (10.45)	52.28 (10.01)	.48	.36
<b>FM (kg)</b>	19.99 (7.15)	21.04 (7.71)	.41	.15
<b>FM %</b>	27.09 (7.98)	28.46 (8.59)	.33	.09
<b>Leg fat (kg)</b>	7.26 (2.72)	6.92 (2.72)	.46	.95
<b>Arm fat (kg)</b>	2.58 (1.07)	2.79 (1.19)	.28	.11
<b>Trunk fat (kg)</b>	9.27 (4.13)	10.40 (4.51)	.12	.06
<b>% FM in Legs</b>	36.96 (7.41)	33.42 (6.55)	.005	.006
<b>% FM in Arms</b>	12.75 (1.97)	12.97 (2.05)	.52	.64
<b>% FM in Trunk</b>	45.18 (7.04)	48.33 (6.88)	.01	.02

Values are unadjusted means (SD). DEXA, dual x-ray absorptiometry; FM, fat mass; LBM, lean body mass; WC, waist circumference.

<sup>1</sup>Data was adjusted for effects of sex, age, and height, except for the data of weight, BMI, FM, LBM, and FM % which were adjusted for age and sex.

Table 17. Pearson correlations between CAD risk factors and regional adiposity in type 1 diabetes - The Pittsburgh Epidemiology of Diabetes

Complications Study (N= 163)

	% FM in Legs		% FM in Arms		% FM in Trunk	
	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Age (years)	-.004	.956	.105	.186	.002	.975
Diabetes duration (years)	.009	.905	.132	.097	-.061	.445
Agatston CAC Score <sup>‡</sup>	<b>-.216</b>	<b>.016</b>	.059	.518	<b>.186</b>	<b>.038</b>
AER (µg/min)*	<b>-.345</b>	<b>&lt;.001</b>	.099	.219	<b>.311</b>	<b>&lt;.001</b>
Serum Creatinine (mg/dL)*	<b>-.261</b>	<b>.001</b>	-.069	.405	<b>.293</b>	<b>&lt;.001</b>
HbA <sub>1c</sub> (%)	-.095	.235	.090	.262	.042	.603
eGDR (mg/kg/min)	<b>.517</b>	<b>&lt;.001</b>	<b>-.170</b>	<b>.034</b>	<b>-.480</b>	<b>&lt;.001</b>
Resting Heart Rate (bpm)	-.136	.089	-.010	.896	.153	.054
SBP (mmHg)	<b>-.300</b>	<b>&lt;.001</b>	.038	.640	<b>.303</b>	<b>&lt;.001</b>
DBP (mmHg)	<b>-.318</b>	<b>&lt;.001</b>	-.143	.075	<b>.312</b>	<b>&lt;.001</b>
Total Cholesterol (mg/dL)	-.038	.637	.054	.503	.071	.375
LDL (mg/dL)	<b>-.230</b>	<b>.008</b>	-.029	.736	<b>.246</b>	<b>.004</b>
HDL (mg/dL)	<b>.366</b>	<b>&lt;.001</b>	.049	.542	<b>-.324</b>	<b>&lt;.001</b>
Non-HDL (mg/dL)	<b>-.255</b>	<b>.001</b>	.029	.717	<b>.266</b>	<b>.001</b>
Triglycerides (mg/dL)*	<b>-.337</b>	<b>&lt;.001</b>	.101	.237	<b>.358</b>	<b>&lt;.001</b>
BMI (kg/m <sup>2</sup> )	<b>-.419</b>	<b>&lt;.001</b>	<b>.164</b>	<b>.039</b>	<b>.541</b>	<b>&lt;.001</b>
Waist Circumference (cm)	<b>-.649</b>	<b>&lt;.001</b>	.150	.062	<b>.722</b>	<b>&lt;.001</b>
Waist-Hip Ratio	<b>-.696</b>	<b>&lt;.001</b>	.105	.191	<b>.667</b>	<b>&lt;.001</b>
% FM in Legs	--	--	<b>-.327</b>	<b>&lt;.001</b>	<b>-.937</b>	<b>&lt;.001</b>
% FM in Arms	<b>-.327</b>	<b>&lt;.001</b>	--	--	.149	.048
% FM in Trunk	<b>-.937</b>	<b>&lt;.001</b>	.149	.048	--	--

\*Log-transformed before statistical testing.

<sup>‡</sup> Log-transformed +1 before statistical testing

**Table 18. Univariate odds ratios for relationship between regional adiposity and presence of coronary artery disease in type 1 diabetes - The Pittsburgh Epidemiology of Diabetes Complications Study (N= 163)**

<i>Variable</i>	<i>Odds Ratio</i>	<i>95% Confidence Interval</i>
% FM in Legs	.929	.882 - .979
% FM in Arms	1.058	.892 – 1.255
% FM in Trunk	1.067	1.015 – 1.122
FM (kg)	2.020	.974-1.068
FM %	1.021	.979 – 1.065

**Table 19. Association of CAD risk factors and regional adiposity measures with presence of CAD in type 1 diabetes - The Pittsburgh Epidemiology of Diabetes Complications Study (N= 163)**

<b>Variable</b>	<i>Model 1</i>			<i>Model 2</i>			<i>Model 3</i>			<i>Model 4</i>		
	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>
<b>Age (years)</b>	.135	.034	<.001	.135	.035	<.001	.135	.035	<.001	.136	.035	<.001
<b>HDL (mg/dL)</b>	-.033	.015	.027	-.029	.015	.046	-.033	.015	.027	-.031	.015	.039
<b>History of Smoking</b>	.259	.414	.532	.347	.423	.414	.259	.414	.531	.317	.419	.575
<b>Male Sex</b>	1.034	.646	.109	.486	.709	.493	1.031	.648	.112	.618	.620	.993
<b>Height (cm)</b>	-.086	.032	.006	-.082	.032	.011	-.087	.032	.006	-.082	.032	.011
<b>eGDR (mg/kg/min)</b>	-.027	.103	.791	.048	.113	.673	-.028	.104	.788	.025	.111	.820
<b>FM (kg)</b>	.027	.032	.397	.005	.034	.894	.027	.032	.396	-.003	.038	.929
<b>FM in Legs (%)</b>				-.078	.042	.062						
<b>FM in Arms (%)</b>							-.006	.110	.954			
<b>FM in Trunk (%)</b>										.062	.043	.145
<b>AIC</b>	169.252			167.497			171.249			169.070		

Table 20. Association of CAD risk factors and regional adiposity measures with presence of CAD in type 1 diabetes by gender - The Pittsburgh

Epidemiology of Diabetes Complications Study (N= 163)

