ADIPOSITY IN TYPE 1 DIABETES

by

Baqiyyah Nilija Conway

BS, Weimar College, 1992

BS, Andrews University, 1999

MA, University of Birmingham, 2000

MPH, University of North Texas Health Science Center at Fort Worth, 2004

Submitted to the Graduate Faculty of

the Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2008

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

This dissertation was presented

by

Baqiyyah Nilija Conway

It was defended on

October 31, 2008

and approved by

Rhobert Evans, PhD, Associate Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Linda Fried, MD MPH, Associate Professor, Department of Medicine
School of Medicine, University of Pittsburgh

Sheryl Kelsey, PhD, Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Dissertation Advisor

Trevor Orchrd, MBBCh M.Med.Sci, Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Trevor J Orchard, MBBCh Med.Sci

ADIPOSITY IN TYPE 1 DIABETES

Baqiyyah N Conway, PhD

University of Pittsburgh, 2008

Background: Increases in the prevalence of overweight and obesity states, and in their associated adverse health outcomes, have been well described in the general population. However, in type 1 diabetes (T1D), a disease traditionally characterized by a lean phenotype, time trends in overweight and obesity and the role of adiposity on complications in TID have not been well investigated. We therefore investigated time trends in overweight and obesity and the association of adiposity with mortality and coronary artery calcification (CAC), a subclinical marker of coronary artery disease in the Pittsburgh Epidemiology of Diabetes Complications cohort of childhood onset T1D.

Methods: Participants were first seen in 1986-1988 and followed biennially thereafter. Mortality was censored at January 1, 2007. Body mass index (BMI) was defined as kg/m² and Waist circumference (WC) was measured. CAC, visceral adiposity (VAT) and subcutaneous adiposity (SAT) by electron beam tomography. Free fatty acids (FFA) were determined by in vitro colorimetry.

Results: After 18 years of follow-up, the prevalence of overweight increased by 47%; the prevalence of obesity increased 7-fold. BMI demonstrated a quadratic relationship with mortality. Adjustment for waist circumference eliminated the increased risk in the obese. Weight gain was positively related to intensive insulin therapy and inversely with mortality.

There was a positive relationship between the presence of CAC and adiposity measures, however the degree of CAC was not associated with any adiposity measure, except negatively with SAT in women. Finally, FFA were not associated with any adiposity measure and showed no association with CAC.

Conclusion: Adiposity is increasing in T1D and shows a complex association with coronary artery disease and mortality. These results have great public health significance by suggesting that avoidance of overweight, per se, in type 1 diabetes should not be a major priority; rather attention should focus on maximizing glucose control even though it may result in weight gain.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

It was about a year ago that I was sitting in Dr. Orchard's office going over descriptive statistics of Epidemiology of Diabetes Complications (EDC) Study participants whom we had collected data on skin fluorescence. Although generally in epidemiology the descriptive data we look at are means and standardized deviations about the mean, it was a small sample so we were looking at the each characteristics of each participant, such as age, years of type diabetes, number of diabetes complications, complications at baseline and number of complications at baseline, and whether the participant was free of any long term diabetes complications. Looking at this population that by definition had had diabetes since childhood, a couple of the participants jumped out at him. In a moment of quiet awe and surprise he said, "We have participants with 60 years of diabetes duration! We have someone in this population pushing 70 years of age!" It was then that I realized that he was far more intricately wrapped up in this population than I had imagined. This had been more than a career for him. It had been a life's work. Although this was still a middle aged population and the average age of the deceased at death was still in the early to mid-forties, so was the average age of those still alive, representing more than 75% of the original cohort. These few participants, now senior citizens and one approaching old age, although extremes in the population, were tell tale signs of things to come. We were standing on the brink of witnessing the extension of life in an entire population to a full lifespan. [And he

had been a primary mover behind it.] It was then that I understood the value of the EDC study. Although its focus was neither type 1 diabetes etiology nor cure, in a different way it was seeking a similar end: the normalization of a population and a full lifespan. And as reflected in this dissertation, in a single generation, despite the wasting nature of the disease, we have witnessed a characteristically lean/thin population achieve weight gain to the point that the prevalence of overweight and obesity are no different from the general population with an effect on mortality similar to that in the general population when an protective effect would have been expected. Sitting in his office that day looking at those extremes in the population, we really did appear to be witnessing the normalization of a population. What more can one ask for out of one's life's work. The physician and the researcher had been one. To Dr. Orchard I would like to say thank you for your work for people with type 1 diabetes, thank you for the training you have given me, and thank you for your extreme patience with my personality.

I would also like to thank the participants of the EDC study for their participation in this research for the past 20 years. Despite the suffering and morbidity imposed by this disease words on paper can not do justice to, and even the cynicism that some of them have expressed that there will ever be a cure, they continue to participate in hopes that they are making life better for future children. This characteristic has amazed me. Like Dr. Orchard, they too have an intricate relationship with the study. It really is a relationship.

I would also like to thank my other committee members. Thank you to Dr. Evans, who had a very quiet way of strongly motivating and whose classes were not only eclectic, but were among the most interesting I have taken at this university. How he managed to do that with biochemistry classes speaks highly of him. Thanks also for training me in the assaying of free fatty acids.

Thank you to Dr. Fried, who although by far one of the most intelligent people I have known somehow managed not to intimidate or hurt my shallow doctoral self-esteem. Somehow I always knew her criticisms were about my work, not me. She was also very helpful.

Thank you to Dr. Kelsey, who strengthened my skills base in statistical analyses. Thanks to her I can now do time-varying, in addition to time-dependent, survival analyses, generalized linear modeling, repeated measures analyses, and intraclass correlations on relatively large populations. Good job market skills. I still have a few books she 'lent' me. But you know the saying, "Ever lend send somebody a book and got it back?"

Thank you to my parents for whom at this moment this PhD means more to than me.

Thank you to my stepfather John, who did not survive the fight with this disease, but who did motivate me to achieve high academically. He helped me more than I could help him.

Finally, thank you to Glenn, whose friendship has been a real source of support during my doctoral experience and who helped guide me in this research, and the meaning behind the research, in only ways that he could. And for other reasons you are my hero.

1.0 INTRODUCTION

While the increase in overweight and obesity and associated adverse outcomes have been well documented in the general population, time trends in populations with pre-existing disease, such as type 1 diabetes (T1D), have not been as thoroughly investigated. The relevance of this is emphasized by studies showing that within populations with pre-existing disease, obesity associations are often opposite of that seen in the general population, a phenomenon termed the obesity paradox (1). In T1D, further complexity is added in that the primary mediator of energy absorption, the anabolic hormone insulin, is either severely deficient or absent and must be exogenously administered. Without this hormone, T1D results in a progressive wasting to death. With initiation of insulin therapy, there is a relative normalization of weight. With intensification of insulin therapy there is (often) further weight gain and a reduction in, or delayed progression to, long term complications of the disease.

Given the widespread adaptation of intensive insulin therapy in the mid 1990s after the release of the results from the Diabetes Control and Complications Trial (DCCT) showing a reduction in microvascular complications (2, 3), investigating the association of weight gain, overweight, and obesity with complications of T1D is critical. This is particularly so since intensive insulin therapy in the DCCT was also associated with excessive weight gain (4, 5), and the association of tightened glycemic control with coronary artery disease, the leading cause of death in type 1 diabetes, continues to yield conflicting results (6-12).

This dissertation focuses on adiposity, that is, the excess of adipose tissue, or the degree of "fatness", in individuals with type T1D. The two main components that will be the focus of this dissertation are overall adiposity (as well as trends over time) and its distribution. The impact of both of these factors on heart disease and mortality in T1D will be examined.

2.0 BACKGROUND

"Some people are born to fatness. Others have to get there." –Les Murray

2.1 TIME TRENDS

Obesity is the result of a chronic positive energy imbalance. Marti el al (13)define obesity as 'a pathological condition accompanied by an excessive fat deposition as compared to expected values for a given stature, sex, and age.' It is an excess accumulation of body fat, operationally defined as a body mass index (BMI) greater than or equal to thirty.

Until the 1960s, obesity was relative rare. It increased by 10% between 1961 and 1978 (14). Since 1984, the prevalence of obesity in the United States has doubled (14). Today nearly one-third of all Americans are obese (15). This epidemic rise in overweight and obesity is not limited to the United States, nor to developed countries. Developing countries are beginning to see a marked rise in obesity, with countries such as Samoa having an obesity prevalence of greater than 50% (16). Over forty% of Kuwait and French Polynesian women are obese (16).

Worldwide, approximately 1 in 6 people are overweight (17). As of 2002, 65% of the U.S. adult population was either overweight or obese and 30% were clinically obese, compared to 47 and 15%, respectively, in 1980 (15). In the United States, obesity accounts for approximately 7% of the health care budget (18), with an economic impact greater than 100 billion dollars annually (19).

Theories for the rising prevalence of overweight and obesity are numerous, including both extrinsic and intrinsic factors. Extrinsic factors include technology, transportation, change in the energy demands of employment, change in the family structure, decreased cost and increased availability of food, a change in the energy-density (14, 20, 21), and a change in the biochemical make-up of food, i.e. the switch from glucose to fructose, and its different metabolic routing, as the primary added sweetener in food (21-25).

Putative intrinsic risk factors may evolve around the "thrifty-gene" hypothesis. The thrifty-gene hypothesis postulates that the relative ability to gain weight and store fat is a result of selective survival (26-28). Until recently, mankind was plagued with periodic famines (26). Limited food availability was a survival pressure, and those who were able to more efficiently store excess energy were preferentially able to survive. As a result, man evolved the ability to more efficiently store fuel for times of energy deprivation (26). As Per Bjorntorp (26) points out, "adipose tissue development is a necessary survival characteristic of species which do not have constant access to food." The reason for this selected survival may not have been limited to periods of famine, but to plague and illness as well. Those with greater energy reserves may have been better able to handle the illness-induced starvation state of disease. They may also have had greater immune responses, i.e. increased secretion of inflammatory proteins, to disease. Body fat gain may have been an adaptive mechanism for survival (28), not only against famine,

but also illness. Weighed against the more imminent survival, the effects of long-term exposure to obesity, e.g. dyslipidemia, hypertension, and insulin resistance, was relatively unimportant (28). The comorbidities of obesity are generally diseases of longevity, a relatively recent phenomenon.

The present environment of abundant, easily accessible food, and reduced energy demands in the face of genes selectively survived to efficiently store energy may be the etiologic cause of the rapid rise in overweight and obesity over the last few decades. This rising prevalence of overweight and obesity seems to be due to a maladaptation of man to his changing environment (27).

2.2 COMPLICATIONS OF OBESITY

Evidence of this maladaptation can be observed from the morbidity and mortality associated with increased weight and fat mass. A study reported in a 1999 issue of JAMA (29) found that obesity was responsible for approximately 300,000 deaths annually, deaths largely due to the comorbidites of obesity, i.e. disease for which obesity is a major risk factor. While this assertion has been recently challenged (30), by putting the number at approximately 112,000 excess deaths, the impact of obesity on mortality is still substantial.

The evidence implicating a role of overweight and obesity in chronic diseases such as cardiovascular disease and T2D is strong. A study by Rimm et al (31) found that BMI, waist-to-hip ratio, and weight gain in adulthood were associated with increased risk of coronary heart disease (CHD). Overweight middle-aged men (BMI 25.0-28.9) had a 75% greater risk of

developing CHD than men with BMIs below 23. Men with BMIs >33 were three-and-a-half times more likely to develop CHD as the lean men (BMI<23). A meta-analysis of 11 studies found a 2.71 and 2.80 relative risk for coronary heart disease in obese women and men, respectively (32), and found that a weight gain in adulthood of 15 kg was associated with a 46% increased risk of a coronary event in men and an 83% increased risk in women. Cardiovascular disease is the leading cause of death in the United States (33), accounting for approximately 700,000 deaths (15) in 2002.

Along with the rise in the prevalence of overweight and obesity in the past few decades, there has also been a similar rise the prevalence of diabetes, particularly T2D, behind which obesity seems to be the driving force (34, 35). The relationship between obesity and diabetes is so strong that it has often been termed diabesity (36, 37). Although most obese individuals do not develop diabetes, 90 percent of type 2 diabetics are obese (38). Diabetes is especially high among the morbidly obese, with as many as 30% having diabetes (39). Even in far lesser degrees of obesity, a substantial dose-response relationship is evident. For example, the Nurses health study found that those with a BMI of 30 were 35 times more likely to develop T2D than those with a BMI of 21 (40). The Male Health Professionals Study found similar results, with men with a BMI ≥ 35 (compared to <22) forty-two times more likely to develop T2D (41). There is an approximate 25% increased relative risk in diabetes for every unit rise in BMI above 22 (39). The following table (Table 2-1) lists studies showing relationships between adiposity and diabetes risk.

Table 2-1. Studies on Diabetes and Obesity.

Investigator	Population	Outcome
Colditz et al, 1995 (40)	The Nurses' Health Study N=114, 824	Diabetes risk in women significantly associated with both BMI and weight gain in adulthood independent of BMI. RR=5 BMI 24.0 to 24.9 compared to BMI<22.
Chan et al, 1994(41)	Male health professionals n=51,529	Obesity associated with increased diabetes risk. BMI ≥ 35 compared to <22, RR=42.1 Weight gain during adulthood associated with increased risk
Ohlson et al, 1985 (42)	Swedish men n=558	BMI and WHR independent of BMI positively significantly associated with diabetes risk
West et al, 1971 (43)	10 different populations around the world	Prevalence of diabetes strongly associated with prevalence of obesity
Resnick et al, 2000 (44)	Cohort of overweight nondiabetic men n=1929	Diabetes incidence increased by 26.2% for a BMI≥37. For each kilogram weight gain per year, diabetes RR=1.49. For each kilogram weight loss, there was a 33% decreased risk of NIDDM.
Boykyo et al, 2000(45) (36)	Japanese American cohort	Visceral obesity associated with later development of diabetes
Kern et al, 1997(46)	In vitro study	Impaired insulin signaling by TNF-α
Boden et al, 1999 (47)	Diabetic and nondiabetic subjects	Infusion with lipid and heparin resulted in reduction in glucose uptake into peripheral tissues

The pathophysiology of increased adiposity begins with an excess in fat mass on organs, particularly visceral organs, an alteration in adipokine and inflammatory peptide secretion, an increase in free fatty acid (FFA) production, and a modification in hemodynamics and lipid metabolism (48). Hypertension and dyslipidemia are a common part of the sequelea of obesity. Elevated levels of triglycerides, small low dense lipoprotein (LDL), and apoliprotein B, but depressed levels of high dense lipoprotein, are seen in obesity, putting the obese at a greatly increased risk of cardiovascular disease (CVD), the leading cause of death in the United States. In fact, obesity is the second leading modifiable risk factor in CVD (49, 50). Obesity is also associated with an increased risk of respiratory diseases, some cancers, T2D, other major chronic diseases, and mortality (30, 50, 51). Table 2-2 lists studies showing the impact of adiposity on various chronic diseases.

Table 2-2. Obesity's Association with Common Chronic Diseases.

Investigator	Population	Outcome
Kenchiah et al, 2002 (51)	Framingham Heart Study N=5881	Obesity associated with increased heart failure risk. HR=2.12 for men and 1.90 for women. Increase in BMI category and heart failure HR=1.46 for women and 1.37 for men
Anderson and Konz, 2001 (32)	Meta-analysis	CAD RR=2.71 and 2.80 in obese men and women, respectively
Zhou et al, 2002 (52)	10 cohorts in China, n=9213	BMI an independent risk factor for coronary heart disease (CHD) and stroke, CHD RR=1.23 and stroke RR=1.09 for each 2-unit increase in BMI Association found at lower BMI levels than in Western countries
Rimm et al, 1995 (31)	The Health Professionals Follow-up Study n=29,122	Increasing RR of CHD with increasing BMI in men. Men aged 40-64, BMI ≥33 RR=3.44 compared to BMI< 23. Men aged ≥ 65, WHR a strong predictor of CHD
Manson et al, 1990 (53)	The Nurses' Health Study n=115,886	Adjusting for smoking, BMI positively related to CHD starting at BMI ≥21. BMI≥29 three –fold increase in CHD. 70% of CHD in the group (BMI≥29) attributed to adiposity
Rexrode et al, 1997 (54)	The Nurses' Health Study n=116,759	RR for ischemic stroke increased with increase in BMI category, starting at BMI ≥27 compared to BMI <21
Tishler et al, 2003 (55)	The Cleveland Family Study n=286	Increasing BMI significantly associated with sleep-disordered breathing. Increased apnea hypopnea index OR=1.14 for each unit increase in BMI
Baik et al, 2000 (56)	Male and female health professionals n=104,491	Obesity associates with increased risk of pneumonia. RR=2 for weight gain >40 pounds in adulthood
Calle et al, 2003(57)	The Cancer Prevention Study II n=900,053	Overweight and obesity accounted for 14% and 20% of cancer mortality in men and women, respectively
Huang et al, 1997 (58)	The Nurses' Health Study cohort n=92256	Weight gain after age 18 associated with increased breast cancer risk after menopause. P=.006
Murphy et al, 2000 (59)	The Cancer Prevention Study II n=875,406	Obesity highly associated with colon cancer mortality in men. Dose response relationship evident in men. A weaker positive, but significant association seen in women.

Although not the focus of this dissertation, the cluster of risk factors mentioned above, namely obesity, specifically abdominal obesity, hypertension, and an abnormal lipid profile, along with disturbed glucose tolerance has often been called the metabolic syndrome (60-62)a concept also referred to as the insulin resistance syndrome (63) as reflected in the WHO criteria (64). The metabolic syndrome is a cluster of risk factors used to identify individuals at increased risk of CVD (60, 61). Those with this condition are at greatly increased risk of CVD death (65) There is an ongoing discussion about whether the central feature is insulin resistance or central adiposity, as reflected in the recent JDF criteria (61). Central to this concept of the metabolic syndrome are insulin resistance and the role of adipose tissue. Recently Reaven (35) suggested that inflammation might be another link between the metabolic syndrome and CVD.

2.3 ADIPOCYTE PATHOPHYSIOLOGY

The adipocyte is a metabolically active cell, functioning not only as a lipid depot, but as an endocrine organ as well (66, 67). It releases the hormone leptin, tumor necrosis factor (TNF)-α, visfatin, resistin, C-reactive protein (CRP), plasminogen activator inhibitor 1 (PAI-1), and other cytokines, complement proteins, adiponectin, prothrombotic agents, enzymes such as angiotensinogen, and substrates such as free fatty acids (FFA) (13, 68, 69). In obesity, there is an alteration of secretions of adipokines, with an over expression of some and a suppression of others (13, 70).

Specifically addressing FFAs, FFA production is increased in obesity, particularly from visceral fat (21, 71). The elevated FFAs seen in obesity may lead to hepatic insulin resistance via (72). Elevated levels of FFAs reduce glucose uptake in the peripheral tissues and increase hepatic glucose production, resulting in insulin resistance (38, 71). Insulin resistance in type 2 diabetes and in the general population is associated with a markedly increased risk of CAD (60, 61). Additionally, FFAs have been postulated to be in the biological pathway between insulin resistance and cardiomyopathy (73).

TNF-α also plays a role in insulin resistance (74). It induces a reduction in insulin receptors and it also increases lipolysis and the subsequent release of FFAs. In addition to its role in insulin resistance, it increases the expression of leptin, IL-6, and other inflammatory mediators and reduces the expression of adiponectin (73), thereby potentially increasing the risk of cardiovascular disease. Increased levels of this proinflammatory adipokine are seen in obesity (73, 74).

The following diagrams (Figure 2-1) demonstrate how increased fat stores, particularly visceral fat stores, may play a role in heart disease.

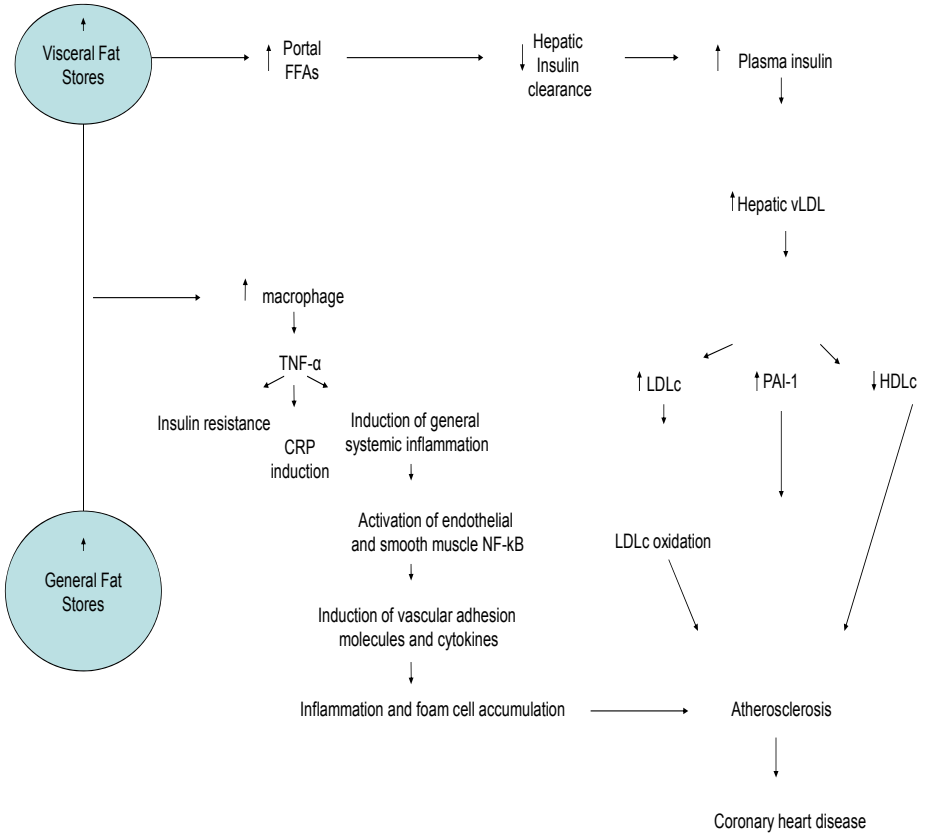

Figure 2-1. Adiposity's pathological route to atherosclerosis

Adapted from Bray G (75) and Raz I (76)

IL-6 is a proinflammatory cytokine also secreted from the adipocyte, among other sources, and overexpressed in obesity, particularly visceral obesity. There is evidence that this cytokine increases hepatic gluconeogenesis and stimulates the release of glucagon and cortisol (77).

Leptin, the recombinant protein encoded by the ob gene, is secreted almost exclusively by adipocytes. Leptin up-regulates inflammatory responses (13), implicated in the pathogenesis of atherosclerosis in chronic low-level inflammation (78). It also stimulates platelet aggregation (79). A case-control study conducted by Wallace et al (79) found that a one standard deviation increase in circulating leptin was associated with a 20% increase in coronary events, independent of BMI and other classic risk factors.

Plasminogen activator inhibitor-1 (PAI- 1), produced in the liver, endothelial cells, thrombocytes, like many other markers of inflammation, is also produced in adipose tissue (80-83). PAI-1 is also associated with many of the components of the metabolic syndrome and CVD (81, 84). Elevated levels of PAI-1 are found in obesity, particularly in visceral obesity, being preferentially synthesized in visceral relative to subcutaneous adipose tissue (81, 82). Elevated levels are also found in insulin resistance, hypertension, elevated levels of triglycerides and low density lipoprotein (LDL), and smoking (80-83). Due to the association of PAI-1 with factors of the metabolic syndrome, impaired fibrynolysis has been included in Reaven's expanded definition of the metabolic syndrome (80, 81, 85). In fact, most of PAI-1's association with CVD appears to be due to the metabolic syndrome (82).