<i>FEMALES</i>												
Variable	<i>Model 1</i>			<i>Model 2</i>			<i>Model 3</i>			<i>Model 4</i>		
	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>
Age (years)	.129	.049	.002	.137	.053	.010	.126	.049	.010	.143	.055	.009
HDL (mg/dL)	-.030	.021	.114	-.023	.021	.279	-.030	.021	.156	-.023	.021	.283
History of Smoking	.496	.655	.920	.686	.694	.322	.499	.652	.445	.683	.703	.332
Height (cm)	-.116	.052	.107	-.119	.054	.029	-.118	.052	.023	-.117	.055	.034
eGDR (mg/kg/min)	.038	.173	.464	.162	.194	.401	.050	.175	.778	.176	.196	.371
FM (kg)	.062	.048	.572	.021	.054	.695	.058	.048	.230	-.006	.060	.915
FM in Legs (%)				-.119	.060	.047						
FM in Arms (%)							.106	.163	.514			
FM in Trunk (%)										.148	.070	.034
AIC		78.479			75.794			80.048			75.224	
<i>MALES</i>												
Variable	<i>Model 1</i>			<i>Model 2</i>			<i>Model 3</i>			<i>Model 4</i>		
	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>	$\beta$	$\pm SE$	<i>p</i>
Age (years)	.140	.048	.003	.139	.048	.003	.147	.049	.003	.147	.049	.003
HDL (mg/dL)	-.039	.022	.082	-.039	.022	.078	-.039	.023	.086	-.039	.023	.086
History of Smoking	.083	.553	.881	.105	.558	.859	.097	.553	.861	.097	.553	.861
Height (cm)	-.067	.042	.108	-.065	.042	.102	-.071	.043	.102	-.071	.043	.102
eGDR (mg/kg/min)	-.065	.128	.611	-.046	.142	.544	-.078	.131	.549	-.078	.131	.549
FM (kg)	-.006	.044	.887	-.011	.046	.818	-.002	.044	.963	-.002	.044	.963
FM in Legs (%)				-.021	.066	.795						
FM in Arms (%)							-.108	.157	.293			
FM in Trunk (%)										-.108	.157	.493
AIC		98.776			100.676			100.300			100.751	

## 10.0 FINAL DISCUSSION

To our knowledge, this is the first investigation examining the associations between direct assessments of IR (i.e. hyperinsulinemic-euglycemic clamp) and prevalent CAD and renal disease in a T1D population. These data document no difference in IR between prevalent states of CAD and renal disease in T1D, even after adjustment for lean body mass and steady-state plasma insulin during the insulin clamp procedure. Although DEXA-assessed regional adiposity measures did not significantly add to the prediction of IR beyond more simplistic clinical measures of adiposity (i.e. WC and BMI), these analyses were the first to examine DEXA-assessed regional adiposity, specifically gluteal-femoral fat stores, and their associations with directly measured IR in T1D. Important negative associations were observed between IR and all absolute measures of regional adiposity (i.e. leg FM, arm FM, trunk FM, and total FM) in T1D, particularly in women; associations that remained after adjustment for other traditional IR risk factors. Other clinical factors that were associated with IR in T1D were diabetes duration, daily insulin dose, presence of overt nephropathy, and waist circumference. Despite lower levels of overall adiposity than the non-diabetic controls, individuals with T1D displayed more severe states of IR suggesting that although general obesity was strongly associated with IR in T1D, IR may occur at lower levels of obesity in T1D than in other populations. Even though evidence exists supporting a metabolically protective effect of lower body adiposity against IR in other populations, these associations were not found in T1D. Not only was leg fat not protective in

T1D, but these results suggest that increasing amounts of fat in all regions (i.e. arms, legs, and trunk) may be unfavorably associated with IR. It has been suggested that the metabolically protective effect of lower body fat storage may strengthen with increasing levels of obesity, thus the absence of this association in T1D may potentially be attributed to the lower relative levels of adiposity in T1D. Our finding of a protective effect of lower body adiposity in obese T1D is consistent with this hypothesis. Due to small sample sizes, these associations could not be fully explored in this investigation and further exploration is warranted.

The final analyses investigating the role of DEXA-assessed regional adiposity and the association with CAD risk are also, to our knowledge, the first to show independent and opposite associations of leg and trunk fat with CAD in a T1D population. Lower body adiposity was independently and negatively associated with the presence of CAD. Interestingly, this association was only observed in females with T1D. Since females have been shown to have greater proportions of overall and lower body adiposity compared to men, these findings add support to previous findings suggesting that the intensity of the cardio-protective effect of lower body adiposity increases with increasing adiposity. However, how this protective association of lower body adiposity relates to the increased risk for CAD in women with T1D is unclear.

It is with the use of the insulin clamp technique that IR was initially found to occur in T1D [1]. Subsequent clinical investigations have provided evidence that this state may be an exacerbating factor for T1D complications, particularly renal disease [71, 259, 317, 318]. Previous studies have also drawn connections between IR and increased risk for the development of cardiovascular disease [319, 320] but the available literature focuses on non-diabetic [321, 322] and T2D [323, 324] populations with no direct exploration of these associations in T1D. With the increased difficulty related to the direct assessment of IR in insulin-dependant

populations, particularly in individuals with long-standing diabetes and complicated medical histories, this deficiency in available insulin clamp data is not surprising. In light of these difficulties, the majority of the available evidence exploring insulin-resistance related complications in T1D has relied on the use of the eGDR formula to identify individuals at risk for IR. The Estimated Glucose Disposal (eGDR) equation, validated by hyperinsulinemic-euglycemic clamp studies, is a method of estimating IR in T1D [9] and has been used in a number of epidemiologic T1D investigations [10-13]. This equation which employs readily obtainable clinical assessments (waist:hip ratio, HbA<sub>1c</sub>, and presence of hypertension) has been shown to identify patients with T1D who are likely to have IR, with an R<sup>2</sup> of 0.63. Results from investigations using the eGDR formula, including those from the EDC where it was developed, have shown eGDR to be a strong predictor of cardiovascular disease and cardiovascular morbidity [13, 78, 325] and the development of renal disease [85, 326, 327] in T1D. Although based on surrogate measures of insulin sensitivity, the strength of these associations has generated inquiries into the potential for more prominent insulin resistant states to exist in T1D individuals who have developed both complications. And since the majority of data on IR in these disease states was not directly measured, the use of the insulin clamp technique to investigate these associations was highly desirable.