CRP is an acute-phase reactant protein synthesized by hepatocytes. Elevated levels of this inflammatory marker correlate with BMI, weight, visceral adiposity, and insulin resistance (86). Elevated levels of CRP are also associated with increased CVD morbidity and mortality.

Furthermore, CRP has been shown to provide further prediction of atherosclerosis and CVD beyond the traditional key risk factor, LDL (87). In the Women's Health Study, baseline CRP levels were more predictive of CVD events than LDL (87). Other studies have also demonstrated the predictive power of CRP in CVD. In the Cholesterol and Recurrent Events trial, those in the highest quartile of CRP levels were 75% more likely to experience a recurrent myocardial infarction (MI) than those in the lowest quartile (87). The Honolulu Heart Study, the odds of MI rose with increasing CRP levels over a course of twenty years (87).

Adiponectin is a 3-kDa monomer hormone secreted by adipocytes and found to be cardioprotective in the general population (86). Adiponectin increases insulin sensitivity, inhibits glucose production, is anti-atherogenic and anti-inflammatory (86, 88). Levels of this hormone are inversely associated with adiposity. Although lower levels of this cardioprotective peptide are seen in the obese, higher levels are observed in those with T1D (89), a population at much higher risk for CVD. De Block et al (90) found adiponectin to be negatively correlated with waist-to-hip ratio and waist circumference and positively correlated with HDL cholesterol and A1C in individuals with T1D. Although they did not investigate the association of adiponectin with CAD in their population, they did look at its relationship with retinopathy and neuropathy and found no differences in adiponectin levels in those with and without these complications. However, Costacou et al (91) did find adiponectin to be predictive of CAD in T1D and observed an inverse correlation between adiponectin levels and ($p=0.07$) and waist-to-hip ratio ($p=0.003$). Increased levels of inflammatory markers, including adiponectin, were observed cross-sectionally in those with proliferative and nonproliferative retinopathy in the EURODIAB study (92). In this same population, BMI was also positively associated with

retinopathy. Since the adipocyte is a primary source of the inflammatory markers investigated, increased adiposity may have played a role in the development of retinopathy in this population.

Finally, visfatin, or pre-B-cell colony-enhancing factor, another insulin sensitizer, is a protein mainly expressed in bone marrow, liver, and muscle (93). It was termed visfatin when it was discovered that higher levels of this hormone were observed in visceral rather than subcutaneous adipose tissue. Visfatin seems to play a paradoxical role in adiposity. Counterintuitively, although visfatin expression is increased in visceral adiposity, rather than increasing insulin resistance, visfatin acts as an insulin mimetic and increases insulin sensitivity and glucose uptake into tissues. This may be an adaptive mechanism of the body to counteract the effects of obesity-induced insulin resistance. In type 1 diabetes, a state of absence of endogenous insulin, increased expression of this insulin mimetic may play a protective role in hyperglycemia.

In summary, the pathophysiology of increased adiposity begins with an alteration in adipokine and inflammatory peptide secretion, an increase in FFA production, and an excess in fat mass on organs, particularly visceral organs. The elevated production and secretion of peptides from adipose tissue may result in increased muscle insulin resistance, leading to increased insulin requirements and pancreatic insulin secretion, looping back to increased insulin resistance. Raised plasma FFA circulation leads to insulin resistance, ultimately resulting in pancreatic beta cell failure and T2D. Elevated visceral adiposity leads to an increase in portal FFAs followed by a decrease in insulin clearance, and an increase in plasma insulin. This increase in plasma insulin upregulates Na+ reabsorption and the sympathetic nervous system activity, finally resulting in hypertension. Increased levels of portal FFAs also cause an upregulation of hepatic vLDL production resulting in an increase in LDL cholesterol and LDL

oxidation, an increase in HDL cholesterol, and an increase in PAI-1. A final sequelae is atherosclerosis and coronary heart disease.

In order for obesity to be a risk factor for, and play an etiologic role in, a disease it has to exhibit temporality. For diseases like CVD or T2D, where obesity is a risk factor for the disease, this temporal relationship may be relatively clear. However, for diseases in which obesity occurs within the context of another preexisting disease, this relationship becomes more complex. One such disease is type 1 diabetes (T1D), the focus of this proposal.

2.4 TYPE 1 DIABETES

2.4.1 Epidemiology of Type 1 Diabetes

T1D is the most frequently occurring type of diabetes in children and adolescents. In the U. S., T1D affects about 1:400-600 children and adolescents (94), making it one of the leading chronic diseases in this age group (95, 96). In the year 2000, the global prevalence of T1D was 4.9 million, i.e. about 0.09% of the world's population (97). The highest prevalence of T1D is found in Europe, affecting approximately 1.27 million, followed by North America, with a prevalence of approximately 1.04 million(97).

T1D results from an autoimmune destruction of pancreatic beta cells (98). Acute complications of T1D are severe hypoglycemia and diabetic ketoacidosis (98, 99). The major long term complications of T1D include retinopathy, neuropathy, nephropathy, early CAD and cerebrovascular disease, peripheral arterial disease, and early mortality (98, 99). Diabetic

retinopathy is the leading cause of blindness in the U.S. (99) and diabetic nephropathy the leading cause of renal failure (99), of with type 1 diabetes accounts for approximately half of all cases (100). Cardiovascular disease occurs several decades earlier in T1D and is the leading cause of death in this population (100). The excess mortality in T1D is 2 to 4-fold compared to age-matched non-diabetics (101, 102).

2.4.2 Complications in Type 1 Diabetes

Recent studies in Scotland and Pittsburgh have shed light on the incidence of major T1D complications. The mortality rate in 1997 for T1D in Tayside, Scotland was 14.6 per 1000 (103). The Pittsburgh Epidemiology of Diabetes Complications (EDC) Study population, with an average age of eight years at diagnosis, depending upon the calendar year of diagnosis, had mortality rate ranging from 23 to 39% after thirty years of disease (104). Annual incidence rates of major diabetes complications in the two populations are as follows (age-unadjusted for the Scotland cohort). The annual incidence rates of proliferative retinopathy and registered blindness was 5.4% and 0.1 % in the EDC and Scotland populations, respectively. The incidence rate for peripheral vascular disease was 0.5% in the Scotland population (no data available for the EDC cohort). The incidence rates for distal symmetric polyneuropathy and symptomatic autonomic neuropathy were 1.5% and 0.8% per year, respectively, after 30 years of disease duration in the EDC cohort (no data available for the Scotland population). The annual incidence rate for end stage renal failure was 0.6% in both populations. The incidence rate for myocardial infarction was 0.9 % in the Scotland cohort, while it was lower in the EDC cohort, at 0.3% for myocardial infarction and fatal coronary artery disease (CAD). The incidence rate for

total CAD was 0.4% in the EDC cohort (data not available for Scotland cohort), and was the leading cause of death in this population.

2.4.3 Overweight and Obesity in T1D Complications

While there appears to be some association of overweight and obesity with T1D complications, it is unclear as to the extent of overweight/obesity's impact on complications in T1D. A recent study by De Block et al (90) found that being overweight was cross-sectionally associated with increased retinopathy and neuropathy in those with T1D and that higher BMI l was associated with greater severity; however, these overweight individuals were also older than the non-overweight. Overweight was not independently associated with neuropathy and after blood pressure was excluded from the model, was no longer significantly associated with retinopathy either. Insulin resistance was not observed in this study as the insulin dose/kg bodyweight was similar in both weight groups. In the Diabetes Control and Complications Trial (DCCT), Zhang et al (105) found that baseline BMI positively predicted retinopathy (OR=1.1, p=0.04) in T1D. Dorchy et al (106) also found an association with overweight and progression from background to proliferative retinopathy in T1D.

In the EDC cohort of T1D, Stuhldreher et al (107) also found WHR cross-sectionally to be positively associated with proliferative retinopathy and peripheral vascular disease univariately in both genders and neuropathy and nephropathy in males. After controlling for hypertension, LDL and total cholesterol, and fibrinogen, WHR was only significantly associated with peripheral vascular disease in females and neuropathy in males. In looking at the impact of weight gain in the EDC population, Williams et al (108) observed that after seven years of follow-up, although the prevalence of overweight was lower than in the general population, the

incidence of overweight was similar. Those who gained the most weight had the greatest improvement of glycemic control. Weight gain with improved glycemic control was associated with an improved lipid and blood pressure profile, i.e. CAD risk factors, while weight gain in the absence of improved glycemic control was associated with a worsening of lipid and hemodynamic factors. Overall, although weight gain was associated with an improvement in glycemic control, it was also associated with an increased incidence of hypertension and overt nephropathy.

These data concerning the interaction between weight gain and improvement in glycemic control with contrast to the results from the DCCT which showed that participants on intensive insulin therapy who gained the most weight had a worse cardiovascular lipid risk profile at the end of follow-up (5), suggesting that they might be at increased risk of CAD. A similar more detailed observation was made by Sibley et al (109) in a subgroup of the DCCT participants five years after the close of the trial. An atherogenic lipid profile was observed in those with central adiposity.

Adiposity, particularly visceral adiposity, has been found to be associated with coronary artery calcification (CAC), a subclinical marker of atherosclerosis. Allison and Wright (110) found higher calcification to be associated with higher abdominal visceral fat and higher quartiles of BMI in both men and women. CAC in the EDC population was shown to be highly predictive of future clinical CAD (111).

Stuhldreher et al (112) cross-sectionally found WHR, a marker of visceral adiposity, to be positively associated with total and LDL cholesterol and triglycerides, i.e., CAD risk factors, in the EDC cohort, implicating WHR in insulin resistance in this population. The mechanism whereby overweight and obesity may increase CAD risk is via an altered lipid and adipokine

profile, increased secretion of FFAs, and insulin resistance. Higher triglyceride and FFA production may result in increased lipid deposition in the coronary arteries. Additionally, higher levels of adipokine secretion and other inflammatory markers may also lead to atherosclerotic plaque build-up as well as to insulin resistance. Coronary artery disease is the leading cause of death in type 1 diabetes. In the EDC population, adiposity has been related to complications (9, 113, 114), in particular, WHR with CAD.

As stated just above, CAD is the number one cause of mortality in T1D. Although adiposity appears to be associated with many chronic diseases, there is some evidence that this relationship may not be so straightforward, particularly in the most chronic complication of them all, mortality. Jarret and Shipley (115) found BMI to be nonsignificantly negatively associated with coronary heart disease mortality (OR=0.29, 0.7-1.1) after ten years in 168 middle-age civil service men, more than one-third of whom had T1D. Similarly, in the EDC population, there was a non-significant inverse relationship between BMI and mortality in both men and women (men: OR=0.89, 0.7-1.2; women: OR=0.82, 0.6-1.2) during six years of follow-up (116). A revisit of the CDC study looking at annual excess deaths associated with obesity found that being overweight (BMI 25.0-29.9) was actually associated with 86,094 fewer deaths than being of normal weight (BMI 18.5-24.9). Furthermore, contrary to expectations, being in the obesity class 1 category (BMI 30.0-34.9) was associated with fewer deaths than being underweight (30). The relationship between BMI and mortality was not linear, but rather had a U-shaped distribution.

The previously mentioned studies demonstrating an effect on BMI or WHR with complications of T1D were not designed specifically to do so; therefore a non-linear relationship may not have been accounted for. Going back to the ‘thrifty-gene’ hypothesis, nonsignificant

inverse relationship between BMI and mortality in those with diabetes and the U-shaped distribution in the general population seem to indicate that the adiposity-disease relationship is muddled, particularly in the context of a pre-existing disease. Those 'thrifty genes' may not be entirely outdated.

2.5 CONCLUSION AND RATIONALE

Obesity's impact on disease is not clear. The ability and manner in which overweight and obesity affects health may be different in T1D, where the key hormone, insulin, has to be systemically administered. Furthermore, the association between obesity and complications in T1D may be complicated by the effect of intensive insulin therapy. Many of the studies looking at obesity in T1D have been cross-sectional. Additionally, it is not clear whether overweight and obesity are associated with an increased mortality in T1D. While overweight/obesity in general is a 'maladaptation to overnutrition of genes selected to survive undernutrition' (27), it is not known whether the same is true in type 1 diabetes (T1D) or whether it is the proper adaptation to a chronic emergency state. Perhaps those with T1D who are 'well-adapted to privation' are better able to survive when privation is less of a problem.

Although there is some evidence that the prevalence of overweight and obesity are increasing in T1D, time trends in weight change have not been well studied in this population nor whether overweight and obesity are associated with an altered risk of diabetes complications. Additionally, the impact of overweight and obesity on overall survival in T1D has not been investigated. Therefore, the purpose of the proposed study is to investigate these aspects of

overweight and obesity in T1D, namely the prevalence, incidence, and time trends, and the associations with complications, specifically coronary artery disease and mortality. A further component will focus on possible mediators, e.g. FFA.

3.0 SPECIFIC AIMS

The proposed research was to investigate the role of overweight and obesity in the complications of type 1 diabetes in a cohort of 658 individuals with childhood onset childhood diabetes from the Pittsburgh Epidemiology of Diabetes Complications Study. Specifically, this research proposed:

1. To assess time trends in overweight and obesity and predictors of weight gain in T1D.

The following null hypotheses were tested:

Hypothesis 1: Overweight is not increasing in T1D.

Hypothesis 2: Obesity is not increasing in T1D.

Hypothesis 3: Overweight and obesity are not increasing in T1D after controlling for intensive insulin therapy.

2. To determine whether overweight or obesity was associated with mortality in type 1 diabetes. The following null hypotheses were tested:

Hypothesis 4: Overweight is not associated with risk of mortality in T1D.

Hypothesis 5: Obesity is not associated with risk of mortality in T1D.

Hypothesis 6: Any association of overweight/obesity with mortality in T1D is not independent of standard correlates of overweight/obesity, including blood pressure, dyslipidemia, and intensive insulin therapy.

3. To determine the role of free fatty acids in explaining any association of adiposity with coronary artery calcification in T1D. The following null hypothesis was tested:

Hypothesis 7: There is no significant difference in the commonly used measurements of overweight and obesity and their association with coronary artery calcification.

Hypothesis 7: Free fatty acids do not attenuate the association of adiposity with coronary artery calcification in T1D.

4.0 METHODS

4.1 STUDY POPULATION

In order to answer the above questions, data from the Pittsburgh Epidemiology of Complications (EDC) Study, Phases I and II were investigated. The EDC study is an observational cohort study of 658 individuals with childhood onset type 1 diabetes first diagnosed at Children's Hospital in Pittsburgh between 1950 and 1980. The study began in 1986 with baseline exams between 1986 and 1988 and has involved continuous biennial follow-up. At study baseline, mean age and duration were 28 and 19 years, respectively. As of July, 2006, a total of 132 subjects had died, 21 had requested no further contact, and 36 had been lost to follow-up. The study was in its 18th-year of follow-up.

4.2 SPECIFIC AIM 1: TIME TRENDS IN OVERWEIGHT AND OBESITY IN TYPE 1 DIABETES

Study design and hypotheses

The purpose of Specific Aim 1 was to assess time trends in overweight and obesity and predictors of weight gain in T1D. The following null hypotheses were tested:

Hypothesis 1: Overweight is not increasing in T1D.

Hypothesis 2: Obesity is not increasing in T1D.

Hypothesis 3: Overweight and obesity are not increasing in T1D after controlling for intensive insulin therapy.

Population, Study Design, and Time Period

The population for Specific Aim 1 consisted of the entire EDC cohort. This was a longitudinal study cohort design. The study time period was from 1986-2007.

Method of data collection

Time trends in weight change and BMI category from study baseline to 18 years of follow-up were investigated. Correlates of baseline BMI category and risk factors for change in BMI category were assessed. In this substudy, BMI categories were defined as follows:

BMI<20: Underweight. 20≤BMI<25: Normal weight. 25≤BMI<30: Overweight.

BMI≥30: Obese.

All data were collected by biennial surveys and medical exams. Before attending each cycle of examinations, information was collected by questionnaire concerning demographic characteristics, medical history, and health care behaviors. At each cycle, both a standardized medical history and clinical examination were performed by a trained internist to document complications of diabetes.

Anthropometric data

During biennial exams, participants were weighed in light clothing on balance beam scale. Height was measured using a wall-mounted stadiometer. Body mass index was calculated as the weight in kilograms divided by the square of the height in meters. For the first ten years of follow-up, all height and weight were measured. Beginning in 1998, exams were limited to certain subgroups, so measured height and weight data were not fully available. Between 2004 and 2007, an eighteen year follow-up exam was again made available to all participants. Self-reported data from the medical history questionnaire were used when measured data were not available.

Lipid, Glucose, and Hemodynamic data

Fasting blood samples were assayed for lipids, lipoproteins, hemoglobin (HbA1), creatinine, and Hct. High-density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure, a modification of the Lipid Research Clinics method (117). Cholesterol was measured enzymatically (118), as were triglycerides. Low-density lipoprotein (LDL) cholesterol levels were calculated from measurements of the levels of total cholesterol, triglycerides, and HDL cholesterol. Stable glycosylated HbA1 was originally measured in saline-incubated samples by microcolumn cation exchange chromatography (Isolab, Akron, Ohio, USA). On October 26, 1987, the method was changed to high-performance liquid chromatography (HPLC) (Diamat, Bio-Rad Laboratories, Hercules, CA, USA). The two methods were highly correlated (r = 0.95; Diamat HbA1 = 0.18±1.00 Isolab HbA1). Beginning in 1998, HbA_{1c} was measured using the DCA2000 analyzer. Original HbA_1 (1986 to 1998) and A_{1c} (1998 to 2004) were converted to Diabetes Control and Complications Trial (DCCT) aligned HbA_{1c} values using regression formulas derived from duplicate analyses (DCCT HbA_{1c} = [0.83 *

EDC HbA_1] + 0.14; DCCT HbA_{1c} = [EDC HbA_{1c} − 1.13]/0.81). Blood pressure was measured by a random-zero sphygmomanometer according to a standardized protocol (119) after a 5-minute rest period. Blood pressure levels were analyzed, using the mean of the second and third readings.

Variables

The variables used and how they were analyzed are listed in Table 4-1 below.

Table 4-1. Variable List for Specific Aim 1.

Dependent Variables	
	Type
BMI (weight in kg/ht in m^2)	Continuous/ Categorical
Independent Variables	
	Type
Age (years)	Continuous/Categorical
Diabetes Duration (years)	Continuous
HbA1, HbA1c (%)	Continuous
Insulin Injections (#)	Continuous/Categorical
Insulin Dose (U/kg/dy)	Continuous
SBP (mm Hg)	Continuous
DBP (mm Hg)	Continuous
Hypertension	Categorical
HDLc (mg/dL)	Continuous
non-HDLc (mg/dL)	Continuous
Estimated Glucose Disposal Rate (mg/kg/min)	Continuous
Kilocalories (kcal/wk)	Continuous
History of Smoking	Categorical

Statistical Analysis

All software analyses were performed using SAS software (version 9.1; SAS Institute, Cary, NC) with a significance level of $p < 0.05$. Simple percentages were used to determine the prevalence and incidence of overweight and obesity. Pearson's and Spearman's correlations Cochran-Mantel-Haenszel chi-square tests were used to test for trends in continuous and categorical variables, respectively, across BMI categories for a given time period. Simple linear regression, generalized linear models, and Cox proportional hazards models were used to determine the predictors of weight change.

4.3 SPECIFIC AIM 2: OVERWEIGHT AND OBESITY AND COMPLICATIONS IN TYPE 1 DIABETES

Population, Study Design, and Time Period

The population for Specific Aim 2 consisted of the entire EDC cohort. This substudy used a longitudinal cohort design. The study time period was from 1986-2007.

Method of data collection

Methods for the collection of body mass index, blood glucose, and hemodynamic measurements were described above. Waist circumference measurements were taken by a standard medical measuring tape, measuring from the mid-point of the iliac crest and the lower

costal margin in the mid-axillary line. Waist circumference measurements were taken at the 1st and 2nd, 5th through 9th (18-year follow-up) medical exams. Creatinine clearance and albumin excretion rate was estimated from timed urine collections.

Nephropathy status was be determined based on consistent results from at least two of three timed urine collections (24-hour, overnight, random timed post-clinic) and urine and albumin excretion rate (AER). Urinary albumin was determined immunonephelometrically. Overt nephropathy was defined as an AER ≥200μg/min or end-stage renal disease (ESRD; renal dialysis or transplant). CAD was defined as myocardial infarction, ischemia (Minnesota Codes 1.1-1.3, 4.1-4.3, 5.1-5.3 and 7.1), or fatal CAD verified from death certificates, autopsy and/or medical records using standard criteria, revascularization, or EDC clinic diagnosed angina. Proliferative retinopathy was determined by stereoscopic fundus photographs taken in fields 1, 2, and 4 and read and classified by the Fundus Photography Center as the University of Wisconsin-Madison. Lesions were classified by the Arlie House system. Symptomatic autonomic neuropathy was determined by an abnormal heart rate response to deep breathing (expiration-inspiration ratio less than 1.1) and two or more symptoms of autonomic neuropathy assessed in the physician's exam based on the DCCT standardized clinical protocol (120). Confirmed distal symmetric polyneuropathy was determined based on the DCCT protocol for distal symmetrical polyneuropathy (121) and confirmed by an abnormal age-specific vibratory threshold. Lower extremity arterial disease was defined as an ankle-brachial index <0.8, claudication, or amputation. All deaths and hospitalized cardiovascular events were investigated by obtaining autopsy, coroner, or medical records. Deaths were clarified by two physicians (TO, JF) according to DERI procedures. Supplementary data were obtained on some CVD hospitalized events.

Variables

The variables used and how they were analyzed are listed in Table 4-2 below.

Table 4-2. Variable List for Specific Aim 2.

Dependent Variables	
	Type
Mortality	Dichotomous
Independent Variables	
Independent Variables	Type
BMI (kg/ m²)	Continuous/ Categorical
Waist Circumference (cm)	Continuous/ Categorical
Coronary Artery Disease	Dichotomous
Overt Nephropathy	Dichotomous
Lower Extremity Arterial Disease	Dichotomous
Proliferative Retinopathy	Dichotomous
Symptomatic autonomic Neuropathy	Dichotomous
Distal Symmetrical Polyneuropathy	Dichotomous
Age (years)	Continuous/Categorical
Diabetes Duration (years)	Continuous
HbA1, HbA1c (%)	Continuous
Insulin Injections (#)	Continuous/Categorical
Insulin Dose (U/kg/dy)	Continuous
SBP (mm Hg)	Continuous
DBP (mm Hg)	Continuous
Hypertension	Categorical
HDLc (mg/dl)	Continuous
non-HDLc (mg/dL)	Continuous
Physical activity (kcal/ week)	Continuous
History of Smoking	Categorical

Statistical Analyses

T-tests and chi-square frequencies were used to test for baseline differences in continuous and categorical variables, respectively, by the prevalence of overweight and obesity. Time-dependent Cox Models were used to determine the relationship of BMI category and waist circumference with mortality.

4.4 SPECIFIC AIM 3: FREE FATTY ACIDS: THEIR IMPACT ON CORONARY ARTERY DISEASE IN TYPE 1 DIABETES

Population, Study Design, and Time Period

The population for Specific Aim 3 consisted of 315 participants in the EDC study who completed medical exams during the 16-year and 18-year follow-up period, i.e. 2000-2007. This substudy utilized a cross-sectectional study design.