As noted, the current findings suggest no differences in IR between individuals with prevalent coronary artery disease and/or overt nephropathy in T1D; findings which contrast earlier reports from the EDC study [78, 195, 235, 249] which indicated that eGDR assessment of IR predicted incident cardiovascular and renal complications. In the current T1D sample, the further examination of all available risk factors determine the best predictors of IR to be diabetes duration, WC, ON status, and daily insulin dose. The regression formula that most closely

determined IR from the selected variables is the following:  $GDR=9.92+.08(\text{Duration})-.06(\text{WC})+2.26(\text{ON})-3.79(\text{Insulin Dose})$ . Although these variables selected have been previously found to be risk factors associated with increased risk for IR in T1D [85, 252, 259, 317, 327, 328], the directionality of both the diabetes duration and ON status coefficients contradict previous reports concerning these risk factors. In this formula, diabetes duration and ON status are both positively associated with IR. A possible explanation for these contradictory findings may be the selection criteria for the current study based on the invasive nature of the insulin clamp study. In order to participate in the current investigation, participants were required to have normal blood counts in order to safely undergo the blood draws required for the insulin clamp procedure. Since anemia has been well-documented as a complication of chronic kidney disease and is associated with adverse renal outcomes [329-333], the recruitment of participants who have both overt nephropathy and normal blood counts may have biased the selection of participants in this group to include a healthier and more insulin sensitive sample compared to the majority of individuals with ON and long standing diabetes complications. A final factor that can not be overlooked when comparing the present findings to other reports on the determinants of IR in T1D is that the majority of epidemiologic investigations rely on indirect assessments of IR to examine associated risk factors. Indirect methods, such as the eGDR equation, are calculated based on previously identified risk factors associated with direct measurement of glucose disposal. So the question still remains as to whether IR, per se, or the risk factors that characterize it, best predict complications in T1D.

Three different direct assessments of glucose disposal rates (GDR) and an indirect assessment of GDR (eGDR) calculated in the current investigation. The direct assessments include GDR adjusted for only body weight ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ), GDR adjusted for lean body mass

( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}$ ), and GDR adjusted for lean body mass and plasma insulin ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}\cdot\text{uUmL}^{-1}$ ). The indirect method, eGDR, was calculated based on the IR risk factors measured during the insulin clamp procedure and this measure was used for estimation of IR when insulin clamp data was not available. When comparing the association between the direct and indirect measures of IR, there are some striking differences (Table 21). All direct measures of GDR calculated from the current investigation were strongly correlated; however, the direct measures of IR exhibited very weak associations with eGDR calculated at the time of the clamp assessment. Since the validation of the eGDR formula nearly a decade ago, major advancements in the therapeutic management of T1D have evolved which may influence the interpretation of this assessment. The increasing use of angiotensin converting enzyme inhibitors (ACE) and/or angiotensin II receptor blockers (ARB) for blood pressure control and/or renal protection has challenged the historically held association between hypertension, IR, and renal disease in T1D [43]. Although previous studies that examined the effect of ACE inhibitors on insulin sensitivity in T2D have yielded conflicting results [334-338], more recent examination of calcium antagonist and ACE inhibitor therapy have associated their use with better metabolic profiles and similar reports have provided evidence that ACE use may improve IR [250, 339]. Over two-thirds of the current participants studied with the insulin clamp technique were taking ACE and/or ARB medication at the time of testing (n=21) and withdrawing participants with cardiac and renal complications from blood pressure for the purpose of this study may have compromised patient safety. No differences in severity of IR were observed between the disease groups in the current study and statistically accounting for the use of these medications in the current investigations did not significantly alter the main findings. Nevertheless, the cross-

sectional nature of the current findings is limited and detailed examination of the influence of these medications on IR requires further investigation, particularly in T1D.

In addition to changes in T1D management guidelines, another issue that may be responsible for the variation in results between the current study and the findings from the previous insulin clamp studies used to validate the eGDR equation [9] may be the methodological differences between the two insulin clamp protocols. Since the use of DEXA for the computation of lean body mass (LBM) was not available for the computation of GDR, the measurements of insulin sensitivity in the original study were only adjusted for overall body weight. It is currently accepted that skeletal muscle is responsible for the vast majority of glucose disposal during clamp studies and the increasing availability of DEXA for the assessment of body composition has made adjustment for LBM a more standard practice in insulin sensitivity assessment. Since large variation exists between individuals in amount of LBM relative to overall body weight, this technical difference could greatly influence the final computation of glucose disposal. Additionally, the original clamp investigation was performed at an insulin dose of 60 mU/m<sup>2</sup>/min compared to the current dose of 40 mU/m<sup>2</sup>/min. Insulin utilization by lean body mass has been well documented to occur increasingly in a dose-responsive manner [340-342] and the insulin dose of 40 mU/m<sup>2</sup>/min was chosen for the current investigation because it is commonly used as an optimal dose for the assessment of peripheral IR[343]. Although the difference in insulin dose between the two studies may seem minimal, the potential influence of dose variation on quantity of glucose uptake can not be ignored.

One final note on the use of indirect vs. direct comparisons of IR in T1D should be made. Although the in-vivo response to insulin under controlled clinical settings is often superior in specificity and validity in T1D research, these assessments do not diminish the utility of the

indirect assessment IR for the identification of individuals at risk for the development of IR. Each measure has its strengths and limitations which should be considered in relation to the overall investigational goals. It is important that the community of diabetes research use both clinical and epidemiologic methods to examine risk factors associated with IR in order to most accurately direct future research and public health efforts.

T1D populations are at increased risk for the development of many conditions which may also be exacerbated by IR, particularly cardiovascular disease, and since evidence exists suggesting that obesity may be increasing in T1D due to improvements in improvements in diabetes therapy, the more specific exploration of adiposity distribution as a characteristic of disease risk in T1D is required. The earlier use of waist circumference and waist-hip ratio was the foundation for the belief that excess storage of abdominal fat poses more risk than general obesity, and similar findings emerged from T1D investigations [197, 300-303]. Building on these data, increasing investigations have employed more sophisticated techniques (e.g. MRI, or CT) to examine these associations from a multi-compartment perspective (i.e. fat mass vs. lean body mass) but noticeably little attention has been paid to T1D populations. Similarly, the use of DEXA to differentiate both compartmental and regional fat storage has suggesting that lower body fat mass is favorably associated with measures of insulin sensitivity [141, 267] and cardiovascular risk [21, 24, 344] but examinations of this “metabolic protection” have not looked at T1D populations. Despite extensive examination of the influence of adiposity in non-diabetic and T2D populations suggesting that regional adiposity is a more important determinant of IR than body size alone [104, 107-109, 112, 134, 135], the current findings suggest that both total adiposity (total FM, BMI) and central adiposity (trunk FM, WC) are strong predictors of IR in

T1D and that each of these measures convey a similar contribution to risk of IR and IR complications.