Method of data collection

The data for Specific Aim 3 was collected in the same manner as that for Specific Aims 1 and 2. Additionally coronary artery calcification and direct measurements of abdominal adiposity (visceral and total adipose tissue surface area) were measured by electron beam tomography scanning (Imatron C150). Scans of abdominal adiposity were taken between the fourth and fifth lumbar regions. FFAs from stored blood samples, collected at 16 year follow-up exam, were analyzed to determine their association with adiposity and coronary artery

calcification in T1D. FFAs were assayed using the in vitro colorimetric method using the NEFA C test kit (Wako Pure Chemical Industries, Ltd) in the Heinz Laboratory of the University of Pittsburgh, Graduate School of Public Health.

Variables

The variables used and how they were analyzed are listed in Table 4-3 below.

Table 4-3. Variable List for Specific Aim 3.

Variables	Measurement	Test
Independent		GLM/Logistic regression
Visceral abdominal adiposity (cm^2)	Categorical	
Subcutaneous abdominal adiposity (cm^2)	Categorical	
Body mass index (kg/m^2)	Categorical	
Waist Circumference (cm)	Dichotomous/Categorical	
Free fatty acids (mmol/L)	Continuous	
Dependent		
CAC (volume score)	Continuous/Dichotomous	
Covariates		GLM/Logistic regression/student's T/ Wilcoxin's rank
Age (years)	Continuous	
Diabetes Duration (years)	Continuous	
HBA1c (%)	Continuous	
Insulin Injections (#)	Continuous/	
Insulin Dose (U/kg/dy))	Continuous	
Estimated glucose disposal rate	continuous	
SBP (mm Hg)	Continuous	
DBP (mm Hg)	Continuous	
HDL (mg/dl)	Continuous	
non-HDLc (mg/dL)	Continuous	
History of Smoking	Categorical	

Statistical Analysis

SAS software (version 9) was used to conduct the statistical analysis. The Student's *t* test was used to compare normally distributed variables and Wilcoxon's rank test was used to calculate non-normally distributed variables. ORs and 95% confidence intervals were calculated using Logistic regression. Generalized linear models and logistic regression were used to assess the association of adiposity with coronary artery calcification.

5.0 PAPER 1: TIME TRENDS IN OVERWEIGHT AND OBESITY IN TYPE 1 DIABETES

Under Review

Baqiyyah Conway[1], Rachel Miller[1], Tina Costacou[1], Linda Fried[2], Sheryl Kelsey[1], Rhobert Evans[1], Trevor Orchard[1]

[1]University of Pittsburgh, Department of Epidemiology, Graduate School of Public Health

[2]University of Pittsburgh, School of Medicine

5.1 ABSTRACT

Background: Time trends in overweight and obesity in the general population have been well documented; however, such temporal patterns in populations with pre-existing disease, such as type 1 diabetes (T1D), have not been as thoroughly investigated.

Methods: We therefore assessed the time trends in overweight and obesity and predictors of weight change in 655 individuals from the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study, a cohort of childhood onset (age <17 years) type 1 diabetes. Participants were first seen in 1986-1988, when mean age and diabetes duration at study baseline were 28 and 19 years, respectively, and biennially thereafter for up to 18 years. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Overweight was defined as a BMI between 25 and 29.9kg/m². Obese was defined as a BMI less than 30 kg/m².

Results: At baseline, the prevalence of overweight and obesity were 28.6% and 3.3%, respectively. After 18 years of follow-up, the prevalence of overweight increased by 47% while the prevalence of obesity increased 7-fold. Seven percent of the population was on intensive insulin therapy (3+ insulin injections per day or on insulin pump) at baseline; by 2004-2007, this had increased to 82%. Predictors of weight change were a higher HbA1c at baseline,

symptomatic autonomic neuropathy (inversely), overt nephropathy (inversely), and going onto intensive insulin therapy during follow-up.

Conclusion: These data demonstrate dramatic weight gain in type 1 diabetes and underscore the complexity of weight change in this disease.

5.2 INTRODUCTION

Time trends in overweight and obesity in the general population have been well documented (1, 2, 3); however, such temporal patterns in populations with pre-existing disease, such as type 1 diabetes (T1D), have not been as thoroughly investigated. Traditionally, the phenotype of T1D was normal or underweight; however, there is evidence that this may be changing. A lower prevalence of overweight and obesity in the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study, relative to the general population has been reported, although the incidence (12%) in both populations was similar over a mean of 7 years of follow-up (4).

The effect of weight gain on cardiovascular risk factors in T1D diabetes has been noted to interact with intensive insulin therapy and improved glycemic control. Reports from the Diabetes Complications and Control Trial/ Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) (5) and the EDC (4) study have demonstrated an adverse lipid and hemodynamic profile with increased adiposity as well as cross-sectional associations with long-term complications (6). However, the EDC study also showed that excess weight in association with improved glycemic control was less harmful in terms of cardiovascular disease risk profile (4). As T1D is a disease characterized by insulin deficiency leading to an abnormal fuel utilization and acute complications such as ketoacidosis, weight loss, or a lower weight, may

partially reflect inadequate insulin therapy. Furthermore, some of the long term complications like neuropathy and nephropathy may also be associated with anorexia, wasting, and weight loss. Thus the causes, and consequences, of weight change in T1D may be different than in the general population, and add complexity to the concern about a rise in overweight and obesity in T1D.

The purpose of this report was therefore to extend our previous EDC follow-up and determine the prevalence and incidence of overweight and obesity over eighteen years of follow-up, as well as to identify potential risk factors for weight gain in T1D. In an accompanying report we describe the impact of adiposity and change in adiposity on mortality.

5.3 METHODS

The EDC study is a prospective study of a well-defined cohort (n=658) with childhood-onset (<17 years) type 1 diabetes, first diagnosed between January 1, 1950 and May 30, 1980 at Children's Hospital of Pittsburgh. Participants were first seen for baseline examination between 1986 and 1988 and biennially thereafter for ten years, after which they were followed by survey with exams limited to certain subgroups. Between 2004 and 2007, an eighteen year follow-up was conducted for all participants. The design and methods of the study have been previously described (7). Participants were followed for up to eighteen years. For this report, because of the variability introduced by the growth spurt, and differing body compositions in children and adolescents, we focus on the population 18 years and older (n=589 at baseline).

Before each cycle of examinations, information was collected by questionnaire concerning demographic characteristics, medical history, and health care behaviors as previously reported (7). Participants were weighed in light clothing and without shoes on a balance beam scale. Height was measured using a wall-mounted stadiometer. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Beginning in 1998, where height and weight data were less available from follow-up clinical exams, self-reported data from the medical history questionnaire were used, representing approximately 20% of the data for this time period. Overweight was defined as a BMI $\geq$25 kg/m^2. Obesity was defined as a BMI $\geq$30 kg/m^2. Weight change was defined as the difference between baseline BMI and BMI at last follow-up. Only measured height and weight were used to determine weight change.

Fasting blood samples were assayed for lipids, lipoproteins, and glycosylated hemoglobin. Stable glycosylated hemoglobin A_1 (HbA_1) was originally measured in saline-incubated samples by microcolumn cation exchange chromatography (Isolab, Akron, Ohio, USA). On October 26, 1987, the method was changed to high-performance liquid chromatography (HPLC) (Diamat, Bio-Rad Laboratories, Hercules, CA, USA). The two methods were highly correlated (r = 0.95; Diamat HbA_1 = 0.18$\pm$1.00 Isolab HbA_1). Beginning in 1998, HbA_{1c} was measured using the DCA2000 analyzer. Original HbA_1 (1986 to 1998) and A_{1c} (1998 to 2004) were converted to DCCT aligned HbA_{1c} values using regression formulas derived from duplicate analyses (DCCT HbA_{1c} = [0.83 * EDC HbA_1] + 0.14; DCCT HbA_{1c} = [EDC HbA_{1c} − 1.13]/0.81). Insulin dose was defined as the total daily units of insulin divided by the body weight in kilograms. Intensive insulin therapy was defined as having three or more insulin injections per day or using an insulin pump. A family history of type 2 diabetes was defined as

diabetes diagnosed after the age of 30 in a first degree relative without immediate insulin use. Physical activity was determined by number of flights climbed per day, city blocks or equivalent walked per day, and mets calculated from sports/exercise*minutes of participation*number of times per week. Physical activity is expressed in kilocalories.

5.4 STATISTICAL ANALYSES

The validity of the reported weight was assessed by conducting a Pearson's correlation test between the measured and reported weight of all participants providing both. Self-reported and measured BMI were highly correlated in both 2000-2004 (intraclass correlation=0.92, 0.10-0.94), median difference=0.4 kg/m²) and 2004-2007 (intraclass correlation=0.82, 0.05-0.85), median difference 0.7 kg/m^2).

Mixed models analyses were used to determine the association of age group cohort with average weight change. Weight change was defined as BMI at last follow-up controlled for baseline BMI. Generalized linear models were used to assess the determine predictors of weight change. All analyses were adjusted for follow-up time. Cox proportional hazards models were used to determine predictors of time to the first gain of 5 kg/m² or loss of 2 kg/m² loss, while controlling for baseline BMI. Results are expressed as per standard deviation change in continuous independent variables. Generalized linear models were used to determine differences in continuous variables by BMI category and to test for a linear trend across BMI category. Linear regression analysis with stepwise selection was used to determine the independent predictors of final BMI. Chi square analyses were used to determine differences in dichotomous

variables by BMI category and to test for a linear trend across BMI category. All analyses were conducted using SAS version 9.1 (Cary, North Carolina).

5.5 RESULTS

Participants were followed for a median of 18 years (range: 1.5-20.6 years), with follow-up data for 95% of the original cohort. Mean and median increase in BMI over the course of follow-up was 2.6 and 2.2 kg/m², respectively, ranging from a change of -6.7 to a change of 29.3 kg/m². This did not vary by sex (p=0.96). One hundred and eighty-three participants gained five or more kg/m² over the course of follow-up. Twenty-six participants had a gain of ten or more kg/m². Seven percent of the population was on intensive insulin therapy (IIT) at baseline; by 2004-2007, this had increased to 82% and median insulin injection frequency among non pump users increased from 2 to 3.7 per day.

Overall 3.3 % of the participants aged 20 years and older were obese in 1986-1988. By 2004-2007 this had risen seven-fold to 22.7%. The prevalence of being overweight, but not obese, rose from 28.6% in 1986-1988 to 42.0% in 2004-2007, a 47% increase. The combined prevalence of overweight and obesity increased by an average of 1.8% per year. Figures 5-1 and 5-2 show the time trends in the prevalences of being overweight and obese and the use of IIT. As these data maybe influenced by the aging of the cohort and survivorship, age specific prevalences of underweight, normal weight, overweight, and obese were also examined (Appendix C) for age-specific groupings by time-period.

As an example, Table 5-1 presents these data for the 40-49 year-old age group, 25% of whom were overweight or obese in 1986-1988. By 2004-2007, 68.2 % in that age group were

overweight or obese. Appendix C shows both full age and gender prevalences by time period. Time trends were similar in men and women. Mixed models analyses of variance revealed no age related effect on average BMI over the eighteen years (p=0.51, 0.10, 0.32 for those aged <20. 20-29, 30-39 respectively, relative to those aged 40-49) (Appendix C).

The incidence of overweight and obesity were also examined. In those with a BMI <25 in 1986-1988, the incidence of being overweight or obese was 49.1%, while the incidence of obesity, for those with a BMI <30 at baseline, was 20.9%, during the 18 years of follow-up. Twenty percent of the population gained at least 5 kg/m² over the course of follow-up; seven percent loss at least 2 kg/m².

The baseline predictors (and subsequent use of intensive insulin therapy) of change in BMI from baseline to last follow-up are shown in Table 5-2. A higher HbA1c at baseline and the subsequent use of intensive insulin therapy during follow-up were predictive of weight gain, while overt nephropathy and symptomatic autonomic neuropathy predicted weight loss. In multivariable linear regression analyses with stepwise selection, intensive insulin therapy was reduced to marginal significance (p=0.07) and overt nephropathy was not selected as an independent predictor (Table 5-3).

Baseline predictors of a 5 kg/m² gain or a 2 kg/m² loss are presented in Appendix C. After multivariable adjustment, independent predictors of a 5 kg/m² gain were low annual household income (HR=1.63, 1.08-2.47) and alcohol consumption (HR=0.53, 0.31-0.91). Independent predictors of a 2 kg/m² loss were female sex (HR=3.57, 1.57-8.14), HbA1c (HR=1.62, 1.13-2.32), overt nephropathy (HR=327, 1.58-6.75), symptomatic autonomic neuropathy (HR=5.80, 2.13-15.78), and smoking (HR=2.79, 1.28-6.06).

Tables 5-4 and 5-5 present selected cardiovascular and mortality risk factors by BMI category at baseline and the 18th year follow-up exam. At baseline there was a significant linear trend by BMI category for use of intensive insulin therapy, systolic and diastolic blood pressures, HDL (inverse) and non-HDL cholesterol, and smoking (inverse). These trends for intensive insulin therapy, systolic blood pressure, and smoking were no longer evident at the 18th year of follow-up, although it remained for diastolic blood pressure. At the 18th year of follow-up, a trend for age emerged, although this appeared to be due to the significantly higher age in the underweight. Additionally, although a significantly higher HDL and lower non-HDL cholesterol were observed in the overweight relative to the normal weight at baseline, this had disappeared by the 18th year of follow-up, although the obese were at increased at the 18th year of follow-up. Finally, at the 18th year of follow-up, systolic blood pressure in both the overweight and the obese were significantly higher than the normal weight, although no linear trend was observed as systolic blood pressure in the underweight was similar to that of the overweight and obese.

5.6 DISCUSSION

Several important findings emerge from these analyses of long-term time trends in weight change in type 1 diabetes. First, the prevalence of being overweight or obese is increasing in type 1 diabetes. Second, we have shown that the percentage on intensive insulin therapy increased nearly 10 fold during the course of follow-up, an increase which parallels that of being overweight or obese. Finally, we have shown that traditional predictors of weight change, that is, smoking, and socioeconomic status appear to be less operant in type 1 diabetes while diabetes complications have a major impact on weight change.

We have previously shown a lower prevalence of overweight/obesity in the EDC study relative to the general population, although the incidence (12%) in both populations was similar after a mean of 7 years of follow-up (4). The rise in adiposity in EDC appears to be independent of aging itself as our data have demonstrated similar increases in overweight and obesity in age-specific strata (Table 5-1). After 18 years of follow-up, the prevalence of overweight in type 1 diabetes appears to have increased at a faster pace than in the general population. At baseline (1986-1988), the combined prevalence of overweight and obesity in our population was much lower than the general population (31.9 vs. 55.9) (8); by 2004-2007 there was no difference in the two populations (64.6 vs. 66.3, EDC vs. NHANES 2003-2004). Similarly, the prevalence of obesity in our population increased 7-fold, from 3.3 % to 22.7%. In the DCCT, weight gain was also apparent, with mean BMI rising from approximately 23 kg/m² to approximately 26 kg/m² over nine years of follow-up (9).

There are several possible reasons for the rising prevalence of overweight and obesity observed in our population. First of all, there is in the total cohort a healthy survivor effect. By the end of follow-up, 22% of the population had died. Thus, as overt nephropathy and symptomatic autonomic neuropathy are associated with weight loss, the survivors are biased toward weight gain. It is likely that those remaining (n=379) in 2004-2007 represents a substantially survival biased cohort.

Insulin itself promotes weight gain in that it stimulates lipogenesis, inhibits protein catabolism, and slows down basal metabolism. This, in combination with the abnormal physiological route of insulin via its peripheral insulin administration in those with T1D, which is also associated with a reduced energy metabolism (10,11), is likely to relate to the well known weight gain with the advent of intensive insulin therapy. This seems to be evidenced in our data

by the marked increase in obesity after 1996-1998 (Figure 5-1) reflecting a dramatic increase in intensive insulin therapy post DCCT results. At baseline, only 7.2 % of our participants were on intensive insulin therapy, but by 2004-2007 82% were on intensive insulin therapy (Figure 5-2). The United Kingdom Prospective Diabetes Study (UKPDS), aimed at assessing the benefits of lowering glycemia in type 2 diabetes, also demonstrated that those in the intensive treatment arm gained an average of 3 kg more than those in the conventional treatment arm during the ten years of follow-up (12).

Weight gain associated with insulin therapy in type 1 diabetes might be traditionally viewed as a normalization of weight, i.e. the correction of glucosuria, diuresis, and/or catabolism (wasting) with the initiation of insulin therapy. Consistent with this, we found that a higher baseline HbA1c was predictive of a 2kg/m² weight loss, independent of complications associated with wasting and poor glycemic control, namely overt nephropathy and symptomatic autonomic neuropathy. However, the association of HbA1c with weight change in our population was also a positive predictor of weight gain, a finding consistent with the DCCT (5). We have also previously shown that those with the worst glycemic control at baseline showed the greatest glycemic improvement after an average of 7 years. This group also gained the greatest amount of weight (4), indicating a reversal of the catabolism. Nevertheless, the anabolic role of insulin itself appears to be quite strong as others have reported an association between weight gain and daily insulin dosage (13).

Income, education, physical activity, and smoking, all inverse risk factors for obesity in the general population (14,15,16), were not associated with absolute weight change from baseline to last follow-up, although low income was predictive of gaining 5 kg/m² during follow-up. In type 1 diabetes, the advantages that income and education offer in reducing the risk of

overweight and obesity may be offset by the increased access they provide to insulin and intensive insulin therapy. Likewise, the same may be operant in the relationship of smoking with weight change in type 1 diabetes. Smokers may be more likely to be of a lower socioeconomic status and therefore affected by the same competing influences on weight change. Although smoking was not predictive of absolute weight change from baseline to last follow-up, it was predictive of a 2kg/m² weight loss. Physical activity, however, was protective against a 2kg/m² weight loss during follow-up. In type 1 diabetes, physical activity may reflect both health and morbidity. That is, it may reflect increased leisure time activity in sports or other types of exercise. Conversely, it may reflect increased morbidity from complications that are part of its natural history, suggested its inverse relationship with a 2 kg/m² weight loss. Finally, alcohol consumption, though not associated with absolute weight change from baseline to last follow-up, was protective against excessive (5 kg/m²) weight gain. In the general population as well, inconsistent associations between alcohol consumption and weight gain are observed (17, 18, 19).

Weight change in type 1 diabetes is thus complex. The increasing prevalence of overweight and obesity may reflect both the anabolic effects of insulin and the "normalization" of the population that intensive insulin therapy allows, i.e., allowing it to follow the temporal trend occurring in the background population. This increasing prevalence occurred in the face of both overt nephropathy and symptomatic autonomic neuropathy at baseline, complications part of the natural history of type 1 diabetes and associated with subsequent weight loss. Indeed, although twenty-two % of our population demonstrated a negative weight change, reflecting the negative impact that these complications have on weight change in type 1 diabetes, mean weight gain in this population was still 2.6 BMI units over an 18 year period.

Finally, we have shown, in every BMI category, an attenuation over time of the risk posed by some CVD and mortality risk factors and, with the exception of diastolic blood pressure, a loss of the trend in risk across BMI category. This is suggestive of better health care overall, in every BMI category. It is possible that the concomitant rise in overweight and obesity in intensive insulin therapy in type 1 diabetes is simply mirroring better health care in this population and that better health care is allowing a normalization of the type 1 diabetes population, such that the same phenomenon occurring in the background population, i.e. a rapid rise in overweight and obesity, is also occurring in type 1 diabetes. However, as we have also shown linear trends in the use of intensive insulin therapy by BMI category and that although not significant at last follow-up, 90% of the obese were on intensive insulin therapy, the role of insulin itself still appears very strong.

A limitation of these analyses was the inclusion of reported height and weight data in the determination of adiposity time trends during the last ten years of follow-up, representing approximately 20% of the data for this time period. Although the R^2 for the reported and measured weight in this population was very high, BMI was underreported by about a half of a BMI unit. Therefore, the prevalence of overweight and obesity for this time period was likely underestimated. However, in the determination of weight change, only measured BMI was used. Another major limitation of this study is the substantial survival bias in the population during the last two examination periods, a time associated with a marked increase in the prevalence of overweight and obesity and the widespread adaptation of intensive insulin therapy. However, as type 1 diabetes is characterized by a very early mortality, this could not be avoided and age specific analyses confirmed a major weight gain.

Taken as a whole, the results of this study may appear quite alarming as we have demonstrated a marked increase in the prevalence of overweight and obesity in type 1 diabetes, which is probably greater than in the general population. However, as overweight and obesity increased with time, overweight and obesity may also be a marker of survival. The effect of adiposity and weight gain on mortality, further explored in the accompanying paper, suggests that weight gain and the overweight state may not be harmful. Thus despite the rise of overweight and obesity in type 1 diabetes, caution should be used in admonishing patients to lose weight or maintain an ideal body weight (BMI <25), particularly in light of the role that insulin plays in weight gain and the reduction of complication risk.

5.7 CITED WORKS

1. Ogden C, Caroll M, Curtin L, McDowell M, Tabak C, Flegal K. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006; 295:1549-1555.

2. Flegal K, Carroll M, Ogden C, Johnson C. Prevalence and Trends in Obesity among US Adults, 1999-2000. *JAMA* 2002; 288:1723-1727.

3. Mokdad A, Serdula M, Dietz W, Bowman B, Marks J, Koplan J. The spread of the obesity epidemic in the United States, 1991-1998. *JAMA* 1999; 282:1519-1522.

4. Williams K, Erbey J, Becker D, Orchard T. Improved Glycemic Control Reduces the Impact of Weight Gain on Cardiovascular Risk Factors in Type 1 Diabetes. *Diabetes Care* 1999; 22:1084-1091.

5. Purnell J, Hokanson J, Marcovina S, Steffes M, Cleary P, Brunzell J. Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure. *JAMA* 1998; 280:140-146

6. De Block CE, De Leeuw IH, Van Gaal LF. Impact of overweight on chronic microvascular complications in type 1 diabetic patients. *Diabetes Care* 2005; 28:1649-55.

7. Orchard T, Dorman J, Maser R, Becker D, Ellis D, LaPorte R, Kuller L, Wolfson S, Drash A. Factors associated with the avoidance of severe complications after 25 years of IDDM: Pittsburgh Epidemiology of Diabetes Complications Study I. *Diabetes Care* 1990; 13:741-747.

8. Ogden C, Yanovski S, Carroll M, Flegal K. The Epidemiology of Obesity. *Gastroenterology* 2007; 132: 2087-2102.

9. DCCT Research Group. Weight gain associated with intensive therapy in the Diabetes Control and Complications Trial. *Diabetes Care* 2001; 241:1711-1721.

10. Nair K, Halliday D, Garrow J. Increased energy expenditure in poorly controlled type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1984; 27:13-16.

11. Charlton M, Nair K. Role of hyperglucagonemia in catabolism associated with type 1 diabetes. Effects of leucine metabolism and the resting metabolic rate. *Diabetes* 1998; 47: 1748-1756.

12. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; 352:837-853.

13. Ferriss J, Webb D, Chaturvedi N, Fuller J, Idzior-Walus B, EURODIAB Prospective Complications Group. Weight gain is associated with improved glycaemic control but with adverse changes in plasma lipids and blood pressure in Type 1 diabetes. *Diabet Med* 2006; 23:557-564.

14. Wang Y, Beydoun M. The Obesity Epidemic in the United States-Gender, Age, Socioeconomic, Racial/Ethnic, and Geographic Characteristics: A Systematic Review and Meta-Regression Analysis. *Epidemiol Rev* 2007; 29:6-28.