When exploring any research question where assessment of adiposity is required, it is important to distinguish between whether associations of interest exist regarding “relative” adiposity or “absolute” measures of body fat. Relative measures are used to determine whether a propensity to store body fat in a particular region is associated with disease states while absolute measures of fat mass assist in distinguishing whether the quantity of adipose tissue plays an important role. When this distinction is clear, the available literature and future investigations can be separated into investigations of whether total fat in a region or the preferential storage of fat in a region has metabolic influence. Whether examining total fat mass or the proportion of fat stored in the region, results from the current investigation do not support the hypothesis that lower body adiposity is favorably associated with measures of insulin sensitivity in T1D. Since absolute measures of leg, trunk, and arm fat were all negatively associated with IR, these findings propose that excess accumulation of body fat in any region of the body may be metabolically detrimental to glucose metabolism in T1D. Measures of regional adiposity were all positively associated with waist circumference, except % FM in legs which was negatively associated with WC (Table 25) and since WC had an independent negative association with insulin sensitivity, these findings add further evidence that the previously established associations between IR and abdominal obesity are also evident in T1D. Another interesting finding is that total fat mass is the DEXA adiposity assessment that was most strongly associated with IR. Since total fat mass was also highly correlated with all regional adiposity measures in this sample, it is hypothesized that the influence of general obesity on IR may overpower the influence of regional adiposity distribution in T1D. Furthermore, when compared to non-

diabetic individuals, the increased IR observed in T1D was not explained by differences in regional adiposity. These findings suggest that although general obesity influences IR in T1D, additional risk factors not directly associated with adiposity may also contribute to altered glucose metabolism in this population and require further investigation. Nevertheless, whether excess lower limb adiposity has a metabolically protective effect in other populations (e.g. T2D or obese), or whether it is merely reflective overall or central adiposity, which is likely the case in this T1D sample, requires further examination.

Since evidence exists supporting the protective role of lower body adiposity on glucose metabolism in non-diabetics [21, 24], the investigation into whether the distribution of leg fat was similar in T1D compared to non-diabetic individuals and whether these differences could account for differences in IR was important. It is first interesting to note that whole body glucose disposal was significantly lower in T1D compared to non-diabetic controls which is additional evidence for the existence of IR in T1D. Secondly, compared to non-diabetic individuals with similar levels of BMI, non-diabetic individuals had more overall adipose tissue but a similar proportion of body fat stored in the trunk when compared to individuals with T1D. Individuals with T1D also exhibited lower levels of absolute leg fat and a lower proportion of total fat stored in the legs. Despite these differences in adiposity, neither regional nor total adiposity accounted for the increased IR in individuals with T1D. As individuals with T1D have lower levels of adiposity but increased states of IR, additional factors beyond general adiposity may be responsible for these insulin resistant states in this population which require further exploration. Studies have shown that more specific measures of muscle and adipose tissue quality (e.g. intramuscular lipid content, muscle attenuation) have been associated with IR in diabetic and non-diabetic populations [5, 23, 75]. The examination of the balance between

levels of absolute fat storage and efficient utilization of intramuscular fat stores has allowed a better understanding of dysregulated glucose metabolism in non-diabetic and T2D populations, therefore, it is conceivable that these associations could exist in T1D but have yet to be examined. Since DEXA assessment of adiposity largely quantifies subcutaneous adipose tissue, the influence of deeper leg fat depots or the quality of leg fat on IR in T1D could not be explored in the current investigation.

Although it was not possible in the present investigation, a more specific exploration of the various compartments of abdominal adipose tissue may also help to explain differences in IR between T1D and non-diabetic individuals. There are data demonstrating that adipose tissue stored deep in the visceral abdominal regions is more sensitive to the breakdown and mobilization of fat compared to other fat stores [143, 144]. Previous investigations have also found elevated free fatty acid (FFA) circulation in individuals with T1D [345, 346]. If the relative systemic flux of FFAs is higher in individuals with increased accumulation of abdominal fat, as in T1D, it is conceivable that excess accumulation of abdominal fat in the visceral area may be occur in T1D by the recurring need for FFAs as an alternate energy source during recurrent hypoinsulinemic states. A propensity to store fat in visceral compartments may be a metabolically “efficient” phenotype for FFA mobilization in T1D, but this type of excess fat storage could explain the excess risk for IR and CAD in this population. Few investigations have examined more specific assessments of visceral adiposity in T1D [82, 296, 347], none of which have investigated the direct association between visceral fat and IR. Since DEXA quantification of adipose tissue does not distinguish subcutaneous from visceral fat, more specific assessments of abdominal fat distribution as it relates to substrate metabolism could not be examined in the current investigation.

Another interesting finding from the current investigation was that T1D was associated with lower levels of absolute leg fat and a lower proportion of total fat stored in the legs when compared to non-diabetic individuals with similar BMIs. If the trunk region is a preferred location for fat storage, possibly due to a need for more readily available energy substrate, then a propensity to store less fat in the legs in T1D is plausible. A greater proportion of fat storage in the legs was associated with positively associated with insulin sensitivity in the non-diabetics, supporting the hypothesis that the capacity to store excess fat in this depot may be metabolically protective. Previous reports in middle aged [146] and older adults [153] have also suggested that strength of the protective effect of lower body adiposity increases with rising levels of obesity. The protective effect of lower body adiposity may still exist in T1D, but since lower levels of overall adiposity and lower levels of gluteal-femoral fat were observed in this population, this association may not be prominent. Although examined in a small sample size (n=7), our data is very consistent with the hypothesis that lower body adiposity is positively associated with IR in obese individuals ( $BMI \geq 30 \text{ kg/m}^2$ ) (Table 24). Further exploration into these associations in overweight or obese individuals with T1D may provide more evidence for a metabolically protective effect associated with lower body adiposity.

The propensity to store body fat in the legs was associated with favorable cardiovascular profiles and exhibited a protective effect against the presence of CAD in T1D, even after controlling for general obesity. Since lower body adiposity was not independently associated with IR in T1D, and the estimation of IR did not influence the cardio-protective association with leg fat, these findings thus suggest that lower body fat could offer protection against CAD independent of IR. While the biological plausibility of such an association is not readily apparent, further exploration would appear appropriate. Since obesity, in general, is linked with

increased cardiovascular risk, it may seem likely that lower body fat would exert either neutral or deleterious effects compared to that of upper body fat. In contrast, emerging evidence suggests a protective effect of adipose tissue content of the thigh and legs on cardiovascular risk factors such as insulin sensitivity [21-23] and lipid profiles [21, 291, 292]. Although lower body adiposity has generally not been considered a correlate of traditional cardiovascular risk in T1D, the potential cardio-protective effect for a propensity to store fat away from the midsection should be explored. Not surprisingly, investigations into these associations are noticeably missing from T1D literature despite the increased cardiovascular morbidity and mortality reported in this group.

In this investigation, the proportion of fat stored in the legs and trunk were both associated with similar CAD risk factors, such as coronary calcification, AER, blood pressures, HDLc, LDLc, and triglycerides, however, these measures displayed nearly identical, yet opposite, associations with these CAD risk factors. Similar associations in trunk and leg fat have been reported in relation to CVD risk in non-diabetic and T2D populations [264, 267, 348], but these findings are the first to confirm these associations in T1D. With duplicate inverse associations between leg and trunk fat, it is conceivable that that this protective effect of leg adiposity may simply be a product of decreased adiposity stored in the abdomen. Since subcutaneous leg fat as been traditionally thought of as a relatively inert storage depot, this idea is conceivable. However, investigations using techniques to quantify sub compartments of adipose tissue within the thigh suggest that specific leg fat storage depots (e.g. intramuscular lipid content) are more metabolically active, yet detrimentally associated with insulin sensitivity [23, 349-353]. Exploration of distinct leg fat compartments in T1D and their independent

associations with cardiovascular risk and IR may better elucidate the metabolic mechanisms behind these the current findings.

Of particular note in these data is the stronger associations between regional adiposity and both IR and cardiovascular disease observed in women compared with men. Stronger negative associations between absolute fat and IR were observed in females, along with a stronger associations between leg and trunk fat with presence of CAD. Differences in adiposity distribution between the genders have been well described using DEXA and other anthropomorphic techniques and the majority of these investigations provide evidence that women have less relative lean mass and more total and peripheral fat storage, particularly in the hips and legs [268-271]. If the protective associations with lower body fat indeed intensify with increasing levels of obesity, and if women also have higher overall fat compared to men which is likely to be reflected in regional adiposity stores, it is logical that these associations would appear more readily in females. Along with having increased adiposity, women have traditionally been considered to be at a much lower risk of coronary disease mortality than men. Previous reports examining the differences in the incidence of CAD in women and men of similar age suggest that endogenous sex hormones such as estrogen, progesterone, and androgens significantly influence vasculature and may be responsible for the reductions in CAD risk in females [354-356]. Despite this feminine advantage in the general population, it is widely believed that diabetes states "erases" this female advantage through the prevention of the protective effects of female hormones [357, 358]. Although the mechanisms by which diabetes inhibits these cardiovascular protective effects associated with the female sex are not well understood, the identification of protective features in women (i.e. greater lower body adiposity) may be used to better identify individuals who may be at an even greater risk for the

development of CAD. Since the majority of investigations examining gender differences in adiposity and CAD risk focus solely on abdominal adiposity distribution, more extensive exploration into how lower body adiposity may influence the development of CAD independent of abdominal adiposity is warranted, particularly in T1D. While IR did not influence this protective association of lower body adiposity in females in the current investigation, the examination of risk factors jointly associated with IR and CAD may offer insight into these associations.

A final interesting finding related to body composition and CAD was the strong protective effect of height on the presence of CAD in T1D. Even after adjusting for traditional CVD risk factors, gender, and both total and regional adiposity, these analyses suggest that being of shorter stature was associated with an increased risk for the presence of CAD in T1D. These findings are supported by a number of similar reports in a variety of populations [359, 360], but to our knowledge, this is the first report this relationship between height and presence of CAD in individuals with long-standing T1D. In insulin-dependent populations, positive associations between final adult height and age of diabetes onset have been reported suggesting that timing of conventional therapy of diabetic children during the natural growth cycle is associated with impairment of physical growth [361-363]. Inverse associations between stature and severity of eye, kidney, and joint complications have also been reported in insulin-dependent populations [364]. Since the positive association between severity of CAD and diabetes duration has been well-established for decades [365, 366], and if children who develop T1D at an earlier age have a shorter final height, then it would be conceivable that shorter individuals are more likely to develop diabetes complications (i.e. CAD) as a result of a longer duration of diabetes.

## 10.1 STRENGTHS

Since T1D is a disease population that has received a disproportionately low amount of research attention compared to resources expended for T2D investigations, any efforts to improve the quality of life in this high risk populations is an asset to the diabetes research community. This investigation is one of few that have examined IR in a T1D population with long-standing disease and the only study that has examined IR in a sample with prevalent cardiovascular disease and renal disease. Although the use of direct assessment techniques of IR limited the sample size for this measure, data from the insulin clamp procedure are widely held as the superior data information. A relatively large sample size (n=163) was used to examine the association between regional adiposity and CAD; a noteworthy sample for T1D investigations. Since clinical measures from the regular EDC exam were also available for inclusion in the current analyses, a wide range of diabetes-related assessments were examined as they were associated the outcomes of interest in this investigation. This study is one of the few investigations that have used DEXA-assessed body composition assessments to examine adiposity distribution in T1D. Together with data collected from both the insulin clamp and the eGDR formula, this study has allowed a through exploration of the associations between IR and regional adiposity in this cohort. Another main strength of this investigation is a direct comparison of how adiposity storage in T1D may differ compared to non-diabetic individuals. Since comparable, high-quality assessments of insulin sensitivity and regional adiposity were also available in a non-diabetic control sample, this allowed the investigation to add support for the existence of a metabolically protective effect of gluteal-femoral fat storage but that this trend

may not be a feature of T1D. One of the more prominent strengths of this investigation was the use of data collected from the EDC cohort, which is a study population that is both well-characterized and well-documented with regards to diabetes complications. The cardiovascular disease and renal disease status of all EDC participants in the study is thoroughly documented via physician examination, urinalyses, and extensive record confirmation which allowed confident comparison between individuals with and without CAD and renal complications.

## 10.2 LIMITATIONS

The use of the insulin clamp technique for the assessment of IR, although the gold-standard assessment of IR in T1D, resulted in a limited sample size of 30 men and women, which did not allow for proper stratified analyses. The sex-differences in regional adiposity associated and the association with disease outcomes could therefore not be examined properly. These limited sample sizes also did not allow examination of the pervasive use of ACE/ARB medication and its potential affect on IR since a reasonable sample of T1D individuals not taking those medications was not available. Comparisons of risk factors associated with IR in T1D between individuals taking and not taking ACE/ARB medication is necessary and may allow identification of modifiable risk factors that more clearly reflect current treatment standards in this population. Also, the cross-sectional nature of the current analyses does not allow for the establishment of a temporal relationship between IR, regional adiposity, and the development of diabetes complications and therefore these relationships must be assessed in a prospective fashion to evaluate true causality.

Despite the investigation of demographic and anthropometric risk factors associated with IR, a characteristic that was not accounted for in this investigation was level of physical activity. Since many participants in this sample had moderate or severe CAD (n=14), strenuous exercise testing would have caused undue health risk. Previous investigations have demonstrated a relationship between exercise capacity and IR [277, 278], and the lack of control for this lifestyle

factor limits the investigational results. The examination of physical activity levels and its influence on adiposity and IR in T1D is an area requiring further exploration.