15. Erlichman J, Kerbey A, James W. Physical activity and its impact on health outcomes. Paper 2: prevention of unhealthy weight gain and obesity by physical activity: an analysis of the evidence. *Obesity Reviews* 2002; 3:273-288.

16. Fogelholm M, Kukkonen-Harjula K. Does physical activity prevent weight gain-a systematic review. *Obesity Reviews* 2000; 1:95-112.

17. Lewis C, Smith D, Wallace D, Williams O, Bild D, Jacobs D. Seven-year trends in body weight and associations with lifestyle and behavioural characteristics in black and white young adults: the CARDIA STUDY. *Am J Pub Health* 1997; 87:635-642.

18. Wannamethee S, Shaper A. Alcohol, body weight and weight gain in middle-aged men. *Am J Clin Nutr* 2003; 77:1312-1317

19. Gruchow H, Sobocinski K, Barboriak J, Scheller B. Alcohol consumption, nutrient intake and relative body weight among US adults. *Am J Clin Nutr* 1985; 42:289-295.

5.8 FIGURES AND TABLES

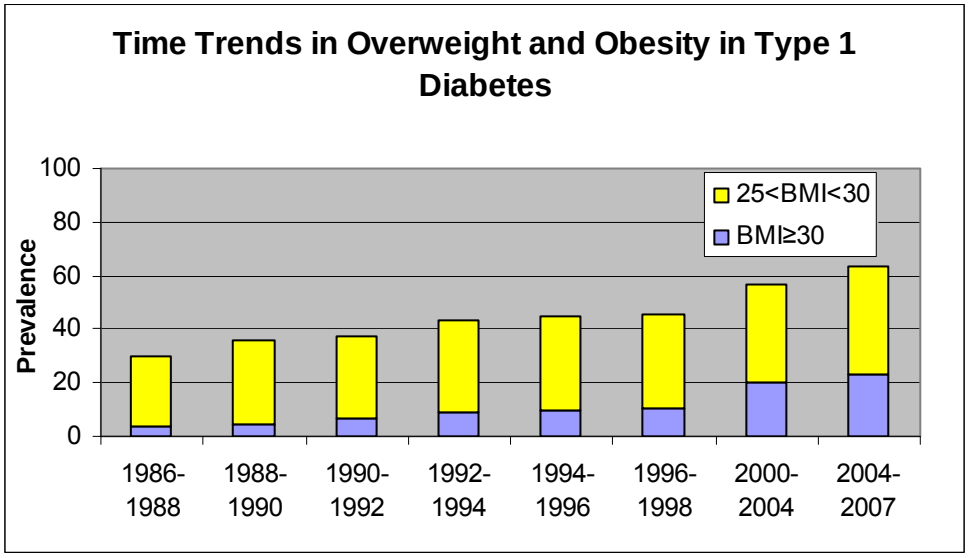

Figure 5-1. Time trends in the prevalence of overweight and obesity in type 1 diabetes.

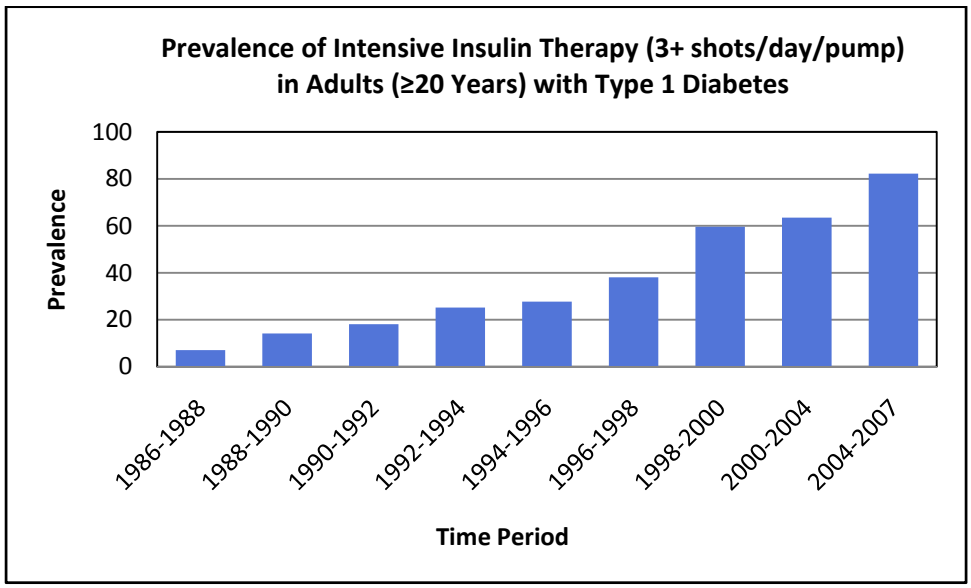

Figure 5-2. Time trends in the prevalence of intensive insulin therapy in type 1 diabetes.

Table 5-1. Age-specific Prevalence of Underweight, Normal Weight, Overweight, and Obese for those Aged 40-49 Years at Each Time Period, n (%).

	<20	20-24.9	25-25.9	≥30
1986-1988	4 (9.1)	29 (65.9)	10 (22.7)	1 (2.3)
1988-1990	3 (5.8)	29 (55.8)	17 (32.7)	3 (5.8)
1990-1992	6 (8.5)	43 (60.6)	18 (25.4)	4 (5.6)
1992-1994	10 (12.2)	39 (47.6)	27 (32.9)	6 (7.3)
1994-1996	14 (11.9)	58 (49.2)	35 (29.7)	11 (9.3)
2004-2007	5 (2.9)	51 (30.0)	77 (45.3)	37 (21.8)

Table 5-2. Predictors of Final Body Mass Index (BMI) Adjusted for Baseline BMI and Follow-up Time in Adults with Type 1 Diabetes

	BMI change	
	β (SE)	p-value
Age (years)	-0.19 (0.15)	0.21
Diabetes duration (years)	-0.12 (0.15)	0.43
HbA1c (%)	0.35 (0.15)	0.03
Insulin dose (U/kg/dy)	0.10 (0.16)	0.54
Insulin injections/day*	-0.02 (0.15)	0.88
Physical activity (kcal/week)*	0.15 (0.16)	0.35
Sex *(female)*	-0.16 (0.30)	0.58
Coronary artery disease	-0.21 (0.58)	0.72
Overt nephropathy	-0.78 (0.35)	0.03
Proliferative Retinopathy	0.10 (0.33)	0.76
Symptomatic autonomic neuropathy	-1.41 (0.61)	0.02
Distal symmetrical polyneuropathy	-0.55 (0.34)	0.11
Lower extremity arterial disease	-0.53 (0.55)	0.34
Intensive insulin therapy:		
Incident during follow-up vs Never	0.77 (0.37)	0.04
Family history of T2D	0.39 (0.43)	0.36
Annual Household Income		
<$20,000	0.34 (0.34)	0.31
Some college	0.006 (0.33)	0.98
Current smoker	0.08 (0.36)	0.83
Alcohol consumption: 3+gl/wk	-0.36 (0.36)	0.31

BMI units=kg/m²*Natural-logarithmically transformed before analysis.

Table 5-3. Predictors of Final Body Mass Index in Adults with Type 1 Diabetes: Multivariable Adjusted Linear Regression Model.

	Standardized regression coefficient (SE)	P-value
HbA1c, %	0.43 (0.18)	0.02
Initiating intensive insulin therapy after baseline	0.77 (0.42)	0.07
Symptomatic autonomic neuropathy	-1.49 (0.67)	0.03
Baseline BMI	1.01 (0.06)	<0.0001
Follow-up time	0.16 (0.04)	0.0006
Intercept	-5.62 (2.91	0.05

Stepwise selection also allowed for age, insulin dose/kg of body weight, and overt nephropathy.

Table 5-4. Characteristics by Body Mass Index (BMI) Category in Adults 18 Years and Older in 1986-1988, % (n), mean (SD), or median (IQR).

Characteristics	BMI <20	20≤BMI<25	25≤BMI<30	BMI ≥30	p-trend
Sex (female), %	57.6 (34)	49.7 (171)	42.8 (71)	70.0 (14)	0.37
Age (years)	30.2±6.2	29.1±7.1	28.8±6.5	28.8±6.9	0.39
HbA1c (%)	8.8±1.7	8.8±1.5	8.4±1.3†	8.6±1.1	0.24
Intensive insulin therapy (%)	0.0 (0)	6.8 (22)	9.4 (15)	10.5 (2)	0.02
Systolic blood pressure (mm Hg)	109.2 (17.7)†	114.5 (16.8)	117.4 (14.0)	118.6 (16.5)	0.01
Diastolic blood pressure (mm Hg)	70.1 (12.3)	72.7 (10.7)	76.6 (10.6)¶	76.4 (12.0)	0.005
Hypertension (%)	13.6 (8)	17.4 (60)	19.4 (32)	30.0 (6)	0.12
HDL cholesterol (mg/dL)	55.9 (13.3)	54.8 (13.0)	51.1 (11.8)‡	50.4 (8.4)	0.03
Non-HDL cholesterol (mg/dL)	128.8 (35.0)	137.1 (40.8)	146.7 (47.2)†	162.2 (47.4)†	0.0007
AER* (μg/min), median (IQR)	42.2 (5.6-423.8)	17.4 (7.3-241.4)	22.7 (9.4-297.9)	42.2 (5.8-423.8)	0.65
Current Smoker (%)	28.8 (17)	27.0 (93) ‡	15.1 (25)	10.0 (2)	0.001

AER=albumin excretion rate HDL=high density lipoprotein *Natural logarithmically transformed before analysis

significantly different than 20≤BMI<25 at p<0.05. ‡ significantly different than 20≤BMI<25 at p<0.01.

¶ significantly different than 20≤BMI<25 at p<0.001 § significantly different than 20≤BMI<25 at p<0.0001

Table 5-5. Characteristics by Body Mass Index (BMI) Category in Adults 18 Years and Older in 2004-2007, % (n), mean (SD), or median (IQR).

Characteristics	BMI <20	20≤BMI<25	25≤BMI<30	BMI ≥30	p-trend
Sex (female), %	69.2 (9)	53.7 (65)	49.1 (78)	51.2 (44)	0.34
Age (years)	50.4 (6.7) ‡	44.6 (8.1)	44.6 (7.3)	44.9 (7.1)	0.02
HbA1c (%)	7.9 (2.2)	7.7 (1.9)	8.0 (1.6)	7.8 (1.6)	0.93
Intensive insulin therapy (%)	66.7 (8)	81.1 (86)	80.3 (118)	89.6 (69)	0.06
Systolic blood pressure (mm Hg)	118.8 (14)	111.6 (16.5)	119.1 (15.1) ¶	119.2 (17.6) ‡	0.47
Diastolic blood pressure (mm Hg)	62.4 (15.6)	62.6 (10.0)	68.1 (9.6)¶	68.5 (12.0)¶	0.02
Hypertension (%)	40.0 (4)	26.5 (26)	37.1 (52)	45.7 (37)	0.02
HDL cholesterol (mg/dL)	65.3 (20.8)	61.9 (16.1)	58.1 (15.8)	54.7 (16.1)‡	0.03
Non-HDL cholesterol (mg/dL)	106.0 (23.4)	108.1 (32.3)	117.4 (36.8)	128.4 (39.2) ¶	0.04
AER* (μg/min), median (IQR)	4.9 (4.4-7.1)	6.6 (3.8-26.5)	9.2 (4.8-75.7)	12.1 (5.1-57.5)	0.58
Current Smoker (%)	23.1 (3)	10.0 (12)	12.0 (19)	5.9 (5)	.16

AER=albumin excretion rate HDL=high density lipoprotein *Natural logarithmically transformed before analysis

significantly different than 20≤BMI<25 at p<0.05. ‡ significantly different than 20≤BMI<25 at p<0.01.

¶ significantly different than 20≤BMI<25 at p<0.001 § significantly different than 20≤BMI<25 at p<0.0001

6.0 PAPER 2: ADIPOSITY AND MORTALITY IN TYPE 1 DIABETES

Under Review

Baqiyyah Conway[1], Rachel Miller[1], Tina Costacou[1], Linda Fried[2], Sheryl Kelsey[1], Rhobert Evans[1], Trevor Orchard[1]

[1]University of Pittsburgh, Department of Epidemiology, Graduate School of Public Health

[2]University of Pittsburgh, School of Medicine

6.1 ABSTRACT

Background: In the general population, adiposity exhibits a J- or U-shaped relationship with mortality; however, in catabolic states this relationship is often inversely linear. We have recently documented an age-independent increase in overweight/obesity in the Pittsburgh Epidemiology of Diabetes Complications study (EDC) of type 1 diabetes (T1D). As intensified insulin therapy (IIT) may promote weight gain, the impact of weight gain in T1D is critical to assess. We therefore assessed the association of adiposity with mortality in 655 EDC participants, followed for up to twenty years.

Methods: Individuals were categorized as underweight (BMI <20), normal (20≤BMI<25), overweight (25≤BMI<30), or obese (BMI≥30). Cox models were constructed using BMI and covariates at baseline, updated means during follow-up, time-varying (reflecting most recent status), and change during adulthood as predictors of mortality.

Results: During follow-up, the prevalence of IIT (3+ insulin shots daily and/or pump) increased from 7% to 82%. The prevalence of overweight increased by 47%, while the prevalence of obesity increased 7-fold. There were 146 deaths. In unadjusted models BMI (modeled continuously) demonstrated a quadratic relationship with mortality ($p=0.002$, <0.0001, <0.0001 for baseline, updated mean, and time-varying models, respectively). However, only in the time-varying model were the obese significantly different from the normal weight. In both the updated mean and time varying models, the underweight were at significantly greater risk than the normal weight ($p<0.0001$ both models), while the baseline model revealed no specific differences by BMI category. The nonlinear relationship of adiposity with mortality remained even after adjustment for diabetes complications, biological, or socioeconomic/lifestyle risk factors, with the exception of baseline socioeconomic/lifestyle risk factors where a linear

association emerged. Adjustment for waist circumference eliminated the risk in the obese. Finally, weight gain was inversely associated with mortality.

Conclusion: The relationship of adiposity with mortality in T1D now appears to resemble that of the general population, albeit with a marked increased risk in those underweight. While obesity shows some relationship with mortality, weight gain appears protective and BMI in the overweight, non-obese range does not predict death. Further study is needed before optimal weight goals can be set in T1D.

6.2 INTRODUCTION

Mortality in type 1 diabetes (T1D) is greatly accelerated, occurring several decades earlier than in the general population (1, 2, 3, 4, 5). Although adiposity is associated with increased risk of many chronic diseases in the general population (6,7,8,9), there is some evidence that this relationship may not be so straightforward, particularly for mortality, where U- and J-shaped relationships are often observed (10,11,12,13,14). Furthermore, within diseased populations, increased adiposity is often associated with longer survival (15, 16, 17).

Within type 1 diabetes, coronary artery disease (CAD) is the leading cause of death overall, although renal disease, especially at shorter and medium term durations of diabetes, is also a major cause (1, 18, 19). Chronic complications such as these are part of the natural history of type 1 diabetes and thus may confound the relationship of adiposity with mortality. Furthermore, in T1D the association of overweight and obesity with mortality may be further complicated, as intensive insulin therapy is associated with both weight gain and a reduction in complications.

Few studies have fully investigated adiposity as a risk factor for mortality in T1D. In the studies in which it was considered, adiposity has not been demonstrated to be a risk factor for mortality (19, 20, 21), with the exception of Roy et al (22) in which adiposity was associated with longer survival. However, with the marked increase in overweight and obesity and therefore a much wider range in adiposity in T1D, this situation may be changing. This paper investigates the association of adiposity with mortality above and beyond the known risk factors for mortality in T1D. To both serve the needs of the practicing clinician and to account for confounding and address reverse causation, adiposity is investigated as both a baseline predictor and as a function of BMI change over an 18-20 year time period.

6.3 METHODS

The Pittsburgh Epidemiology of Diabetes Complications Study is a prospective study based on a well-defined cohort of individuals with childhood-onset (<17 years old) type 1 diabetes mellitus. There were 658 eligible subjects (325 women and 333 men; 98% Caucasian) diagnosed between January 1, 1950, and May 30, 1980, who were first seen between 1986 to 1988; 654 provided BMI and some mortality follow-up data. Mortality follow-up was censored January 1, 2008.

At biennial cycles of examinations, information was collected concerning demographic characteristics, medical history, and health care behaviors as previously described (23,24). At each cycle, both a standardized medical history and clinical examination were performed by a trained internist to document complications of diabetes.

Participants were weighed in light clothing and without shoes on a balance beam scale. Height was measured using a stadiometer. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. For the first ten years of follow-up, all height and weight were measured. Beginning in 1998, exams were limited to certain subgroups, so measured height and weight data were not fully available. Between 2004 and 2007, an eighteen year follow-up exam was again made available to all participants. Self-reported data from the medical history questionnaire were used when measured data were not available, representing 83%, 33%, and 19% of the data from the 14^{th}, 16^{th}, and 18^{th} year follow-up periods. The validity of the reported height and weight has been reported (Paper 1). Underweight was defined as a BMI <20kg/m²; normal weight a BMI ≥ 20 kg/m² < 25 kg/m²;

overweight a BMI ≥25 kg/m² < 30 kg/m²; obesity a BMI ≥30 kg/m². Weight change was defined as BMI at the 10-year follow-up exam minus baseline BMI.

Fasting blood samples were assayed for lipids, lipoproteins, glycosylated hemoglobin (HbA1), creatinine, and HCT. High-density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure, a modification of the Lipid Research Clinics method. Cholesterol was measured enzymatically (25). Stable glycosylated hemoglobin A_1 (HbA_1) was originally measured in saline-incubated samples by microcolumn cation exchange chromatography (Isolab, Akron, Ohio, USA). On October 26, 1987, the method was changed to high-performance liquid chromatography (HPLC) (Diamat, Bio-Rad Laboratories, Hercules, CA, USA). The two methods were highly correlated (r = 0.95). Beginning in 1998, HbA_{1c} was measured using the DCA2000 analyzer. Original HbA_1 (1986 to 1998) and A_{1c} (1998 to 2004) were converted to Diabetes Control and Complications Trial (DCCT) aligned HbA_{1c} values using regression formulas derived from duplicate analyses (DCCT HbA_{1c} = [0.83 * EDC HbA_1] + 0.14; DCCT HbA_{1c} = [EDC HbA_{1c} − 1.13]/0.81). Blood pressure was measured by a random-zero sphygmomanometer according to a standardized protocol (26) after a 5-minute rest period. Blood pressure levels were analyzed, using the mean of the second and third readings. Insulin dose/kg body weight was defined as the total daily units of insulin divided by the body weight in kilograms.

Intensive insulin therapy was defined as having three or more insulin injections per day or using an insulin pump. Physical activity was determined by number of flights climbed per day, city blocks or equivalent walked per day, and mets calculated from sports/exercise*minutes of participation*number of times per week, and is expressed in kilocalories. Complications were assessed as previously described (23, 24) and overt nephropathy (ON) defined as albumin

excretion rate >200 μg/min in 2 or 3 timed urine samples (27) or a history of renal dialysis or transplant. All procedures were approved by the Institutional Review Board of the University of Pittsburgh.

Statistical analyses

The student's *t* test and chi-square tests were used to examine univariate associations of BMI category with mortality risk factors. Cox proportional hazards modeling was used to determine the independent predictive ability of BMI category on mortality, with normal weight being used as the reference. Risk factors were grouped into three categories (complications, biological risk factors, and socioeconomic/lifestyle risk factors) and models fitted separately for each group as predictors of mortality. The number of participants included in different statistical models varied due to item nonresponse. Preliminary analyses revealed that the relationship of BMI category with mortality was essentially similar in each sex; therefore sex-specific analyses were not conducted. Children younger than 18 years old (n=66) were excluded from baseline analyses. Cox models with baseline risk factors were used to determine the association of baseline BMI category on mortality. Cox models with updated mean covariates were also used to determine the association of average BMI status during follow-up with mortality. The updated mean was determined by taking the average value of a risk factor during follow-up. For dichotomous variables, the updated variable was entered as the number of years with the given risk factor. The forward selection procedure was used to identify the most predictive risk factors; BMI category was forced into all models. Cox modeling was also used to determine the association of the residuals of weight change with mortality in adults at least 18 years old at baseline. Variables are expressed as per standard deviation change in the continuous variable.

All tests were two-tailed and a p-value <0.05 was considered significant. All analyses were conducted using SAS version 9.1 (Cary, North Carolina).

6.4 RESULTS

Body mass index data were available on 655 participants and 99.8% provided some follow-up data. Participants (mean age and diabetes duration 28 and 19 years, respectively) were followed for a median of 18.2 years (range: 0.2-20.6 years). There were 146 deaths (22%).

Baseline characteristics of the participants (aged 18 years and older) by BMI category are described in Table 6-1. At baseline, compared to normal weight participants, obese participants had a higher non-HDL cholesterol. Overweight participants also had, compared to normal weight, a lower HbA1c, were on more insulin injections per day, had a higher diastolic blood pressure, a lower HDL cholesterol, a lower prevalence of symptomatic autonomic neuropathy, and were less likely to be current smokers.

During follow-up, the prevalence of intensive insulin therapy (IIT-3+ insulin shots daily and/or pump) increased from 7% to 82% (Paper 1). The prevalence of being overweight increased by from 28.6% to 42.0% while the prevalence of obesity increased 7-fold (from 3.4 to 22.7%) over an average of 18 years of follow-up (Paper 1). Figure 6-1 shows the unadjusted association of mortality with baseline BMI category (panel a), updated mean BMI status during follow-up (panel b), and most recent BMI status prior to event or censoring (panel c). Baseline BMI demonstrated a slight U-shape relationship with mortality (p for quadratic term=0.002), such that there was a higher risk in the underweight relative to the normal weight and a more

marked higher level of risk in the obese. However, average BMI during follow-up (updated means model), revealed a reverse J-shaped relationship with mortality, such that those with an average BMI in the obese category were at slightly increased risk of mortality relative to the normal weight, but the greatest risk was in those with an average BMI less than 20 kg/m² (p for quadratic term <0.0001). The BMI nadir for mortality risk fell in the overweight category. In the time-varying model, reflecting most recent BMI status prior to event or censoring, the risk in the underweight and obese appeared to be even stronger, compared to the baseline and updated means models, with the risk in the underweight being 3 times, and the risk in the obese twice, that of the normal weight (p for quadratic term<0.0001).

The results of modeling baseline risk markers by type, namely, complications, biological risk factors, and socioeconomic/lifestyle risk factors are shown in Table 6-2. As sample size with full data available varies for these three risk marker categories, the base models show the relationship of BMI category with mortality, specific for that population, adjusted only for age and sex. The increased risk in the obese was attenuated after adjustment for chronic complications of diabetes, and eliminated after adjustment for biological and socioeconomic risk factors. The multivariable baseline models showed weak U- and J-shaped relationships with mortality.

Table 6-3 shows the relationship of updated mean BMI category with mortality. In contrast to the baseline model, average BMI status during follow-up demonstrated a strong U-shaped relationship with mortality after adjustment for the proportion of follow-up time spent with complications independently predictive of mortality during follow-up. Adjusting for these complications did not account for the increased risk in the underweight or obese. Similarly, compared to the base model, adjusting for updated mean biological risk factors had very little

effect on the BMI relationship with mortality. However, adjusting for socioeconomic lifestyle risk factors, where intensive insulin therapy emerged as a protective factor, eliminated the risk in the underweight while no substantial change in risk of the obese was noted. Comparing both the base and the adjusted updated mean models with baseline, the risk in the underweight appears to have increased while that in the obese to have been reduced, with the exception of the socioeconomic/lifestyle risk factors adjusted analyses, where no change in risk appeared to be observed in the obese.