Data has been collected from the EDC cohort for over 20 years, and though this lengthy follow-up is a true strength of the overall investigation, there is also an inherent limitation to this strength in that remaining participants become a “survivor cohort”. Those individuals recruited for the insulin clamp studies were screened for healthy vasculature and eligibility to safely undertake an insulin infusion and extensive blood draws. Consequently, the recruited sample resulted in a relatively healthy sample of individuals with T1D complications, and therefore, these caution should be taken when generalizing these results to individuals with more advanced T1D complications.

Although DEXA is quickly becoming the gold-standard for the assessment regional adiposity, there are limitations in its ability to distinguish between various fat depots. Due to the high concentration of visceral organs in the trunk region, composition assessment in this area involves substantial prediction rather than measurement. As a result, soft tissue estimation in the trunk area is less accurate than in the limbs. Adipose tissue quantification with this technique is also limited to subcutaneous depots, thus intramuscular fat can not be quantified. DEXA results have also been reported to vary according to body shape and outcome, and little information is available on any limitations in adiposity assessment in T1D cohorts. DEXA may provide useful information on relative fat and lean masses as a single measurement in an individual, particularly with respect to limb lean mass, however, such assessments need the development of normal reference data in order to accurately assess disease populations.

Finally, all participants in the current investigation are Caucasian, thus our results are limited in generalizability to T1D in other races and ethnicities. Although the prevalence of T1D

was low among African Americans relative to Caucasian Americans in the diagnosis years of EDC eligibility, recent reports suggest that an increase in incidence of T1D in the black population may be occurring [367]. The current study sample does not allow examination of the extent to which race may influence the observed associations between IR, regional adiposity, or diabetes complications thus similar investigations are needed in non-white T1D populations. The role of obesity as it may influence IR and diabetes complications is a relatively new area of investigation in the area of T1D, and an under-representation of racial/ethnic groups exists in research area. Since risk factors associated with T2D have been more closely examined in non-white populations, caution should be taken not to over-generalize the findings to T1D populations.

### **10.3 PUBLIC HEALTH AND CLINICAL IMPLICATIONS**

The combined use of the insulin clamp technique and DEXA has never before been used to explore regional adiposity distribution, cardiovascular disease, and IR in T1D; therefore the utility of the current findings is exploratory in nature. Considering the complicated relationship between obesity and cardio-metabolic risk, little is known concerning the modification of insulin resistant states in T1D. Although previous findings in other populations have provided evidence that lower body adiposity may exert a protective effect on IR and IR risk factors, these findings do not support this hypothesis in T1D, but rather that all measures of regional and general obesity are detrimentally associated with IR. Since extensive evidence is available which links IR to the development of diabetes complications, these findings further suggest that excess weight gain in T1D should be avoided to prevent the exacerbation of IR states which may accelerate the development of T1D complications..

While adiposity may have a negative influence on IR in T1D, these data also suggest that the propensity to store adiposity in the legs may be associated with a lower risk for the presence of CAD. Additional findings that leg and trunk adipose tissue storage have independent and opposing effects on CAD risk factors supports the need for further investigation into the biological plausibility of the protective role lower body adiposity in T1D. Regardless of how excess fat is stored in the body, the public health message should remain aimed at the reduction of prevalent obesity and the prevention of weight gain due to excess adiposity, even in T1D. These investigations into the positive benefits of adiposity raise the possibility that an optimal phenotype may exist that is associated with reduced IR and improved cardiovascular risk profile.

If such a phenotype exists, the identification of deviations from this profile may improve the characterization of cardiovascular risk in T1D and other populations, and improve the identification of individuals at high risk for metabolic abnormalities.

Since limited data is available concerning anthropomorphic trends in T1D, a larger collection of information in this area is needed and such assessments should be used for the development of normal reference data in order to accurately assess disease risk in this population. WC was highly correlated with all DEXA adiposity measures in these analyses, particularly with trunk FM, and the both abdominal adiposity measures have been associated with similar independent associations with IR and cardiovascular risk. Since WC has similar associations with cardio-metabolic risk when compared to DEXA-assessed trunk FM, this more simplistic and clinically available measurement, which is able to be easily measured and interpreted in both genders, may therefore be a more clinically relevant measure in identifying risk for IR and cardiovascular disease in long standing T1D. Cross-sectionally, the current DEXA-assessed regional adiposity measures do not assist in the estimation of IR in T1D, however, an independent protective association was observed between leg fat and presence of CAD in this population. Thus, additional prospective analyses are required to more extensively examine the association between regional adiposity and the risk for development of CAD in T1D.

Finally, uncertainty still exists as to the best adiposity measure to use when assessing metabolic risk in T1D. DEXA is fast becoming the preferred technique for the assessment of body fat distribution, but the higher degree of precision afforded by this measurement is also accompanied by increased labor and costs. Although there is clinical utility in using DEXA to quantify regional adiposity, the current investigation suggests that this is not needed for IR estimation in T1D. More general estimates of general obesity (BMI) and abdominal adiposity

(WC) were found to have strong associations with IR and these findings support the continued use of these simple and inexpensive measurements to assess risk for IR in T1D.

## **APPENDIX**

### **SUPPLEMENTAL TABLES AND FIGURES**

**Table 21. Pearson correlation coefficients between glucose disposal rates (GDR) in type 1 diabetes**

	GDR mg·min <sup>-1</sup> ·kg <sup>-1</sup>	GDR mg·min <sup>-1</sup> ·kgLBM <sup>-1</sup>	GDR (mg·min <sup>-1</sup> ·kgLBM <sup>-1</sup> ·uUmL <sup>-1</sup> )	eGDR mg·min <sup>-1</sup> ·kg <sup>-1</sup>
GDR mg·min <sup>-1</sup> ·kg <sup>-1</sup>	--	.88**	.74	-.02
GDR mg·min <sup>-1</sup> ·kgLBM <sup>-1</sup>	.88**	--	.73	-.14
GDR (mg·min <sup>-1</sup> ·kgLBM <sup>-1</sup> ·uUmL <sup>-1</sup> )	.74**	.73	--	-.04
eGDR mg·min <sup>-1</sup> ·kg <sup>-1</sup>	-.02	-.14	-.04	--

\**P* < 0.05, \*\**P* < 0.01.

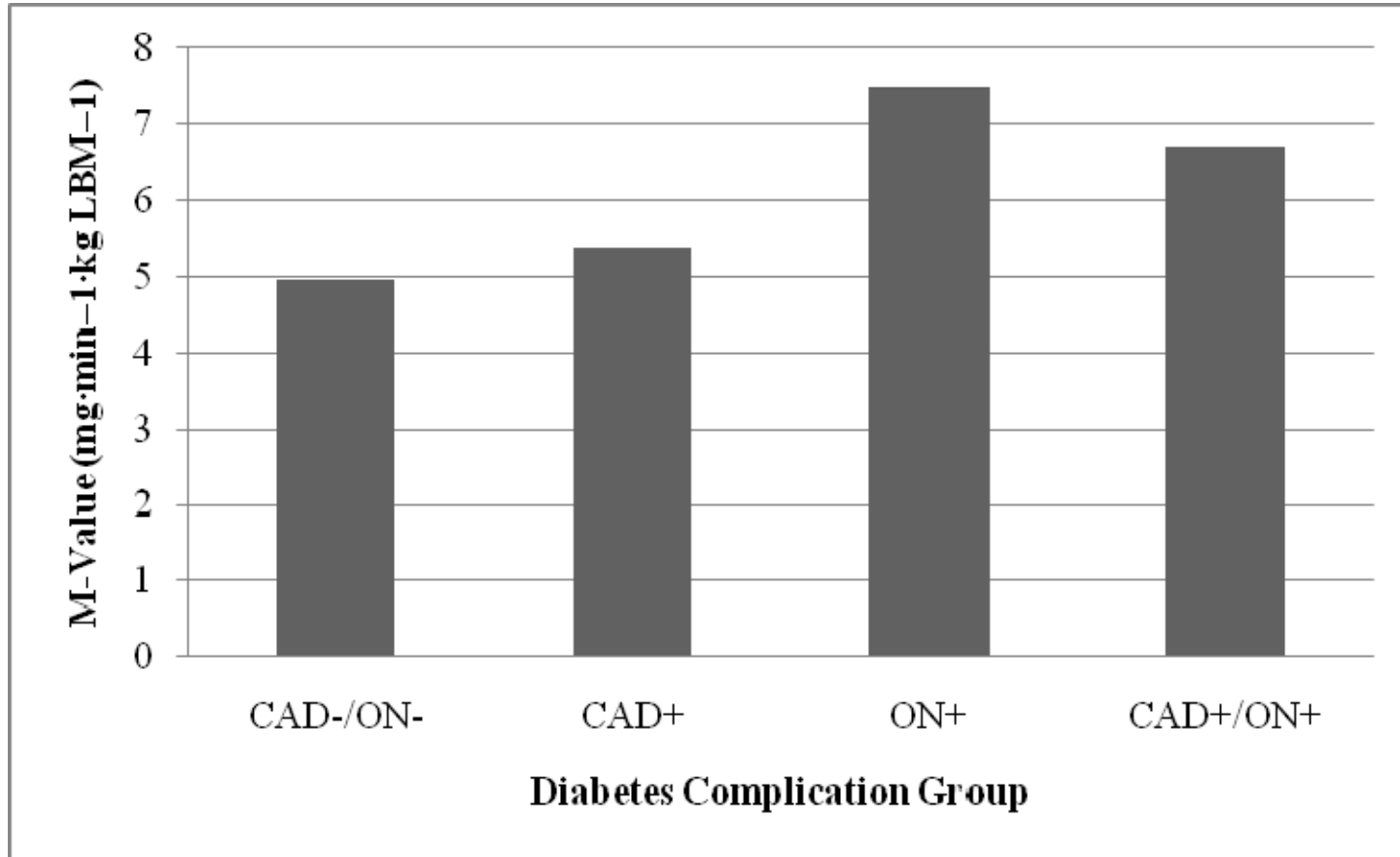


Figure 1. Insulin sensitivity by diabetes complication group in type 1 diabetes: M-value

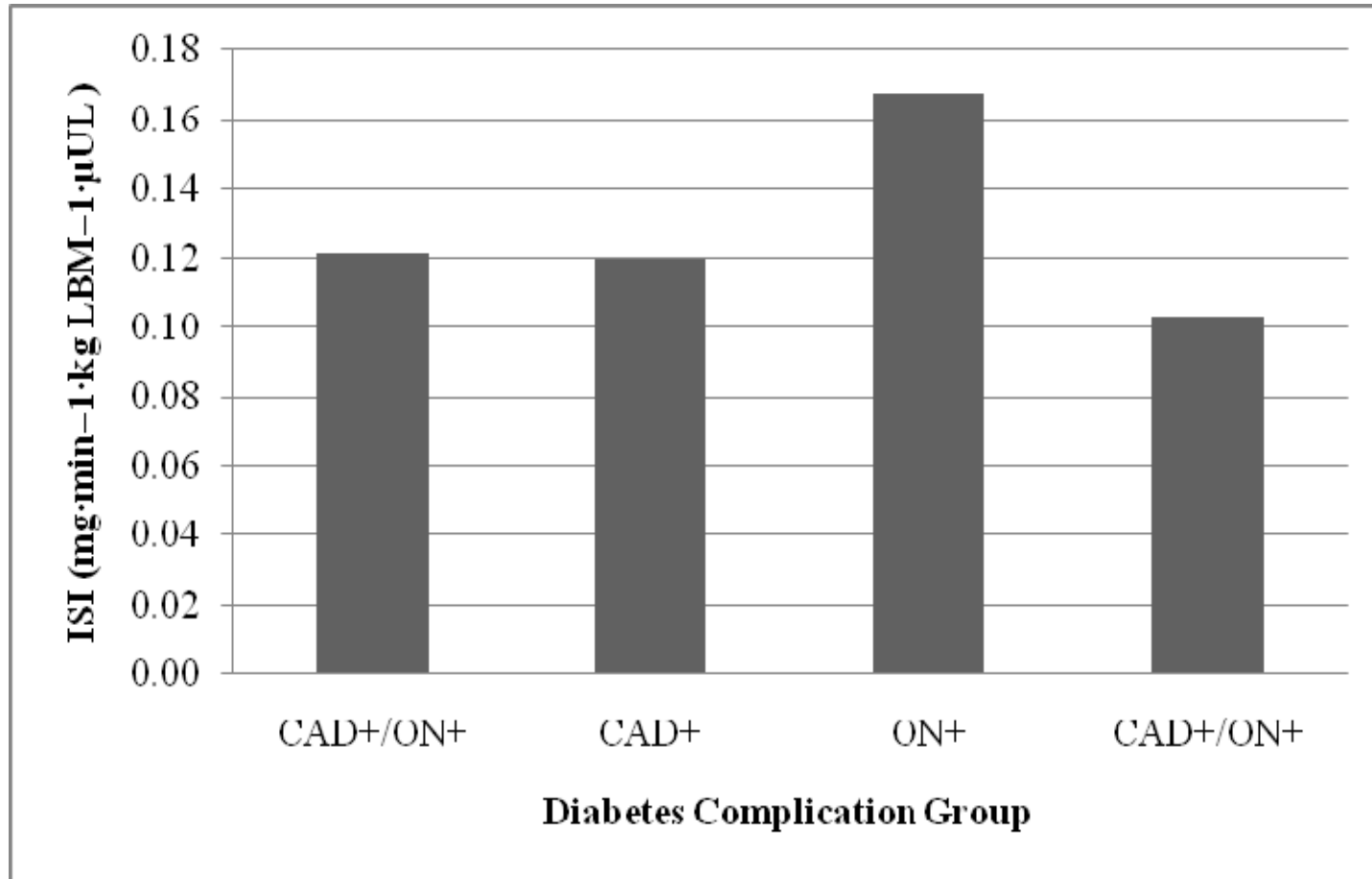


Figure 2. Insulin sensitivity by diabetes complication group in type 1 diabetes: Insulin sensitivity index