Table 6-4 shows the relationship of most recent BMI status with mortality. Thus adjusting for time-varying complication status, appears to have attenuated the risk in the underweight compared to the base model, while adjusting for time-varying biological or socioeconomic/lifestyle risk factors did not appear to have substantial affect the relationship of BMI category with mortality. Compared to the baseline and updated means models, whether adjusting for complications, biological risk factors, or socioeconomic/lifestyle risk factors, base and adjusted time-varying models show a stronger (larger effect size) adverse relationship with being underweight. Base time-varying models show no substantial difference from updated mean base models in obesity's relationship with mortality, but suggest an attenuation in risk compared to baseline base models. This same pattern is not observed for the adjusted models where the time-varying adjusted models appear similar to the baseline adjusted models in the relationship of obesity with mortality.

Figure 6-2 shows the relationship of baseline BMI category, updated means BMI category, and time-varying (most recent) BMI category with mortality after adjusting for waist circumference. Controlling for waist circumference eliminated the relationship of obesity with

mortality in all three time models. Being overweight was also protective in the baseline and updated means models.

Figure 6-3 shows the association of change in BMI in adults during the first ten years of follow-up with mortality during years the subsequent 10 years. BMI change ranged from -6.5 to 11.0 kg/m². There was a significant trend for a positive change in BMI to be associated with a lower mortality, such that for each tertile of change, risk was reduced by approximately one-third (p for trend=0.01). In multivariable analysis in adults 18 years and older, after controlling for baseline BMI, age, and albumin excretion rate, and allowing for intensive insulin therapy and other univariate significant risk factors, each one unit positive change in the residuals of BMI change during the first 10 years of follow-up was associated with a 12% decreased risk of mortality during follow-up years 11-20 (HR=0.88, 0.80-0.97) (Table 6-5).

6.5 DISCUSSION

In this report, we have documented the association of both baseline BMI and BMI measured repeatedly during follow-up with mortality in type 1 diabetes. To our knowledge, this is the first study to document the long term association of adiposity with mortality in type 1 diabetes, where adiposity was the predictor of interest. We have shown that baseline BMI demonstrated a slight U-shaped relationship with 20-year mortality. We have also shown during follow-up the role of underweight as a predictor increases and conversely, we have shown that weight gain in adults with type 1 diabetes is protective against mortality. Finally, we have shown that the role of overweight and obesity in increasing mortality appears to be mediated by waist circumference.

The relationship of BMI with mortality in this population was not linear, neither at baseline nor throughout follow-up. Although Roy et al (22), found BMI to be inversely associated with mortality (HR=0.94, 0.91-0.97) in a large African American population with type 1 diabetes, in general in type 1 diabetes the relationship of BMI with mortality has been reported to be nonsignificant, although apart from the present study, no study has specifically looked at mortality in this population with adiposity as the explanatory variable of interest. The association of BMI with mortality in the general population is usually found to exhibit a U-or J-shaped curve, although some argue that this may be due to failure to exclude for pre-existing disease, smoking, or recent weight loss (28, 29, 30). Against this, others have shown that even after excluding for pre-existing disease, smoking, or recent weight loss, this non-linear relationship persists (13, 31, 32). Excluding for pre-existing disease in type 1 diabetes may be debatable, as type 1 diabetes itself is a pre-existing disease. Furthermore, 55% of our adult population at baseline, with a mean age of 29, had at least one of the long-term diabetes complications at study entry and 23% of our adult population at baseline were smokers; therefore, exclusion of smokers and those with pre-existing disease would not be representative of the type 1 diabetes population. However, we have attempted to account for weight loss, smoking, and long-term complications by looking at them biennially in updated means and time-dependent survival analyses. In our population, after examining the association of BMI on mortality with up to twenty-years of follow-up, BMI failed to show a linear relationship with mortality, demonstrating a similar relationship observed in the general population, with the exception of a much increased risk in those whose average or most recent BMI was in the underweight category.

The issue as to whether complications (or comorbidities) should be adjusted for in analyses of overweight and obesity is complex. It might be argued that it is inappropriate to control for complications such as coronary artery disease or kidney disease as they may be intermediates in the causal pathway between obesity and mortality. However, complications such as these are a part of the natural history of type 1 diabetes. Coronary artery disease risk factors such as hypertension and dyslipidemia also tend to increase with adiposity and as we do not know to what extent they are part of the natural history of obesity within type 1 diabetes, i.e. risk additive to the underlying risk these complications already pose in type 1 diabetes, it is also appropriate to control for them in order to determine the residual or independent effect of adiposity on mortality. In our analyses we have therefore presented multivariate data on all three levels-comorbidities, biological risk factors/mediators, socioeconomic/lifestyle factors in order to serve the multiple objectives of those interested in this use.

A major biologic risk factor for mortality in our population was HbA1c. HbA1c has been shown previously to be a risk factor for mortality in type 1 diabetes (1, 33) and Shankar et al (33) noted that the mortality risk associated with HbA1c was greater at a higher BMI although this was not noticed by Stadler et al (1). Intensive insulin therapy has been shown to reduce the risk of long term diabetes complications, the major causes of death in type 1 diabetes; however intensive insulin therapy is also associated with weight gain and its association with weight gain both in this population and in others raises concern that the gains made in the reduction of microvascular disease may be lost with the increased risk of obesity related complications such as CAD, particularly as this increased weight gain associated with intensive insulin therapy is accompanied by a deterioration in the CAD risk profile, although this has not always been observed (34). In this population, both HbA1c and intensive insulin therapy were positive

predictors of weight gain (Paper 1); however, in the prediction of mortality, while HbA1c was directly predictive, average amount of time spent on intensive insulin therapy during follow-up was protective. After accounting for age and sex, further adjusting for time on intensive insulin therapy during follow-up in the updated means socioeconomic/lifestyle risk factors model appeared to eliminate the risk in the underweight while having no effect on the overweight or obese. Thus it appears the elimination of risk in the underweight after adjustment for intensive insulin therapy may due to treatment by indication where thinner participants in poorer control may have been advised to intensify their insulin therapy due to comorbid conditions.

Other socioeconomic lifestyle risk factors associated with adiposity and mortality are also complex in type 1 diabetes. In our analyses of predictors of weight change from baseline to last follow-up in this population, neither smoking, education, nor income were predictive; however, physical activity was an inverse predictor of a 2 kg/m^2 loss, i.e. the more physically active, the less likely they were to lose weight (Paper 1). In the current analyses of mortality, physical activity was a consistent protective factor against mortality in all three of the time to event models looking at BMI category. In type 1 diabetes, increased physical activity may be intentional and related to a healthier lifestyle; conversely, decreased physical activity may be largely a marker of morbidity associated with the long term complications of the disease. Smoking, associated with lower socioeconomic status, leanness, and mortality in the general population, was a strong predictor of mortality in our population only in the baseline model; however, at baseline in the population with full data on socioeconomic/lifestyle risk factors, the underweight were never different from the normal weight in mortality risk, whether adjusting only for sex and age or for socioeconomic/lifestyle risk factors. Income also was an independent predictor of mortality at baseline and in the time-varying analysis, i.e. analysis assessing BMI

near to the time of event. However, only at baseline was the effect of obesity accounted for by socioeconomic /lifestyle risk factors, suggesting that the closer to the time of event, the more important obesity is as a risk factor itself than as a marker of socioeconomic status.

The effect of change in weight over time is yet another dimension of the complex association of adiposity with mortality in type 1 diabetes. In adults eighteen years and older, we observed a protective effect of weight gain, as assessed by BMI, and mortality, such that with each increasing tertile of change, mortality was reduced by approximately thirty-three percent. While it is not unexpected for weight loss to predict mortality in a population with pre-existing disease, i.e. type 1 diabetes itself, we found that weight gain had beneficial survival effects beyond that of even the relatively weight stable, an observation that should be underscored as average baseline BMI in this population was 23.8 kg/m^2, a value well within the normal weight range. Weight change modeled as a continuous variable was inversely associated with mortality even after adjustment for other factors associated with mortality. Although weight gain in adulthood has been reported to be a positive predictor of mortality (32) and that weight loss is beneficial if volition is taken into account (35, 36), in the main, general population studies have demonstrated an inverse or U-shaped relationship between weight change and mortality (37, 38, 39), even when pre-existing illness and smoking have been taken into account (40, 41). In our population with type 1 diabetes, with a mean age of 28 years at baseline, weight gain appeared to be protective against mortality in middle-age.

A major observation of this study was that waist circumference accounted for the U-shaped relationship of BMI with mortality. Some have hypothesized that, contrary to being a failure to adequately control for smoking, subclinical, or occult disease, the non-linear relationship observed between BMI and mortality may be a consequence of BMI being a

composite of both fat and fat-free mass (42,43,44), not simply a surrogate for overall adiposity. Bigaard et al (44) demonstrated that the U-shaped relationship between BMI and mortality was due to the J-shaped relationship of fat mass and the reverse J-shaped relationship of fat-free mass with mortality. Several studies have shown that adjustment for waist circumference, a surrogate for abdominal adiposity (45, 46, 47, 48), eliminates or attenuates BMI's nonlinear relationship of with mortality (49, 50). In our type 1 diabetes population as well, adjustment for waist circumference also eliminated the U-shaped relationship between BMI and mortality such that both overweight and obesity were protective while the risk in the underweight appeared to be even greater. Beyond suggesting that the effect of obesity on mortality is mediated through central adiposity, this is suggestive of a protective effect for both peripheral body fat and for lean body mass. Consistent findings have also been reported in the literature (51, 52, 42).

Strengths and Limitations

Major strengths of our study include the prospective nature of the design, measured height and weight, repeated assessment of height and weight as well other risk factors over time, and a long follow-up period. As BMI was assessed every two years, we were able to assess the affects of weight change on mortality, demonstrating an increased risk in those who lost weight and a decreased risk in those who gained the most weight, a weight gain well beyond a normalization of weight.

A major strength of this paper could also be one of its limitations. As this study tracked mortality over 20 years, changes in both diabetes treatment and risk factor management associated with obesity may have affected mortality results. However, we have attempted to account for this, as well as weight change, in the time-varying covariate models. Results from

these models did not reveal a major change in the association of obesity with mortality, but a they did reveal a much stronger relationship with being underweight and a reversal of the relationship of being overweight with mortality, even after long-term diabetes complications and intensive insulin therapy were taken into account. This would seem to suggest that with improved diabetes treatment and risk factor management, a modest increased adiposity is actually beneficial, as also suggested by our analysis of weight change.

Conclusion

With the rise in overweight and obesity in type 1 diabetes, and the rise in intensive insulin therapy, the traditional view of type 1 diabetes as a starvation state is clearly outdated. Nevertheless, an interaction between catabolic and anabolic imbalances is evidenced by the increased risk in the obese and the greatly increased risk in the underweight. Although an understanding of the risk associated with obesity is of interest, in terms of a disease traditionally characterized by relative thinness and enhanced catabolism, of greater concern maybe the excess mortality risk due to leanness. Given the wide BMI range associated with minimal mortality (20-29 kg/m^2), weight gain is not necessarily a bad occurrence in type 1 diabetes. Though frank obesity should be avoided, risk factor management may be better focused on glycemia, blood pressure and lipids, and other complication specific risk factors, than on overweight per se.

Acknowledgments

This research was funded by the National Institutes of Health grant DK34818. We are also indebted to the participants of the Pittsburgh Epidemiology of Diabetes Complications Study for their dedication and cooperation to the advancement of knowledge in the scientific community relating to the complications of type 1 diabetes with the ultimate goal of prolonging the survival of those with this disease.

6.6 CITED WORKS

1. Stadler M, Auinger M, Anderwald C, Kastenbauer T, Kramar R, Feinbock C, Irsigler K. Long-term mortality and incidence of renal dialysis and transplantation in type 1 diabetes mellitus. *J Clin Endocrinol Metab* 2006; 91:3814-3820.

2. Sochett E, Daneman D. Early diabetes related complications in children and adolescents with type 1 diabetes. Implications for screening and intervention. *Endocrinology Metab Clin Am* 1999; 28:865-882.

3. Pambianco G, Costacou T, Ellis D, Becker D, Klein R, Orchard T. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. *Diabetes*. 2006; 55(5):1463-9.

4. Nishimura R, LaPorte R, Dorman J, Tajima N, Becker D, Orchard T. Mortality trends in type 1 diabetes. The Allegheny County (Pennsylvania) Registry 1965-1999. *Diabetes Care* 2001; 24:823-827.

5. Dorman J, LaPorte R, Kuller L, Cruickshanks K, Orchard T, Wagener D, Becker D, Cavender D, Drash A. The Pittsburgh insulin-dependent diabetes mellitus (IDDM) morbidity and mortality study. Mortality results. *Diabetes* 1984; 33:271-276.6.

6. Eckel R, Krauss R. American Heart Association Call to Action: Obesity as a major risk factor for coronary heart disease. *Circulation* 1998; 97: 2099-2100.

7. Kenechiah S, Evans J, Levy D, Wilson P, Benjamin E, Larson M, Kannel W,Vasan R. Obesity and the risk of heart failure. *The New England Journal of Medicine* 2002; 347:305-313.

8. Baik I, Curhan G, Rimm E, Bendich A. Willett W, Fawzi, W. A prospective study of age and lifestyle factors in relation to community-acquired pneumonia in U.S. men and women. *Arch Intern Med* 2000; 160: 3082-3088.

9. Calle E, Rodriguez C, Walker-Thurmond K, Thun M. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003; 348:1625-1638.

10. Flegal K, Graubard B, Williamson D, Gail M. Excess Deaths Associated with Underweight, Overweight, and Obesity. *JAMA* 2005; 293:1861-1867.

11. Allison D, Zhu S, Plankey M, Faith M, Heo M. Differential associations of body mass index and adiposity with all-cause mortality among men in the first and second National Health and Nutrition Examination Surveys (NHANES I and NHANES II) follow-up studies. *International Journal of Obesity* 2002;26:410-416.

12. Freedman M, Sigurdson A, Rajaraman P, Michele D, Linet M, Ron E. The mortality risk of smoking and obesity combined. *American Journal of Preventative Medicine* 2006; 31(5): 355-362.

13. Allison D, Fontaine K, Manson J, Stevens J, VanItallie T. Annual deaths attributable to obesity in the United States. *Journal of the American Medical Association* 1999; 282:1530-1538.

14. Calle E, Thun M, Petrelli J, Rodriguez C, Heath C. Body-mass index and mortality in a prospective cohort of U.S. adults. *NEJM* 1999; 341(15): 1097-1105.

15. Nicoletti I, Mariantonietta C, Morando G, Benazzi C, Prati D, Morani G, Rossi A, Zardini P, Vassanelli C. Impact of body mass index on short-term outcome after acute myocardial

infarction: Does excess body weight have a paradoxical protective role? *International Journal of Cardiology* 2006; 107:395-399.

16. Lavie C, Osman A, Milani R, Mehra M. Body composition and prognosis in chronic systolic heart failure: the obesity paradox? *Am J Card Imaging* 2003; 91:891-894.

17. Kalantar-Zadeh K, Horwich T, Oreopoulos A, Kovesdy C, Younessi H, Anker S, Morley J. Risk factor paradox in wasting diseases. *Current Opinion in Clinical Nutrition and Metabolic Care* 2007; 10:433-442.

18. Bosnyak A, Nishimura R, Hughes M, Tajima N, Becker D, Tuomilehtos J. Excess mortality in Black compared with White patients with type 1 diabetes: an examination of underlying causes. *Diabet Med* 2005; 22:1636-1641.

19. Muhlhauser I, Overmann H, Bender R, Jorgens V, Berger M. Prediction of mortality and end-stage diabetic complications in patients with type 1 diabetes mellitus on intensified therapy. *Diabetic Medicine* 2000; 17:727-734.

20. Bulpitt C, Sleightholm M, Hunt B, Fletcher A, Palmer A, Kohner E. Causes of death and risk factors in young and old diabetic patients referred to retinopathy clinic. *Journal of Diabetes and its Complications* 1996; 10 (3): 160-167.

21. Moy C, Songer T, LaPorte R, Dorman J, Kriska A, Orchard T, Becker D, Drash A. Insulin-dependent diabetes mellitus, physical activity, and death. *American Jounal of Epidemiology* 1993;137:74-81.

22. Roy M, Rendas-Baum R, Skurnick J. Mortality in African-Americans with type 1 diabetes: the New Jersey 725. *Diabetic Medicine* 2006; 23:698-706.

23. Orchard TJ, Dorman JS, Maser RE, Becker DJ, Ellis D, LaPorte RE, Kuller LH, Wolfson S, Drash AL. Factors associated with the avoidance of severe complications after 25 years of insulin-

dependent diabetes mellitus: Pittsburgh Epidemiology of Diabetes Complications Study-I. Diabetes Care 1990;13(7):741-7.

24. Orchard TJ, Dorman JS, Maser RE, Becker DJ, Drash AL, Ellis D, LaPorte RE, Kuller LH. The prevalence of complications in insulin-dependent diabetes mellitus by sex and duration: Pittsburgh Epidemiology of Diabetes Complications Study - II. Diabetes 1990;39:1116-1124.

25. Allain C, Poon L, Chan C, Richmond W, Fu P. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20:470-475.

26. Borhani N, Kass E, Langford H, Payne G, Remington R, Stamler J. The Hypertension Detection and Follow-up Program. *Prev Med* 1976; 5:207-215.

27. Ellis D, Buffone G. A new approach to the evaluation of proteinuric states. Clin Chem 1977; 23:666–670.

28. Singh P, Lindsted K, Fraser G. Body weight and mortality among adults who never smoked. *Am J Epidemiol*. 1999; 150(11):1152-64.

29. Trojano R, Frongillo E, Sobal J, Levitsky D. The relationship of body weight and mortality: a quantitative analysis of combined information from existing studies. *Int J Obes Relat Metab Disord* 1996; 20: 63-75.

30. The BMI in Diverse Populations Collaborative Group. Effect of smoking on the body mass index-mortality relation: empirical evidence from 15 studies. *AM J Epidemiol* 1999; 150:1297-1308.

31. Hjartaker A, Adami H, Lund E, Weiderpass E. Body mass index and mortality in a prospectively studied cohort of Scandinavian women: The women's lifestyle and health cohort study. *European Journal of Epidemiology* 2005; 20:747-754.

32. Manson J, Willett W, Stampfer M, Colditz G, Hunter D, Hankinson S, Hennekens C, Speizer F. Body weight and mortality among women. *N Engl J Med* 1995; 333:677-685.

33. Shankar A, Klein R, Klein B, Moss S. Association between glycosylated hemoglobin level and cardiovascular and all-cause mortality in type 1 diabetes. *American Jounral of Epidemiology* 2007; 166 (4): 393-402.

34. Williams K, Erbey J, Becker D, Trevor J. Improved Glycemic Control Reduces the Impact of Weight Gain on Cardiovascular Risk Factors in Type 1 Diabetes. Diabetes Care 1999; 22:1084-1091.

35. Gregg E, Gerzoff R, Thompson T, Williamson D. Intentional Weight Loss and Death in Overweight and Obese U.S. Adults 35 Years of Age and Older. *Ann Intern Med* 2003; 138:383-389.

36. Wannamethee G, Shaper G, Lennon L. Reasons for Intentional Weight Loss, Unintentional Weight Loss, and Mortality in Older Men. *Arch Intern Med* 2005; 165:1035-1040.

37. Dyer A, Stamler J, Greenland P. Associations of weight change and weight variability with cardiovascular and all-cause mortality in the Chicago Western Electric Company Study. *Am J Epidemiol* 2000; 152(4): 324-333.

38. Rosengren A, Wedel H, Wilhemsen L. Body weight and weight gain during adult life in men in relation to coronary heart disease and mortality. A prospective population study. *Eur Heart J* 1999; 20(4):267-277.

39. Yarnell JW, Patterson CC, Thomas HF, Sweetnam PM. Comparison of weight in middle age, weight at 18 years, and weight change between, in predicting subsequent 14-year mortality and coronary events: Caerphilly Prospective Study. *J Epidemiol Community Health* 2000; 54(5):344-8.

40. Nilsson P, Nilsson J, Hedblad B, Berglund G, F Lindgarde. The enigma of increased non-cancer mortality after weight loss in healthy men who overweight or obese. *Journal of Internal Medicine* 2002; 252:70-78.

41. Lee I, Paffembarger R. Change in body weight and longevity. *JAMA* 1992; 268 (15)2045-2049.

42. Allison D, Faith M, Heo M, Kotler D. Hypothesis concerning the U-shaped relation between body mass index and mortality. *Am J Epidemiol* 1997; 146:339-349.

43. Katzmarzyk P, Craig C, Bouchard C. Adiposity, adipose tissue distribution and mortality rates in the Canada Fitness Survey follow-up study. *Int J Obes Relat Metab Disord* 2002; 26:1054-1059.

44. Bigaard J, Frederiksen K, Tjønneland A, Thomsen B, Overvad K, Heitmann B, Sørensen T. Body fat and fat-free mass and all-cause mortality. *Obes Res*. 2004;12(7):1042-9.

45. Lean M, Han T, Morrison C. Waist circumference as a measure for indicating need for weight management. *BMJ* 1995;311:158-161.

46. Han T, McNeill G, Seidell J, Lean M. Predicting intra-abdominal fatness from anthropometric measures: the influence of stature. *Int J Obes Relat Metab Disord* 1997; 21: 587-593.

47. Janssen I, Heymsfield S, Allison D, Kotler D, Ross R. Body mass index and waist circumference independently contribute to the prediction of nonabdominal, abdominal subcutaneous, and visceral fat. *Am. J. Clin Nutr* 2002; 75: 683-688.

48. Snijder M, van Dam R, Visser M, Seidell J. What aspects of body fat are particularly hazardous and how do we measure them? *Int. J. Epidemiol* 2006; 35: 83-92.

49. Kanaya A, Vittinghoff E, Shlipak M, Resnick H, Visser M, Grady D, Barrett-Connor E. Association of total and central obesity with mortality in postmenopausal women with coronary heart disease. *Am J Epidemiol* 2003; 158(12):1161-70.

50. Bigaard J, Tjønneland A, Thomsen B, Overvad K, Heitmann B, Sørensen T. Waist circumference, BMI, smoking, and mortality in middle-aged men and women. *Obes Res* 2003 Jul;11(7):895-903.

51. Heitmann B, Erikson H, Ellsinger B, Mikkelsen K, Larsson B. Mortality associated with body fat, fat-free mass and body mass index among 60-yer-old Swedish men-a 22-year follow-up. The study of men born in 1913. *International Journal of Obesity* 2000; 24:33-37.

52. Lissner L, Cecilia B, Heitmann B, Seidell J, Bengtsson C. Larger Hip Circumference Independently predicts health and longevity in a Swedish Female Cohort. *Obes Res* 2001; 9:644-646.

6.7 FIGURES AND TABLES

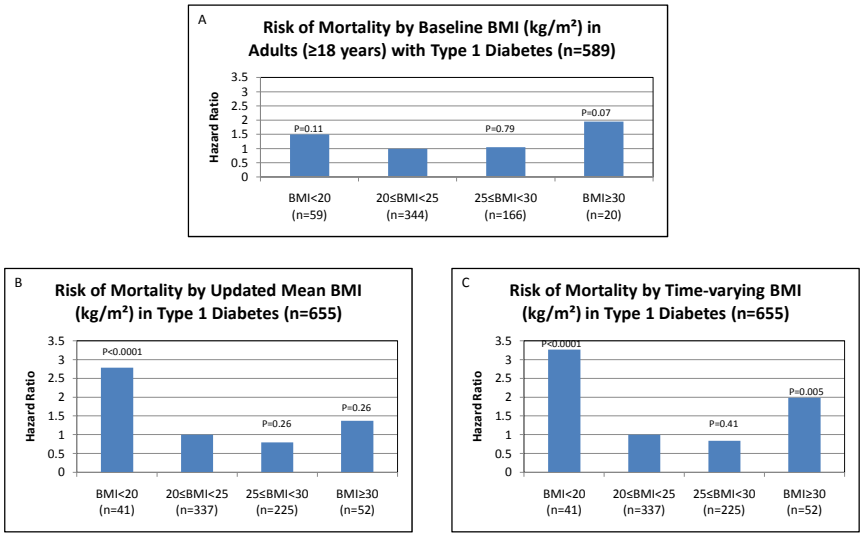

Figure 6-1. Risk of mortality by body mass index (BMI) category.

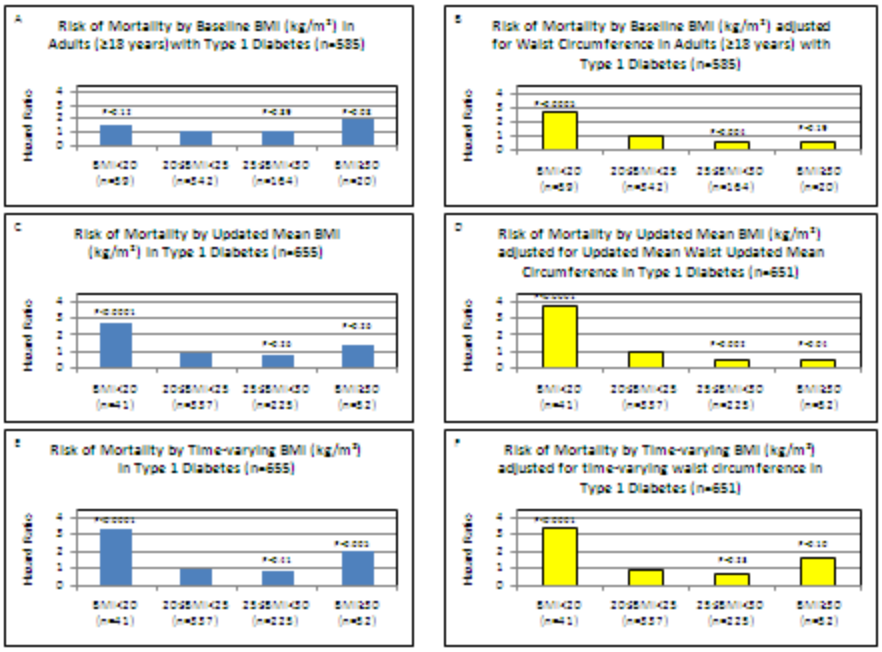

Figure 6-2. Risk of mortality by body mass index (BMI) category, unadjusted and adjusted for waist circumference.

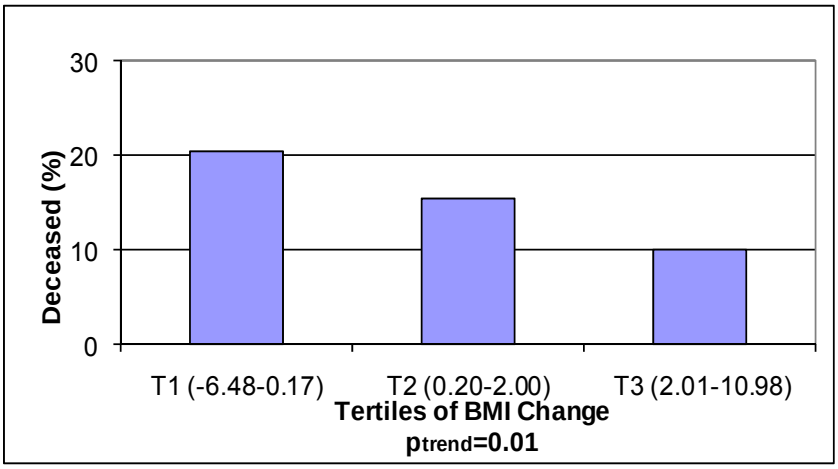

Figure 6-3. Mortality in adults in years 11-20 of follow-up by change in body mass index (BMI) during the first 10 years of follow-up.

Table 6-1. Baseline (1986-1988) Characteristics by Body Mass Index (BMI) Category in Adults 18 Years and Older, mean±SD, % (n), or median (IQR).

Characteristics	BMI <20	20≤BMI<25	25≤BMI<30	BMI ≥30	p-trend
Sex (female), %	57.6 (34)	49.7 (171)	42.8 (71)	70.0 (14)	0.37
Age (years)	30.2±6.2	29.1±7.1	28.8±6.5	28.8±6.9	0.39
Diabetes Duration (years)	21.3±6.2	20.3±7.4	20.3±6.9	21.3±7.3	0.99
BMI (kg/m2)	19.1±0.81§	22.7±1.3	26.9±1.4§	31.6±1.7§	<0.0001
Biological risk factors					
HbA1 (%)	8.8±1.7	8.8±1.5	8.4±1.3‡	8.6±1.1	0.24
Daily insulin dose/kg body weight	0.82 (0.59-0.99)	0.75 (0.58-0.91)	0.77 (0.63-0.93)	0.74 (0.63-0.90)	0.11
Insulin injections per day	1.5 ±0.50	1.6 ± 0.83	1.8± 1.0†	1.9 ± 0.85	0.01
Intensive insulin therapy (%)	0.0 (0)	6.8 (22)	9.4 (15)	10.5 (2)	0.02
Systolic blood pressure (mm Hg)	109.2 ± 17.7	114.5 ± 16.8	117.4 ± 14.0	118.6 ± 16.5	0.01
Diastolic blood pressure (mm Hg)	70.1 ± 12.3	72.7 ± 10.7	76.6 ± 10.6¶	76.4 ± 12.0	0.005
Hypertension (%)	13.6 (8)	17.4 (60)	19.4 (32)	30.0 (6)	0.12
HDL cholesterol (mg/dL)	55.9 ± 13.3	54.8 ± 13.0	51.1 ± 11.6‡	50.4± 8.4	0.03
Non-HDL cholesterol (mg/dL)	128.8 ± 35.0	137.1 ± 40.8	146.7 ± 47.2	162.2 ± 47.4†	0.0007
AER* (μg/min), median (IQR)	31.6 (11.9-178.5)	17.4 (7.3-241.4)	22.7 (9.4-297.9)	42.2 (5.8-423.8)	0.65
WBC (x $10^3/mm^2$)	6.7 ± 2.2	6.6 ± 1.9	6.7 ± 1.9	7.7 ± 2.3	0.05
Complication, %					
Coronary Artery Disease	8.5 (5)	8.5 (29)	8.4 (14)	5.0 (1)	0.78
Overt Nephropathy	27.8 (15)	29.9 (96)	33.3 (50)	42.1 (8)	0.20
Proliferative Retinopathy	39.0 (23)	32.1 (106)	41.1 (65)	42.1 (8)	0.24
Symptomatic Autonomic Neuropathy	10.9 (5)	11.3 (31)	3.7 (5)†	6.3 (1)	0.04
Distal Symmetrical Polyneuropathy	46.4 (26)	32.6 (104)	32.4 (48)	36.8 (7)	0.26
Lower Extremity Arterial Disease	15.3 (9)	8.2 (28)	6.0 (10)	15 (3)	0.23
Sociodemographic/lifestyle risk factors					
Household Income: <$20,000/yr	47.9 (23)	45.7 (122)	36.6 (52)	53.3 (8)	0.23
Education: any college	63.0 (34)	63.4 (203)	65.8 (104)	63.2 (12)	0.69
Physical activity*, median (IQR)	1316 (560-2236)	1414 (616-2752)	1428 (616-2912)	1295(224-2484)	0.40
Alcohol consumption (3+ gl/wk)	23.6 (13)	26.2 (85)	24.2 (38)	5.3 (1)	0.27
Current Smoker (%)	28.8 (17)	27.0 (93)	15.1 (25) ‡	10.0 (2)	0.001

AER=albumin excretion rate WBC=white blood cell count *Natural log transformed before analysis

† significantly different than 20≤BMI<25 at p<0.05 ‡ significantly different than 20≤BMI<25 at p<0.01

¶ significantly different than 20≤BMI<25 at p<0.001 § significantly different than 20≤BMI<25 at p<0.0001

Table 6-2. Independent Baseline Predictors of Mortality in Type 1 Diabetes by Risk Factor Groupings in Adults ≥ 18 Years, Cox Regression Analyses.

	Complications, base and adjusted models (n=465)		Biological Risk Factors, base and adjusted models (n=478)		SES/Lifestyle Risk Factors, base and adjusted models (n=441)	
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Normal weight	Ref	Ref	Ref	Ref	Ref	Ref
Underweight	1.44(0.79-2.63)	1.60 (0.88-2.91)	1.27(0.73-2.21)	1.04(0.58-1.86)	1.15(0.61-2.16)	0.87 (0.45-1.67)
Overweight	1.13 (0.70-1.81)	1.43 (0.86-2.38)	0.99(0.65-1.52)	0.83 (0.54-1.28)	1.15(0.74-1.80)	1.29 (0.83-2.02)
Obese	2.87 (1.21-6.80)	3.37 (1.42-7.98)	2.62(1.12-6.13)	1.70 (0.72-3.98)	3.18(1.42-7.11)	2.25 (0.99-5.13)
Sex (female)	0.69 (0.46-1.05)		0.60(0.42-0.88)		0.58(0.38-0.88)	0.46 (0.30-0.70)
Age (years)	2.15 (1.71-2.72)	1.54 (1.16-2.04)	2.16(1.75-2.66)	1.85 (1.49-2.31)	2.34(1.86-2.94)	2.23 (1.75-2.84)
Overt nephropathy		2.43 (1.52-3.74)				
Symptomatic AN		2.74 (1.66-4.52)				
Distal symmetrical PN		2.28 (1.42-3.66)				
Proliferative retinopathy		1.68 (1.02-2.77)				
HbA1c (%)				1.31 (1.08-1.59)		
AER (µg/min)*				1.48 (1.19-1.84)		
Serum creatinine (mg/dl)				1.40 (1.17-1.67)		
Non-HDLc (mg/dl)				1.26 (1.06-1.49)		
WBC (x $10^3/mm^2$)				1.31 (1.12-1.53)		
Pulse (beats/min)				1.34 (1.15-1.56)		
Physical activity (kcal)						0.74 (0.62-0.88)
Current smoker						1.81 (1.20-2.73)
Low income						2.02 (1.33-3.06)

AN=autonomic neuropathy PN= polyneuropathy AER=albumin excretion rate WBC=white blood cell count *natural logarithmically transformed before analyses Low income= household income <$20,000/yr

Complications model also allowed for coronary artery disease and peripheral vascular disease.

Biological risk factors model also allowed for sex, daily insulin dose/kg of body weight, high density lipoprotein cholesterol, diastolic blood pressure, use of hypertension medications, and hematocrit.

SES/Lifestyle risk factors model also allowed for having some college education, consumption of alcohol ≥3x/wk, and intensive insulin therapy.

Table 6-3. Independent Updated Mean Predictors of Mortality in Type 1 Diabetes by Risk Factor Groupings, Cox Regression Analyses.

	Complications, base and adjusted models (n=519)		Biological Risk Factors, base and adjusted models (n=632)		SES/Lifestyle Risk Factors, base and adjusted models (n=598)	
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	**HR (95% CI)**
Normal weight	Ref	Ref	Ref	Ref	Ref	Ref
Underweight	2.90 (1.60-5.28)	2.66 (1.46-4.85)	2.55 (1.55-4.20)	2.19 (1.30-3.68)	2.07 (1.15-3.72)	1.41(0.78-2.54)
Overweight	0.93 (0.57-1.49)	1.19 (0.73-1.94)	0.72 (0.48-1.08)	0.70 (0.46-1.05)	0.80 (0.53-1.20)	0.82(0.55-1.24)
Obese	2.00 (1.03-3.89)	2.41 (1.22-4.76)	1.73 (0.99-3.02)	1.43 (0.81-2.53)	1.92 (1.07-3.44)	2.18(1.19-4.01)
Sex (female)	0.64 (0.42-0.97)		0.58 (0.41-0.81)		0.56 (0.39-0.80)	0.54 (0.37-0.79)
Age (years)	2.14 (1.73-2.64)	1.43 (1.10-1.87)	2.10 (1.76-2.50)	1.76 (1.45-2.14)	2.16 (1.80-2.60)	1.78 (1.46-2.17)
Overt nephropathy*		3.03 (1.86-4.95)				
Distal symmetrical PN*		3.33 (1.68-6.57)				
Proliferative Retinopathy		2.32 (1.28-4.20)				
Peripheral vascular disease		0.95 (0.90-1.00)				
HbA1c (%)				1.40 (1.16-1.70)		
Serum creatinine (mg/dl)**				1.30 (1.10-1.54)		
AER (µg/min)**				1.67 (1.32-2.13)		
Non-HDLc (mg/dl)**				1.54 (1.28-1.84)		
DBP (mm Hg)**				1.33 (1.09-1.62)		
Hypertension medication*				0.81 (0.74-0.88)		
Physical activity (kcal)						0.64 (0.51-0.80)
Intensive insulin therapy*						0.77 (0.72-0.83)
Alcohol consumption						0.93 (0.89-0.98)

*% of time with this condition AN=autonomic neuropathy PN= polyneuropathy AER=albumin excretion rate DBP=diastolic blood pressure **natural logarithmically transformed before analyses Alcohol consumption=3+ beverages/wk Low income= household income <$20,000/yr

Complications model also allowed for coronary artery disease, proliferative retinopathy, and peripheral vascular disease.

Biological risk factors model also allowed for sex, daily insulin dose/kg of body weight, high density lipoprotein cholesterol, heart rate, and hematocrit.

SES/Lifestyle risk factors model also allowed for having some college education and years of current smoking.

Table 6-4. Independent Time-Varying Predictors of Mortality in Type 1 Diabetes by Risk Factor Groupings, Cox Regression Analyses.

	Complications, base and adjusted models (n=519)		Biological Risk Factors, base and adjusted models (n=632)		SES/Lifestyle Risk Factors, base and adjusted models (n=598)	
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Normal weight	Ref	Ref	Ref	Ref	Ref	Ref
Underweight	4.28 (2.46-7.46)	2.96 (1.78-4.91)	3.40 (2.14-5.42)	3.26 (2.06-5.17)	3.01 (1.78-5.08)	2.49 (1.42-4.16)
Overweight	0.81 (0.47-1.38)	0.89 (0.55-1.42)	0.72 (0.46-1.12)	0.74 (0.47-1.15)	0.85 (0.55-1.33)	0.94 (0.60-1.47)
Obese	2.21 (1.26-3.88)	2.10 (1.26-3.52)	2.01 (1.25-3.24)	1.92 (1.18-3.14)	2.19 (1.33-3.61)	1.92 (1.14-3.23)
Sex (female)	0.59 (0.39-0.89)	0.61 (0.42-0.88)	0.56 (0.39-0.79)		0.55 (0.38-0.79)	0.57 (0.40-0.83)
Age (years)	2.09 (1.69-2.58)		2.10 (1.76-2.50)	1.71 (1.41-2.07)	2.16 (1.79-2.59)	1.99 (1.64-2.42)
Coronary artery disease		2.86 (1.91-4.29)				
Overt nephropathy		2.08 (1.39-3.12)				
Symptomatic AN		2.11 (1.41-3.14)				
Proliferative retinopathy		2.33 (1.37-3.95)				
Peripheral vascular disease		1.57 (1.03-2.38)				
HbA1c (%)				1.32 (1.14-1.53)		
AER (µg/min)*				1.55 (1.28-1.88)		
Serum creatinine (mg/dl)				1.48 (1.31-1.66)		
Non-HDLc (mg/dl)				1.23 (1.08-1.41)		
WBC (x $10^3/mm^2$)				1.16 (1.01-1.35)		
Physical activity (kcal)						0.79 (0.73-0.86)
Alcohol consumption						1.87 (1.28-2.75)
Low Income						3.28 (2.24-4.80)

AN=autonomic neuropathy AER=albumin excretion rate DBP=diastolic blood pressure *natural logarithmically transformed before analyses Alcohol consumption=3+ beverages/wk Low income= household income <$20,000/yr.

Complications model also allowed for distal symmetrical polyneuropathy.

Biological risk factors model also allowed for daily insulin dose/kg of body weight, hematocrit, high density lipoprotein cholesterol, diastolic blood pressure, use of hypertension medications, and heart rate.

SES/Lifestyle risk factors model also allowed for having some college education, low income, smoking, and consumption of alcohol ≥3x/wk.

Table 6-5. Change in Body Mass Index (BMI) in Adults with Type 1 Diabetes in Follow-up Years 1-10 and Risk of Mortality in Years 11-20.

	HR (95% CI)
BMI CHANGE (RESIDUALS)	0.88 (0.80-0.97)
BASELINE BMI	1.10 (1.00-1.21)
AGE (YEARS)	2.10 (1.53-2.87)
ALBUMIN EXCRETION RATE*	2.32 (1.74-3.09)

*Natural logarithmically transformed before analysis.

Forward selection model also allowed for sex, hypertension, HbA1c, intensive insulin therapy, HDL cholesterol, and non-HDL cholesterol.

7.0 PAPER 3: DOUBLE-EDGED RELATIONSHIP BETWEEN ADIPOSITY AND CORONARY ARTERY CALCIFICATION IN TYPE 1 DIABETES

Published December, 2007

Diabetes and Vascular Disease Research, 2007; 4:332-339

Baqiyyah Conway[1], Rachel Miller[1], Tina Costacou[1], Linda Fried[2], Sheryl Kelsey[1], Rhobert Evans[1], Daniel Edmundowicz[2], Trevor Orchard[1]

[1]University of Pittsburgh, Department of Epidemiology, Graduate School of Public Health

[2]University of Pittsburgh, School of Medicine

7.1 ABSTRACT

Background: Coronary artery disease (CAD), a leading cause of death in type 1 diabetes (T1D), often occurs two or more decades earlier in this population. Although CAD generally increases with adiposity, this association is unclear in T1D. We thus examined the associations of adiposity with Coronary Artery Calcium (CAC-a subclinical marker of CAD) in 315 individuals with T1D.

Methods: Mean age and diabetes duration were 42 and 34 yrs, respectively at the time of assessment of CAC, visceral adiposity (VAT) and subcutaneous adiposity (SAT) by electron beam tomography and when BMI and waist circumference (WC) were measured. Chi-square frequencies and generalized linear models were used to compare the presence of any CAC and age-adjusted mean CAC total score, respectively, by tertiles of adiposity.

Results: There was a positive relationship between the presence of CAC and tertiles of VAT, SAT, BMI, and WC in both genders (p trend<0.05). The presence of CAC was positively associated with VAT, SAT, and BMI in men ($p<0.05$) and with all four adiposity measures in women ($p<0.05$). However, the degree of CAC was not associated with any adiposity measure, with the exception of SAT in women. Women in the lowest tertile of SAT had more CAC than those in the second tertile ($p<0.016$).

Conclusion: Adiposity was positively associated with the presence of CAC, but the relationship with its severity was either inverse or nonexistent. This double-edged association, which appears to be more pronounced in women, emphasizes the complex relationship between adiposity and cardiovascular risk in diabetes.

7.2 INTRODUCTION

Coronary artery disease (CAD) is the leading cause of death in type 1 diabetes (1) and often occurs two or more decades earlier than in the general population. Although the risk of CAD tends to increase with increasing BMI in the general population, this association in type 1 diabetes is unclear. Coronary artery calcification (CAC) is a subclinical marker of coronary vascular disease (2) and has been shown to be predictive of future clinical cardiac events (3). The few studies that have investigated CAC in T1D are inconsistent in terms of the relationship between CAC and adiposity. All five studies investigated the association of BMI with CAC. Both Dabelea et al (4) and Colhoun et al (5) reported a positive association between BMI and the prevalence of CAC; in contrast, in the Epidemiology of Diabetes Complications Study (6), the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study (7), and in Starkman et al (8), no association was found between BMI and CAC prevalence. Olson et al (6) and Cleary et al (7) also failed to show an association with CAC severity. Additionally, in a subgroup of the CACTI population reported on by Dabelea et al (4), Snell-Bergeon et al (9) failed to find a difference in BMI when investigating progression of CAC.

Markers of visceral adiposity, thought by many to independently relate to cardiovascular disease risk, have also been studied. Unlike BMI, the association of waist-to hip ratio (WHR) and/or waist circumference (WC) with CAC has been more consistent. With the exception of Colhoun et al (5), who failed to show an association in men, and Starkman et al (8), who did not

investigate WHR /WC, all of the studies found CAC to be positively associated with WHR/WC. Additionally, Dabelea et al (4) using a direct measurement of visceral obesity, also found intraabdominal fat to be positively associated with the prevalence of CAC, although this was not investigated sex-specifically. They also found men to be at higher risk for CAC. As sex differences in adiposity also exist, even for BMI, and not all of the above mentioned studies looked at adiposity sex-specifically (4,7,8), sex specific analyses are warranted. Furthermore, none of the studies investigated subcutaneous abdominal fat (SAT). This is important as SAT has also been suggested to be a major contributor of free fatty acids into both the portal and systemic circulation and thus insulin resistance (10, 11, 12, 13).

Given the above conflicts in the literature and the evidence that being overweight and obese is rising in type 1 diabetes (T1D) (14), concern and further evaluation of the association of adiposity with CAD in this population already at increased risk is warranted. This study therefore sought to determine the following: a) which measure of adiposity best identifies CAC (testing the hypothesis that measures of central obesity will be more strongly associated with CAC), b) whether any associations of adiposity with CAC vary by sex and c) whether any associations differ for the prevalence as opposed to the severity of CAC. Four different indices of body fat, i.e. BMI, WC, VAT, and SAT were investigated.

7.3 METHODS

7.3.1 Subjects

The EDC study is an ongoing cohort study examining the long term complications of T1D in 658 individuals diagnosed before the age of 17 years with T1D at Children's Hospital of Pittsburgh between 1950 and 1980. This current report is based on a subset (n=315) who underwent electron beam tomography (EBT) for CAC between 2000 and 2007. These participants were also scanned for VAT and SAT via EBT scanning.

7.3.2 Clinical Evaluation Procedures

Before attending the clinic, participants completed a questionnaire concerning demographic information, lifestyle, and medical history. An ever smoker was defined as having smoked at least 100 cigarettes in a lifetime. Participants were weighed in light clothing on a balance beam scale. Height was measured using a wall-mounted stadiometer. BMI was calculated as the weight in kilograms divided by the square of the height in meters. Two waist measurements were taken by a standard medical measuring tape, measuring from the mid-point of the iliac crest and the lower costal margin in the mid-axillary line. The average was used for data analysis.

Fasting blood samples were assayed for lipids, lipoproteins, and glycosylated hemoglobin (HbA1c). High-density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure, a modification of the Lipid Research Clinics method (15). Cholesterol and triglycerides were measured enzymatically. Glomerular filtration rate (GFR) was estimated

using the Modification of Diet in Renal Disease (MDRD) formula (16). Sitting blood pressures were measured according to the Hypertension Detection and Follow-up Program protocol (17) using a random zero sphygmomanometer. The mean of the second and third readings was used. Estimated glucose disposal rate (eGDR) was calculated using the equation: eGDR=24.395-12.971 (waist-to-hip ratio)-3.388 [hypertension status (140/90 mm Hg or on hypertension medication)]-0.601 (HbA1c). This formula was derived from a substudy of 24 EDC participants (12 men and 12 women drawn from low, middle, and high age-specific tertiles of insulin resistance risk factors) who underwent euglycemic clamp studies (18).