**Table 22. Multiple linear regression model for dependent variable insulin sensitivity (M-value) ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}$ ) in type 1 diabetes; DEXA-assessed regional adiposity regression coefficients ( $\pm\text{SE}$ )**

	Model 1	Model 2	Model 3	Model 4	Model 5
Diabetes Duration	$0.10 \pm 0.04^*$	$0.12 \pm 0.04^*$	$0.10 \pm 0.04^*$	$0.10 \pm 0.04^*$	$.12 \pm .04^*$
Overt Nephropathy	$2.34 \pm 0.60^{**}$	$2.58 \pm 0.61^{**}$	$2.43 \pm 0.64^{**}$	$2.24 \pm 0.65^{**}$	$2.60 \pm .68^{**}$
Insulin Dose (u/kg)	$-4.50 \pm 1.21^{**}$	$-4.01 \pm 1.24^{**}$	$-4.29 \pm 1.29^{**}$	$-4.72 \pm 1.32^{**}$	$-3.95 \pm 1.41^*$
Sex	$-1.38 \pm .82$	$-1.55 \pm .86$	$-1.35 \pm .84$	$-1.54 \pm .91$	$-1.57 \pm .95$
Height (cm)	$.04 \pm .04$	$.06 \pm .04$	$.04 \pm .04$	$.04 \pm .04$	$.06 \pm .04$
FM (kg)	$-.10 \pm .04^*$	$-.10 \pm .04^*$	$-.11 \pm .04^*$	$-.11 \pm .05^*$	$-.09 \pm .08$
% FM in Leg		$-.02 \pm 0.05$			$-.05 \pm .28$
% FM in Arm			$-.08 \pm 0.15$		$-.02 \pm .26$
% FM in Trunk				$.03 \pm 0.06$	$-.04 \pm .30$
$R^2$	0.74	0.77	0.74	0.74	.77

\* $P < 0.05$ , \*\* $P < 0.01$ .

Sex: Negative value indicates lower GDR in men

**Table 23. Multiple linear regression model for dependent variable insulin sensitivity**

**(M-value) ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}$ ) in type 1 diabetes; regional adiposity regression coefficients ( $\pm\text{SE}$ )**

	Model 1	Model 2	Model 3
Diabetes Duration	$0.10 \pm 0.04^{**}$	$0.08 \pm 0.04^*$	$0.10 \pm 0.04^*$
Overt Nephropathy	$2.47 \pm 0.56^{**}$	$2.27 \pm 0.61^{**}$	$2.34 \pm 0.55^{**}$
Insulin Dose (u/kg)	$-3.90 \pm 1.18^{**}$	$-3.78 \pm 1.41^*$	$-3.22 \pm 1.28^*$
Sex	$-.26 \pm .49$	$.05 \pm .55$	$-.59 \pm .55$
BMI ( $\text{kg}/\text{m}^2$ )	$-.205 \pm .07^{**}$		$-.26 \pm .08^{**}$
Waist Circumference (cm)		$-.07 \pm .03^*$	
Residuals of Waist Circumference			$.14 \pm .30$
$R^2$	0.74	0.68	0.76

\* $P < 0.05$ , \*\* $P < 0.01$ .

Sex: Negative value indicates lower GDR in men

**Table 24. Multiple linear regression models for dependent variable insulin sensitivity (M-value) ( $\text{mg}\cdot\text{min}^{-1}\cdot\text{kgLBM}^{-1}$ ) in type 1 diabetes by obesity status; lower body adiposity regression coefficients ( $\pm\text{SE}$ )**

	BMI < 30.0 $\text{kg}/\text{m}^2$		BMI $\geq$ 30.0 $\text{kg}/\text{m}^2$		
	$\beta\pm\text{SE}$	p		$\beta\pm\text{SE}$	p
Age	0.07 $\pm$ 0.06	.31	Age	0.06 $\pm$ 0.06	.35
Sex*	-3.83 $\pm$ 2.09	.09	Sex*	-3.56 $\pm$ 1.98	.09
Height (cm)	0.06 $\pm$ 0.08	.43	Height (cm)	0.06 $\pm$ 0.08	.48
FM (kg)	-0.22 $\pm$ 0.09	.03	FM (kg)	-0.24 $\pm$ 0.09	.02
% FM in Legs	-17.57 $\pm$ 8.65	.06	% FM in Legs	20.75 $\pm$ 9.22	.04
$R^2$	.33		$R^2$	.36	

\* Negative coefficient denotes lower insulin sensitivity in males

**Table 25. Pearson correlation coefficients for measures of total and regional adiposity in type 1 diabetes (N=29)**

	BMI (kg/m <sup>2</sup> )	WC (cm)	FM (kg)	FM (%)	Leg fat (kg)	Arm fat (kg)	Trunk fat (kg)	FM in Legs (%)	FM in Arms (%)	FM in Trunk (%)
BMI (kg/m <sup>2</sup> )	--	.83**	.88**	.67**	.61**	.82**	.90**	-.49*	.23	.66**
WC (cm)	.83**	--	.74**	.38*	.34	.61**	.86**	-.67	.06	.83**
FM (kg)	.88**	.74**	--	.89**	.85**	.95**	.96**	-.29	.34	.51*
FM (%)	.67**	.38*	.89**	--	.90	.89**	.77**	.01	.40*	.19
Leg fat (kg)	.61**	.34	.85**	.90**	--	.81**	.66**	.23	.33	-.03
Arm fat (kg)	.82**	.61**	.95**	.89**	.81**	--	.89**	-.31	.59	.44
Trunk fat (kg)	.90**	.86**	.96**	.77**	.66**	.89**	--	-.53*	.26	.72**
FM in Legs (%)	-.49*	-.67	-.29	.01	.23	-.31	-.53*	--	.26	-.95
FM in Arms (%)	.23	.06	.34	.40*	.33	.59	.26	-.23	--	.05
FM in Trunk (%)	.66**	.83**	.51*	.19	-.03	.44	.72**	-.95	.05	--

\* $P < 0.05$ , \*\* $P < 0.001$ .

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