CAC was measured using EBT (Imatron C-150, Imatron, South San Francisco, CA). Threshold calcium determination was set using a density of 130 Hounsfield units in a minimum of 2 contiguous sections of the heart. Scans were triggered by ECG signals at 80% of the R-R interval. The entire epicardial system was scanned. CAC volume scores were calculated based on isotropic interpolation (19). Direct measurements of abdominal adiposity (visceral and subcutaneous abdominal adipose tissue surface area) were also taken by EBT scanning. Scans of abdominal adipose tissue were taken between the fourth and fifth lumbar regions, which were located by counting from the first vertebra below the ribs. Two 10 mm thick scans were taken during suspended respiration. The images were then analyzed using commercially available software for all pixels corresponding to fat density in Hounsfield units in the appropriate anatomical distribution (subcutaneous or visceral).

7.3.3 Statistical Analyses

Pearson's correlations were used to assess the association between each of the four adiposity measures. Two stage analyses were performed given the large number of subjects with no calcification and the resulting non-normal distribution. The first analysis evaluated the presence/absence of CAC. The second analysis evaluated the degree of CAC by volume scores in individuals with any CAC. This approach also allows assessment of the third objective, i.e. whether relationships were different for prevalence versus severity.

Differences between groups with and without CAC were evaluated using the Student's *t* test for continuous variables and $\chi 2$ for dichotomous variables. Logistic regression analysis was used to determine the association of adiposity with the presence of CAC. Spearman's correlations were used to determine how well the different adiposity measures correlated with CAC volume scores, given the presence of any CAC. Generalized linear models (GLMs), which are fairly robust in analyses of non-normally distributed data, were used to compare CAC volume scores (CACs) across tertiles of the four adiposity measures.

In order determine whether any adiposity associations with CAC vary by sex, BMI, WC, VAT, and SAT were examined by sex-specific tertiles; sex interactions were also explored. Analyses were performed on the entire cohort and within only those positive for calcification. All non-normally distributed variables were transformed using an appropriate transformation or were tested nonparametrically with the Kruskal Wallis test. One was added to all values of CAC before log-transformation. All odds ratios and parameter coefficients are reported as per one standard deviation change in the continuous variables. Akaike's Information Criterion (AIC) and Pearson's r were used to determine which adiposity measurement best accounted for the

prevalence and severity of CAC, respectively. The criterion for statistical significance was $P < 0.05$. Analyses were conducted using SAS version 9.1 (Cary, North Carolina).

7.4 RESULTS

7.4.1 Adiposity and the Presence or Absence of CAC

Baseline characteristics revealed that although men (n=152) had significantly higher VAT, WC, non-HDLc, and lower HDLc than women (n=161), there was no difference in percent with CAC or median CAC levels (Table 7-1). Figure 7-1 shows the prevalence of CAC by sex-specific tertiles of adiposity. There was a significant direct linear trend between tertile of each adiposity measure and prevalence of CAC in both sexes ($p<0.05$) (data not shown). When the measures of adiposity were analyzed as continuous variables and adjusted for age, the presence of CAC was positively associated with VAT, SAT and BMI in both sexes, and WC in females (effect modification by gender was not observed, $p=0.53$). Further adjustment for other clinically and/or statistically significant risk factors did not alter these associations (ORs range from 1.2 to 3.2), including menopausal status. Model comparisons suggest that BMI was marginally better at accounting for CAC prevalence in both sexes.

7.4.2 Correlation between the Adiposity Measures and CAC

Age-adjusted CACs showed low order positive correlations with each adiposity measure overall in both sexes, which reached statistical significance only for SAT in men (Table 7-2). However, when restricted to only those with some measurable CAC, i.e. excluding those with '0' values, correlations were surprisingly in the inverse direction, but none approached statistical significance (Table 7-3).

7.4.3 Adiposity and the Degree of CAC

Graphical examination of tertiles of adiposity in those with calcification revealed that there was a tendency for an inverse relationship of CACs with each of the adiposity measures in both men and women (p for trend <0.05 for each measure) (Figure 7-2). With the exception of VAT in men, in both sexes and for all measures, the lowest tertile of adiposity had the highest median CAC scores. Comparing the first tertile with second and third combined did not change the results, with the exception of significantly higher CACs in the lowest tertile of BMI for men (data not shown). In order to explore whether other confounding variables may explain this finding, other risk factors were examined by tertile of SAT, where this observation was most striking. No excess of major risk factors were identified in the lowest tertile; however, age, diabetes duration, and smoking were higher in both men and women, albeit nonsignificantly while eGFR was significantly lower and overt nephropathy was significantly higher in women (Table 7-4).

Generalized linear modeling revealed that given the presence of any CAC, there was no significant association of age-adjusted CAC with any of the four adiposity measures, with the exception of SAT in women. Women in the lowest tertile of SAT had more CAC than those in the second tertile ($p<0.016$). After adjustment for age, glomerular filtration rate, having ever smoked, and a history of a renal transplant, being in the lowest tertile of SAT remained significantly associated with CAC in women (Table 7-5) and after further adjustment for menopausal status which was not a strong independent predictor ($p=0.8$). Model comparisons show R^2 ranging from 35 to 42%, suggesting that all four models generally explain variance in CAC to a similar degree.

7.5 DISCUSSION

In this cross-sectional study in which we investigated the association of adiposity with CAC in T1D several important findings are of note. First, we demonstrated that the four different measures of adiposity investigated are similarly associated with CAC, both within and between sexes. We also showed that the direction of the associations differed when looking at the presence of CAC as opposed to the degree of CAC, i.e. a lower level of adiposity had a higher CACs.

Contrary to expectations, central adiposity measures, e.g. VAT and WC, were not better able to identify CAC than the other body morphology parameters. A major hypothesized mechanism by which adiposity is associated with CAD is via increased lipolysis of metabolically active VAT with its consequent release of inflammatory cytokines into the systemic circulation

and excess free fatty acids into the portal vein (20). Cytokines such as Il-6 and CRP are associated with atherosclerosis while increased free fatty acid flux to the liver will increase triglyceride and LDLc and small dense LDLc synthesis and are postulated to be in the causal pathway of insulin resistance (21, 13). The small dense LDL phenotype, associated with insulin resistance, is very atherogenic in high concentrations. Despite these characteristics of visceral adiposity and our previous reports of CAD events being related to WHR (22; 23) and small dense LDL (24), in these current analyses CAC was not preferentially linked with visceral compared to general obesity, suggesting possible differences in these measures in T1D. However, other investigators state that it is elevated SAT, which is correlated with VAT, that is primarily responsible for the elevated systemic levels of FFA associated with VAT (13). Nevertheless, most of the studies investigating CAC in type 1 diabetes have found WHR or WC to be associated with CAC (6, 7, 9), although BMI has been less consistent (4, 5, 6, 7). Biological plausibility notwithstanding, no adiposity parameter appears strikingly better than another in detecting CAC.

That BMI was marginally better able to detect the presence of CAC in both men and women may support the argument that BMI is not so much a measure of overall adiposity as it is a marker/predictor of health status (25). In investigating the relationship of obesity with CAD in type 1 diabetes, it must be borne in mind that adiposity associations observed in non-diseased populations may be very different than that observed in populations with pre-existing disease, such as type 1 diabetes. The inverse relationship of adiposity with severity of CAC in this population appears to lend support to this postulate.

That increasing adiposity was positively associated with the presence of CAC, while it was inversely associated with severity, albeit non-significantly for most parameters, seems to suggest at least two divergent disease processes. A search for confounding by adiposity tertile within those with any CAC revealed that estimated glucose disposal rate (eGDR) in men, age, lipids, and kidney function/disease in women, and blood pressure in both sexes were significantly different for those in the lowest adiposity tertile and who had measurable CAC. As alluded to earlier, obesity correlates such as hypertension, dyslipidemia, inflammation, and insulin resistance, i.e. features of the metabolic syndrome, may be responsible for the increased CAC observed. Our marker for insulin sensitivity in this population, eGDR, was inversely associated with CAC severity and thus consistent with this hypothesis, though the correlation was not significant in women. Despite identifying these potential confounders, the significant SAT difference in women (Table 7-5) remained significant.

The CAC detected, at least in some of the participants, may not be from atherosclerotic plaque, i.e. intimal, as is generally associated with CAD, but rather partially medial (4,5). This cannot be determined by EBCT. In a recent analysis demonstrating medial wall calcification in the EDC population (26), some six years prior to EBT scanning for CAC, a strong association was seen between earlier medial wall calcification, determined six years earlier by ankle x-rays, and CAC, which remained in multivariable analyses unless neuropathy was included as a variable. Thus both processes may be at work in this population.

The majority of the studies looking at CAC in T1D have found age and diabetes duration to be the strongest correlates of CAC. Residual confounding due to factors related to long-term exposure to hyperglycemia, such as advanced glycated end products (AGEs), may also be apart of the pathogenesis of CAC. Long-term exposure to hyperglycemia may result in AGEs

depositing into the extracellular matrix of the arterial wall. These AGES have the ability to stimulate osteoblastic differentiation, leading to vascular calcification. Sakata et al (27) reported increased CML, an AGEs, in the medial wall of the inter-thoracic artery of individuals with type 1 diabetes, while very little of this was noted in those with type 2 diabetes. Although there was no age-adjusted association between glycemic control and CAC in our population, this does not negate the possibility that long duration of hyperglycemia, i.e. diabetes, may be responsible for the more severe CAC, particularly in the older participants, who also happened to be the thinnest. However, increased levels of AGEs are also found in kidney disease.

CAC is a well known to be associated with kidney disease, possibly due to abnormal calcium and phosphorus metabolism (28). Extensive calcification is observed in those in renal failure, even in the young, and CAC is an important predictor of overall mortality in the kidney disease population. Colhoun et al (5) found AER to be associated with the presence of CAC in men with T1D, but not women. Thilo et al (29) observed no association between microalbiminuria and CAC in 71 participants with T1D with a mean age of 48 and disease duration of 26 years. In our population, kidney function was associated with CAC. We observed that GFR tended to increase and overt nephropathy tended to decrease with adiposity in women, suggesting that the more severe CAC observed in women with less body fat might be partially explained by kidney disease. However, this association was not observed in men. Additionally, although renal transplant recipients had the highest levels of CAC, i.e. most severe CAC, they were not more likely to be in the lowest adiposity tertile. Nevertheless, where adiposity failed to be a strong predictor, renal function, as measured by GFR and renal failure were significantly related to CAC severity in this population.

Consistent with the literature in type 1 diabetes, there were no significant differences in CAC prevalence, severity (5,6) or its association of body fat by sex; though the association in men for waist circumference was not significant, there was no gender interaction (p=0.53). However, contrary to expectations, VAT, albeit nonsignificantly, appeared to be better at detecting CAC severity in women than in men. In the general population, men have an average of about twice as much visceral fat as premenopausal women when matched for total body fat (30). We did not observe such a large sex difference in our population. Visceral fat levels were 50% higher in males than premenopausal women in our T1D population (data not shown). This attenuation in the sex difference in VAT in T1D has been observed elsewhere. In the CACTI study, Dabelea et al (4) observed that men with T1D had lower WHRs and much lower levels of VAT, although similar BMI levels, than nondiabetic men. Women with T1D had higher waist-to-hip ratios, WCs, and BMIs, but similar levels of VAT. Dabelea noted that women with T1D "had a more android disposition of adipose tissue" while this was attenuated in men. It appears that VAT is not stored to the same extent in T1D, indicating a more functional role of VAT in T1D. Although many CAD risk factors increase with adiposity in this population, traditional CAD risk factors appear (including menopausal status) to be less operant in the pathogenesis of severe atherosclerosis, particularly in women, an observation noted elsewhere (31).

Study Limitations

Obesity may lead to false CAC detection, that is, the higher prevalence in the more obese could be artifactual; however, our major findings were not different when being positive for CAC was defined as having a CAC score ≥10. A major limitation of this study is that we were unable to follow participants from an earlier time point when participants were free of CAC to

determine if the adiposity measures predict the incidence or severity of CAC. In a population such as this, in which it is defined by its pre-existing disease, complications that are part of the natural history of the disease may be well underway after 16-18 years of follow-up. As CAC, VAT, and SAT were not available in this population at earlier time periods, the adiposity indices measured at the time of EBT may not reflect the adiposity level prior to the development of severe calcification. It is thus not possible to determine the exact causal pathways given the cross-sectional nature of our study. Survival bias may also be at issue in the current study. It is possible that the more obese died before current follow-up. However, being overweight is not a mortality risk factor in this population (14) so disruption of the natural obesity/CAC association by premature loss of the more obese is unlikely. It is also possible that longer exposure to kidney disease may result in weight loss or that increased body fat is merely a marker of better health.

In conclusion, we found that adiposity was related to the presence of calcification irrespective of the measure used. Age, which in this population is also a proxy for diabetes duration, remained, as in many studies, the strongest correlate for both the prevalence and severity of CAC. Although the presence of CAC increased with adiposity, more severe disease, i.e. greater CAC, was inversely associated with body fat, albeit only significantly for SAT in women. This was only partially explained by other risk factors, e.g. renal disease and age. At least two distinct disease processes (atherosclerosis and medial wall calcification) may be operant in the CAC seen in T1D, underscoring the complex relationship of obesity with CAC in T1D. This double-edged association, the association of CAC with both fat and thin, which appears to vary by sex, further highlights that factors other than the standard risk factors are at work in the development of CAD in T1D.

7.6 CITED WORKS

1. Libby P, Nathan D, Abraham K, Brunzell J, Fradkin J, Haffner S, Hsueh W, Rewers M, Roberts B, Savage P, Skarlatos S, Wassef M, Rabadan-Diehl C. Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. *Circulation* 2005; 111:3489-3493.

2. Abedin M, Tintut Y, Demer L. Vascular Calcification: Mechanisms and Clinical Ramifications. *Arterioscler Thromb Vasc Biol* 2004; 24:1161-1170.

3. Kennedy J. Shavelle R, Wang S, Budoff M, Detrano R. Coronary Calcium and Standard Risk Factors in Symptomatic Patients Referred for Coronary Angiography. *AM Heart J* 1998; 135:696-702.

4. Dabelea D., Kinney G, Snell-Bergeon J, Hokanson J, Eckel R, Ehrlich J, Garg S, Hamman R, Rewers M. Effect of Type 1 Diabetes on the Gender Difference in Coronary Artery Calcification: a Role for Insulin Resistance? The Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study. *Diabetes* 2003; 52:2833-2839.

5. Colhoun H, Rubens M, Underwood R, Fuller J. The Effect of Type 1 Diabetes Mellitus on the Gender Difference in Coronary Artery Calcification. *J Am Coll Cardiol* 2000; 36:2160-2170.

6. Olson J, Edmundowicz D, Dorothy B, Kuller L, Orchard T. Coronary Calcium in Adults with Type 1 Diabetes: A Stronger Correlate of Clinical Coronary Artery Disease in Men Than in Women. *Diabetes* 2000; 49:1571-1578.

7. Cleary P, Orchard T, Genuth S, Wong N, Detrano R, Backlund J, Zinman B, Sun W, Lachin J, Nathan D, the DCCT/EDIC Research Group. The Effect of Intensive Glycemic Treatment on Coronary Artery Calcification in Type 1 Diabetic Participants of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study.

8. Starkman H, Cable G, Hala V, Hecht H, Donnely C. Delineation of Prevalence and Risk Factors for Early Coronary Artery Disease by Electron Beam Computed Tomography in Young Adults with Type 1 Diabetes. *Diabetes Care* 2003; 26:433-436.

9. Snell-Bergeon J, Hokanson J, Jensen L, MacKenzie T, Kinney G, Barbelea D, Eckel R, Ehrlich J, Garg S, Rewers M. Progression of Coronary Artery Calcification in Type 1 Diabetes: the importance of glycemic control. *Diabetes Care* 2003; 26:2923-2928.

10. Tanko L, Bagger Y, Alexanderson P, Larsen P, Christiansen C. Peripheral Adiposity Exhibits an Independent Dominant Aniatherogenic Effect in Elderly Women. *Circulation* 2003;107: 1626-1631.

11. Abate N, Garg A, Peshock R, Stray-Gundersen J, Grundy S. Relationships of generalized and regional adiposity to insulin sensitivity in men. *Journal of Clinical Investigation* 1995; 96:88-98.

12. Abate N, Garg A, Peshock R, Stray-Gundersen J, Adams-Huet B, Grundy S. Relationships of generalized and regional adiposity to insulin sensitivity in men with NIDDM. *Diabetes* 1996;45: 1684-1693.

13. Frayn K. Visceral fat and insulin resistance-causative or correlative? *British Journal of Nutrition* 2000; 83:S71-S77.

14. Conway B, Costacou T, Orchard T. Time Trends in Overweight and Obesity in Type 1 Diabetes and Their Association with Mortality. *Diabetes* 55 (S1): A382.

15. National Institute of Health, Department of Health, Education and Welfare. (1978) Lipid Research Clinics Program. Washington, D.C.: U.S. Govt. Printing Office 1975 (NIH pub no. 75-628).

16. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease. Am J Kidney Dis. 2006 May;47(5 Suppl 3):S11-145.

17. Borhani N, Kass E, Langford H, Payne G, Remington R, Stamler J. The Hypertension Detection and Follow-up Program. Prev Med 1976; 5:207-215.

18. Williams K, Erbey J, Becker D, Arslanian S. Can Clinical Factors Predict Insulin Resistance in Type 1 Diabetes? Diabetes 2000; 49: 626-632.

19. Callister T, Cooil B, Raya S, Lippolis N, Russo D, Raggi P. Coronary artery disease: improved reproducibility of calcium scoring with an electron-beam CT volumetric method. Radiology 1998; 208:807-814.

20. Bjorntorp P. 'Portal' adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis 1990; 10:493-496.

21. Arner P. Impact of visceral fat. International Journal of Obesity 1997; 21:S20.

22. Stuhldreher WL, Orchard TJ, Ellis D. The association of waist-hip ratio and risk factors for development of IDDM complications in an IDDM adult population. Diabetes Res Clin Pract 1992;17 :99-109.

23. Orchard T, Olson J, Erbey J, Williams K, Forrest K, Kinder L, Ellis D, Becker D. Insulin resistance-related factors, but not gycemia, predict coronary artery disease in type 1 diabetes. *Diabetes Care* 2003; 26:1374-1379.

24. Soedamah-Muthu S, Chang Y Otvos J, Evans R, Orchard T. Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary

artery disease in Type 1 Diabetes. A prospective report from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetelogia* 2003; 46:674-682.

25. Fontaine K, Allison D. Obesity and Mortality Rates in Bray G, Bouchard C (eds). Handbook of Obesity: etiology and pathophysiology (2nd ed.). New York: Marcel Decker. 2004.

26. Costacou T, Husk N, Edmundowics D, Stolk R, Orchard T. Lower-extremity arterial calcification as a correlate of coronary artery calcification. *Metabolism* 2006; 55(12):1689-96.

27. Sakata N, Takeuchi K, Noda K, Saku K, Tachikawa Y, Tashiro T, Nagai R, Horiuchi S. Calcification of the medial layer of the internal thoracic artery in diabetic patients: relevance of gloxidation. *J Casc Res* 2003; 40:567-74.

28. Stenvinkel P, Pecoits-filho, Lindholm B. Coronary artery disease in end-stage renal disease: no longer a simple plumbing problem. *J Am Soc Nephrol* 2003; 14:1927-1939.

29. Thillo C, Standi E, Knez A, Reiser M, Steinbeck G, HAberl R, Schnell O. Coronary Calcification in Long-term Type 1 Diabetic Patients-A Study with Multi Slice Spiral Computed Tomography. *Exp Clin Endocrinol Diabetes* 2004; 112:561-565.

30. Nicklas B, Penninx B, Ryan A, Berman D, Lynch N, Dennis K. Visceral Adipose Tissue Cutoffs Associated with Metabolic Risk Factors for Coronary Heart Disease in Women. *Diabetes Care* 2003; 26:1413-1420.

31. Schurgin S, Rich S, Mazzone T. Increased Prevalence of Significant Coronary Artery Calcification in Patients with Diabetes. *Diabetes Care* 2001; 24:335-338.

7.7 FIGURES AND TABLES

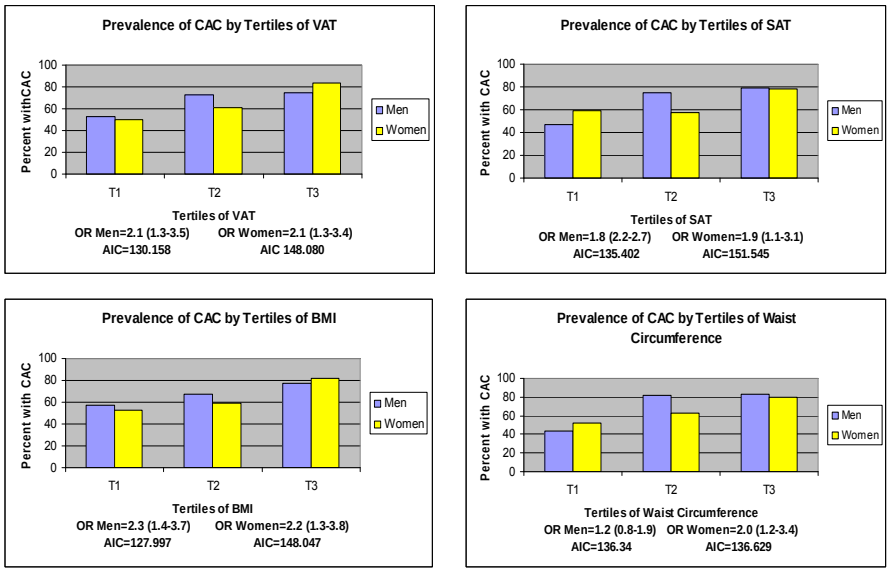

Figure 7-1. The prevalence of coronary artery calcification by sex-specific tertiles of adiposity.

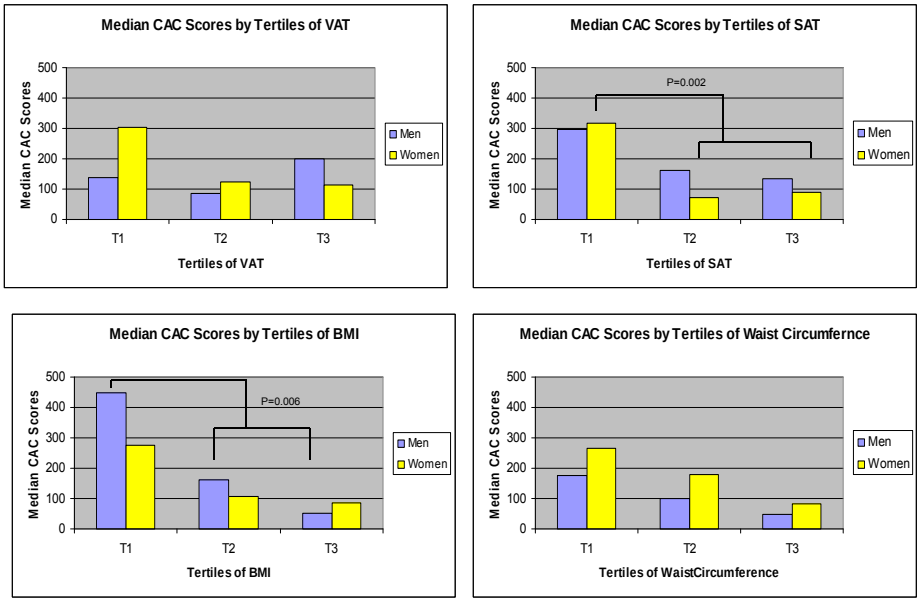

Figure 7-2. Median coronary artery calcification score by sex-specific tertiles of adiposity in those with a calcification score>0.

Table 7-1. Characteristics by Gender. The Pittsburgh Epidemiology of Diabetes Complications Study.

Characteristics	Males (152)	Females (161)	p-value
Age (years)	42.7 ± 6.7	43.2 ± 7.0	0.61
Duration (years)	34.6 ± 7.3	34.6 ± 7.7	0.54
CAC+ (%)	67.1	65.0	0.73
CACs (median)*	22.2 (0-313.3)	8.9 (0-287.3)	0.47
Hba1c (%)	7.8 ± 1.5	7.6 ± 1.3	0.10
Hypertension (%)	37.7	30.2	0.22
Ever Smoker (%)	36.2	36.9	0.82
HDLc (mg/dL)	50.2 ± 11.8	63.9 ± 14.4	0.01
NonHDLc (mg/dL)	132.0 ± 35.0	125.6 ± 28.9	0.02
VAT cm^2*	124.3 ± 68.9	82.0 ± 48.6	<0.0001
SAT cm^2*	224.9 ± 275.9	307.1 ± 446.9	0.0002
BMI	26.8 ± 3.6	26.9 ± 5.6	0.96
WC (cm)	92.8 ± 12.0	84.7 ± 11.8	<0.0001
Menopause* *		39 (24.7)	

*Non-parametrically tested. **n=158

Table 7-2. Age-adjusted Correlations of Adiposity with Coronary Artery Calcification (CAC) in Type 1 Diabetes (R, r), 2000-2007.

Men, n=152						
	SAT	BMI	Waist Circumference	eGDR	Age	CACs
VAT**	0.67	0.67	0.69	-0.42	0.25	0.12
	<0.0001	<0.0001	<0.0001	<0.0001	0.002	0.14
SAT**		0.67	0.67	-0.39	-0.03	0.17
		<0.0001	<0.0001	<0.0001	0.75	0.04
BMI			0.76	-0.28	-0.07	0.11
			<0.0001	<0.0001	0.40	0.19
Waist				-0.46	0.07	0.10
Circumference				<0.0001	0.42	0.32
eGDR					-0.07	-0.24
					0.59	0.006
Age						0.59
						<0.0001
Women, n=161						
VAT*	0.58	0.63	0.77	-0.36	0.17	0.12
	<0.0001	<0.0001	<0.0001	<0.0001	0.03	0.12
SAT**		0.64	0.67	-0.15	0.03	0.06
		<0.0001	<0.0001	0.08	0.66	0.42
BMI			0.74	-0.22	-0.06	0.15
			<0.0001	0.01	0.39	0.07
Waist				-0.35	-0.05	0.15
Circumference				<0.0001	0.39	0.08
eGDR					-0.09	-0.09
					0.26	0.31
Age						0.59
						<0.0001

*Pearson for adiposity measures with each other; Spearman for correlations with CAC **Natural-logarithmically transformed

Table 7-3. Age-adjusted Correlations* of Adiposity with Coronary Artery Calcification (CAC) in those with CAC (R, r), 2000-2007.

	SAT	BMI	Waist Circumference	eGDR	Age	CACs
Men, n=102						
VAT**	0.54	0.66	0.67	-0.45	0.13	0.07
	<0.0001	<0.0001	<0.0001	<0.0001	0.18	0.46
SAT**		0.63	0.61	-0.39	-0.19	-0.08
		<0.0001	<0.0001	<0.0001	0.06	0.40
BMI			0.73	-0.24	-0.25	-0.14
			<0.0001	0.02	0.01	0.15
Waist Circumference				-0.49	-0.12	-0.16
				<0.0001	0.25	0.12
eGDR					0.01	-0.22
					0.90	0.04
Age						0.59
						<0.0001
Women, n=104						
VAT**	0.58	0.63	0.77	-0.37	-0.08	-0.08
	<0.0001	<0.0001	<0.0001	0.0003	0.42	0.37
SAT**		0.64	0.67	-0.12	-0.17	0.18
		<0.0001	<0.0001	0.26	0.09	0.07
BMI			0.74	-0.19	-0.29	-0.05
			<0.0001	0.07	0.08	0.64
Waist Circumference				-0.37	-0.09	-0.06
				0.0003	0.40	0.59
eGDR					-0.09	0.05
					0.40	0.61
Age						0.54
						<0.0001

*Pearson for adiposity measures with each other; Spearman for correlations with CAC. **Natural-logarithmically transformed

Table 7-4. Characteristics of Participants with Coronary Artery Calcification by Tertiles of Subcutaneous Abdominal Adiposity and Gender.

Variable, mean (SD)	Tertile 1	Tertile 2	Tertile 3	p-value	Variable, mean (SD)	Tertile 1	Tertile 2	Tertile 3	p-value
Men					*Women*				
Age (yrs)	47.0 (8.0)	44.0 (6.1)	44.3 (7.4)	0.23	Age (yrs)	48.2 (6.4)	45.2 (7.8)	44.4 (6.6)	0.06
Diabetes Duration (yrs)	38.1 (8.5)	34.8 (6.2)	34.4 (7.2)	0.32	Diabetes Duration (yrs)	38.0 (7.1)	36.0 (8.0)	34.5 (7.0)	0.43
HbA1c (%)	7.5 (1.3)	7.3 (1.6)	7.9 (1.3)	0.17	HbA1c (%)	7.7 (1.4)	7.2 (1.4)	7.4 (1.1)	0.30
eGDR (mg/kg/min)	7.2 (1.8)‡	7.5 (1.8) ‡	5.6 (2.1)	0.0001	eGDR (mg/kg/min)	8.0 (2.5)	8.5 (2.0)	8.1 (1.6)	0.51
HDL (mg/dl)	53.7 (15.2)	48.3 (9.2)	46.9 (11.2)	0.08	HDL (mg/dl)	63.5 (15.0)	63.2 (14.1)	61.7 (12.7)	0.85
Non-HDLc (mg/dL)	123.5 (35.2)	127.6 (35.0)	140.7 (40.7)	0.15	Non-HDLc (mg/dl)	123.2 (31.8)	125.0 (20.2)	138.4 (28.5)	0.04
SBP (mm/Hg)	121.7 (19.9)	125.5 (16.1)	130.5 (16.0)	0.08	SBP (mm/Hg)	120.8 (17.8)	113.5 (14.4)	121.2 (15.4)	0.09
DBP (mm/Hg)	68.9 (9.1) ‡	71.9 (9.9)	75.3 (8.2)	0.03	DBP (mm/Hg)	63.9 (7.7)	63.4 (8.7)	68.8 (9.5)	0.02
eGFR (mg/min)γ	81.7 (22.8)	81.2 (28.3)	80.7 (28.9)	0.87	eGFR (mg/min)γ	62.0 (23.4) ‡	75.0 (19.5)	78.6 (27.6)	0.02
Overt Nephropathy (%)	34.8	43.6	32.5	0.61	Overt Nephropathy (%)	48.4 ‡	29.0	22.0	0.05
Transplant recipient (%)	8.3	15.4	7.3	0.52	Transplant recipient (%)	12.5	12.9	4.7	0.38
Ever Smoker (%)	50.0	36.8	36.6	0.54	Ever Smoker (%)	48.4	32.3	40.5	0.45
CAC, median (IQR) ***	297.5 (48.4-1153.0)	162.6 (19.3-605.3)	135.5 (20.5-492.6)	0.32	CAC, median (IQR) ***	317.1 (124.9-868.8)	73.5 (5.5-376.9)	90.4 (7.5-510.4)	0.0006

*Significantly different from (sdf) Tertile (T) 1, †sdf T2, ‡ sdf T3 at p<0.017 **Natural logarithmically transformed before analysis

***Nonparametrically tested γTransplant recipients excluded

Table 7-5. Generalized Linear Models for the Association of Visceral Abdominal Adiposity (VAT), Subcutaneous Abdominal Adiposity (SAT), Body Mass Index (BMI), and Waist Circumference (WC) with Coronary Artery Calcification by Gender.

	VAT	**SAT**	**BMI**	**WC**
Characteristics	**ß± (p)**	**ß± (p)**	**ß± (p)**	**ß± (p)**
Men				
Adiposity*				
2nd tertile	-0.70 ± 0.44 (0.12)	-0.25 ± 0.46 (0.59)	-0.44 ± 0.45 (0.34)	-0.26 ± 0.52 (0.62)
3rd tertile	-0.08 ± 0.43 (0.85)	-0.32 ± 0.45 (0.48)	-0.37 ± 0.46 (0.43)	-0.45 ± 0.53 (0.40)
1st tertile	N/A	N/A	N/A	N/A
AGE	1.0 ± 0.18 (<0.0001)	1.0 ± 0.19 (<0.0001)	1.0 ± 0.19 (<0.0001)	0.90 ± 0.23 (0.0002)
MDRD	-0.01 ± 0.01 (0.12)	-0.01 ± 0.01 (0.08)	-0.01 ± 0.19 (0.09)	-0.01 ± 0.01 (0.06)
Ever Smoker	0.27 ± 0.18 (0.13)	0.32 ± 0.17 (0.07)	0.32 ± 0.18 (0.07)	0.31 ± 0.19 (0.10)
Transplant	1.6 ± 0.60 (0.01)	1.5 ± 0.61 (0.01)	1.3± 0.64 (0.05)	1.6± 0.78 (0.04)
R²	0.42	0.41	0.41	0.35
Women				
	VAT	**SAT**	**BMI**	**WC**
Characteristics	**ß± (p)**	**ß± (p)**	**ß± (p)**	**ß± (p)**
Adiposity*				
2nd tertile	0.32 ± 0.54 (0.55)	-1.2 ± 0.50 (0.02)	-0.17 ± 0.52 (0.76)	0.06 ± 0.53 (0.91)
3rd tertile	0.19 ± 0.41 (0.71)	-0.76 ± 0.48 (0.12)†	-0.16 ± 0.51 (0.74)	-0.24 ± 0.53 (0.65)
1st tertile	N/A	N/A	N/A	N/A
AGE	1.4 ± 0.23 (<0.0001)	1.3 ± 0.2.2 (<0.0001)	1.4 ± 0.23 (<0.0001)	1.4 ± 0.25 (<0.0001)
MDRD	-0. 01 ± 0.01 (0.18)	-0.01 ± 0.01 (0.39)	-0.01 ± 0.22 (0.18)	-0.01 ± 0.01 (0.27)
Ever Smoker	0.02 ± 0.15 (0.91)	-0.02 ± 0.15 (0.91)	0.03 ± 0.15 (0.85)	-0.04 ± 0.20 (0.83)
Transplant	0.76 ± 0.71 (0.29)	0.79± 0.68 (0.25)	0.71 ± 0.70 (0.31)	0.56 ± 0.70 (0.43)
R²	0.36	0.40	0.36	0.40

*VAT, SAT, BMI, and WC, respectively †Significantly different (lower) from the first tertile at p<0.016.

8.0 DISCUSSION

In 1832, Adolf Quetlet described an index of relative body weight for height in adults with stable weight (122). This index, the weight divided by the square of the height, was later termed the body mass index (BMI) by Alan Keyes (123) and has been found to be one of the best indices of relative adiposity or excess body weight in the association of body composition with mortality. Major putative complications of obesity include cardiovascular disease, hypertension, kidney disease, and type 2 diabetes. Nevertheless, most epidemiological studies have found the relationship of body mass index to be U-shaped (30, 124), J-shaped (125, 126), or reverse J-shaped (127) and, within populations with pre-existing disease, the relationship of adiposity on adverse outcomes, particularly mortality, is often found to be inverse (128-130).

The prevalence of obesity was relatively stable in the U.S. until the 1960s and only rose slightly from the late 1960 to 1980, from 13.3 to 15.1% (131). From 1980 to 1988 it rose from 15.1% to 23% and has continued to rise, reaching a prevalence of 32.2% in 2004 (132). Although secular trends in overweight and obesity in the general population have been well documented (132-134), time trends in populations with pre-existing disease, such as type 1 diabetes, have not been as thoroughly investigated, nor the relationship of adiposity with the long term complications of type 1 diabetes.

At its essence type 1 diabetes is a wasting disease, characterized by severe deficiency or absolute absence of the anabolic hormone, insulin, essential to ensuring intake of energy into

cells and the prevention of muscle and fat catabolism. Without this hormone, the natural history of the disease would be a progressive wasting to death. Upon correction, or relative correction, of wasting via exogenous insulin, an alternate natural history of the disease commences. This alternate natural history includes progressive kidney disease, with its sequelae of hypertension leading to further progression of kidney disease leading to an atherogenic lipid profile, anorexia, and wasting in its final stages. This alternate natural history also includes very early cardiovascular disease, still largely unexplained, particularly in women, autonomic neuropathy with its dysphagia, anorexia, and early satiety, other neuropathies causing muscle wasting or limited mobility via pain, bone deformities, or amputations. It also includes blindness with its limitation on mobility and peripheral vascular disease, the latter also causing limited mobility due to pain or amputations. Finally, this natural history ends with very early mortality, with its obvious limit on the time in which weight gain can occur and its implications for adiposity as in populations with limited longevity increased adiposity tends to be protective. Any effects of or on adiposity within type 1 diabetes must take place within this environment and to the extent that these effects mirror the general population, whether good or bad, represents a major accomplishment in type 1 diabetes.

Within this hostile environment, we observed a 47% increase in the prevalence of being overweight and a 700% increase in the prevalence of obesity over 18 years of follow-up in the Pittsburgh Epidemiology of Diabetes Complications Study adult cohort. Mean weight increased by 2.6 kg/m² over the course of follow-up, with weight gain observed in over two-thirds of the cohort at last follow-up. Although more than one-fifth of the population had died by the end of follow-up, the mean age at death was the same as the mean age of the living at last follow-up, indicating an extension of the lifespan within this population. Adiposity was not however

independently predictive of coronary artery disease (Appendix C), the leading cause of death within this population. Body mass index was not predictive of overt nephropathy (Appendix C), nor complications of overt nephropathy (135), the second leading cause of death within this population. Adiposity demonstrated divergent associations with the prevalence as opposed to the severity of coronary artery calcification, a process associated with both coronary artery disease and overt nephropathy. Adiposity was positively associated with the presence of any coronary artery calcification, but, given calcification was present, inversely associated with the severity of coronary artery calcification. Free fatty acids, a major putative factor in the relationship of adiposity with insulin resistance and cardiovascular disease, and posited to increase with increasing visceral fat, demonstrated no relationship with visceral adiposity or estimated insulin resistance and showed no relationship with coronary artery calcification; however, it was associated with subcutaneous abdominal adiposity, at low levels in men and at both low and high levels in women. In women, free fatty acids were also associated with brachial pulse pressure, a measure of arterial stiffness. Finally, adiposity demonstrated a complex relationship with mortality in this population, with increased risk at both ends of the BMI spectrum and demonstrated an inverse relationship with mortality when accounting for waist circumference, whether looking at baseline BMI, average BMI during follow-up, or BMI closer to the end of follow-up. Weight gain in adulthood was associated with decreased mortality.

Although traditionally viewed as a starvation state, after the discovery of Banting and Best and the subsequent availability of exogenous insulin (136, 137), type 1 diabetes can now be better described as a tug of war between excess peripheral insulin and insulin deficiency. Indeed, studies of mean amplitude of glycemic excursions indicate that even in relatively well controlled individuals with type 1 diabetes this is an ongoing process (138-140). Insulin, the

primary anabolic hormone of nutrient metabolism, suppresses lipolysis, proteolysis, and gluconeogensis, facilitates glucose uptake into muscle and adipose tissue, increases glycogenesis and lipogenesis, and stimulates general protein anabolism, and thus weight gain (141). While acute excess of this hormone in peripheral tissues can result in mild to severe hypoglycemia resulting in increased secretion of counterregulatory hormones and free fatty acids, and in very severe cases permanent brain damage or death, insulin deficiency results in hyperglycemia, and in more severe cases polyuria, dehydration, electrolyte imbalance, again increased free fatty acids, ketoacidosis, and weight loss. Very severe cases of acute insulin deficiency can result in death. Thus, although the anabolic effects of insulin promote adipose and muscle tissue build up and its deficiency promotes weight loss, both extremes of the insulin homeostasis spectrum result in increased FFA production and death.

In this population, we have shown a complex interaction between catabolic and anabolic factors over the approximate two decades of follow-up. Intensification of insulin therapy increased dramatically while HbA1c, a marker of glycemic control, showed a substantial reduction, both factors directly associated with weight gain via a reduction in catabolic tissue breakdown and glycosuria and an increase in adipose tissue storage. They are also indirectly associated with weight gain via a reduction in complications associated with wasting, i.e. nephropathy and autonomic neuropathy (3). Nevertheless, although the progression of the natural history of the disease has slowed, it has not halted, and for coronary artery disease, no improvement has been made (104) (some would argue that cardiac events may have even increased after the institution of intensive insulin therapy (142), and after 18 years of follow-up, the EDC population is considerably older with considerably more complications and thus the wasting process may still be operant. Furthermore, although there was a reduction in many risk

factors associated with complications of diabetes, for blood pressure there was not. Blood pressure, increased overtime and, particularly for systolic blood pressure, across BMI categories. This has important implications for mortality since in the general population hypertension is postulated to be a primary mediator of obesity's impact on mortality (143).

Despite the marked increase in overweight and obesity observed in this population, risk factors for weight gain were largely indeterminate. There is strong biological plausibility for weight gain with intensive insulin therapy use (5, 144) and in our population, with a time lag of approximately 2 years, the increase in overweight an obesity paralleled that of intensive insulin therapy use. Thus, intensive insulin therapy appeared to demonstrate a strong relationship with weight gain and the increasing prevalence of overweight and obesity; nevertheless, this was difficult to demonstrate in our statistical models and therefore ecological fallacy cannot be ruled out. To the extent that increased longevity represents a normalization of the type 1 diabetes population, our observation may simply reflect secular trends in the general population. It is possible that intensification of insulin therapy is allowing for greater dietary freedom and greater ability to simulate the background population in eating habits. It is also possible that better risk factor management overall in those on intensive therapy as well as the reduction in or delayed progression to chronic diabetes complications as a result of intensification of insulin therapy is allowing for normalization of the population and subsequently the ability to mirror the secular trend observed in the general population. This appears to be partially substantiated by our results, as the strongest predictors of weight gain were diabetes complications and smoking, all inverse predictors, while many risk factors for wasting associated complications demonstrated an improvement after 18 years of follow-up, despite the aging of the cohort. However, intensive insulin therapy itself, being a more physiological method of insulin delivery, may itself result in

a relative normalization of the type 1 diabetes population. All of this notwithstanding, direct anabolic effects of insulin appear to be operant; although delayed, the increase in the prevalence of overweight and obesity in our population appeared to be occurring at a faster rate than in the general population.

Intensive insulin therapy, associated with both weight gain (4, 145) and reduction in HbA1c (2, 146) and diabetes complications, loomed large but was relatively silent, statistically speaking, in our population. Nonetheless, for the most important complication of all, mortality, intensive insulin therapy showed a strong protective effect. But this was only when looking at the average duration on intensive insulin therapy and this did not account for the strong relationship of obesity with mortality. Average duration of time on blood pressure medication was also protective of mortality, and in this model average amount of time spent in the obesity BMI range was not predictive, but average blood pressure during follow-up was, as was being underweight for the majority of follow-up. Just as hypertension when defined by both absolute blood pressure cut points and use of hypertension medication can be misleading in its relationship with outcomes, e.g. hypertension is protective of mortality when modeled in place of blood pressure and hypertension medication, similarly it is possible that the assumed relationship of intensive insulin therapy with outcomes can be confounded by its individual components. We (Appendix C) have shown that the reduction in HbA1c with intensive insulin therapy use was actually due to self monitoring of blood glucose and not to intensification of insulin therapy.

Type 1 diabetes is not only an (absolute) insulin deficiency state; it is also an amylin deficiency state as amylin is cosecreted with insulin (100, 147, 148). Thus another possible reason for weight gain with intensification of insulin therapy is that in tight glycemic control, although exogenous insulin may compensate for the glucoregulatory effect of amylin, it cannot

compensate for its satiety inducing effect, i.e. slowing of gastric emptying and reduction of food intake, and thus, knowing when to stop eating. Conversely, in relatively low insulin states it may also result in wasted calories, without necessarily clinically evident wasting, due to its satiety and postprandial glucose regulatory effects, and thus increased postprandial glucose levels which may exceed the renal threshold (~180 mg/dl) (100), resulting in polyuria and thus the traditional lean phenotype in type 1 diabetes. Nevertheless, intensification of insulin therapy may have effects on complications of diabetes beyond that of any effects on adiposity, as our updated means mortality model appeared to demonstrate.

Not only does insulin suppress endogenous glucose output and facilitate glucose uptake, it also suppresses lipolysis. In our population, a low insulin dose at baseline was an independent predictor of the eighteen year incidence of non-fatal coronary artery disease, while HbA1c, body mass index, and waist circumference were not (Appendix C). It is possible that this low insulin dose resulted in increased levels of free fatty acids, shown to be related to adverse cardiac outcomes, such as arrhythmias(149, 150), ischemia (151), and sudden cardiac death (152). While we did not have free fatty acid measurements at baseline, for this thesis they were measured in the sixteen-year follow-up exam population and found to be predictive of pulse pressure in women, a measure of arterial stiffness also associated with arrhythmias (153), ischemic heart disease (154), and sudden cardiac death (1).

The major putative mediator of obesity's relationship with coronary artery disease is abdominal adiposity induced insulin resistance, primarily through free fatty acids. Free fatty acids induce hepatic insulin resistance by inhibiting glycogenolysis, reducing peripheral tissue glucose uptake, and increasing hepatic glucose production. Lipolytically active visceral adiposity, with its direct route to the portal vein, has generally been put forth as the primary

source of insulin resistance inducing free fatty acids. However, it has also been argued that subcutaneous abdominal fat is the major source of both plasma and hepatic free fatty acids, and thus the insulin resistance associated with elevated abdominal adiposity. Although free fatty acids are elevated with obesity in the general population, in our population, free fatty acids did not appear to increase with increasing adiposity overall. They did, however, show a strong relationship with fasting glucose level and in type 1 diabetes, both ends of the glucose homeostasis spectrum may result in insulin resistance due to increased catecholamine and free fatty acid secretion, and in severe hyperglycemia, glucose toxicity.

Although we did not observe a relationship of free fatty acids with pulse pressure in men, in men both visceral and subcutaneous abdominal adiposity were related to aortic pulse pressure. These abdominal fat measures were also associated with pulse pressure in women, although in women this relationship interacted with free fatty acids. However, in both men and women, visceral and subcutaneous abdominal adiposity was associated with coronary artery calcification, a positive relationship with the presence of any coronary artery calcification and, particularly in women, an inverse relationship with its severity given any calcification. In men, there was a suggestion of a U-shaped relationship of visceral adiposity with the severity of coronary. Thus in men, high levels of visceral fat appear to be associated with both pulse pressure and insulin resistance (Appendix A, Paper 3). Just as there is a sexual dimorphism in lipid metabolism and adiposity distribution (155-157), there also appears to be sexual dimorphism in lipid and adipose tissue function. The free fatty acid relationship with insulin resistance in the non-diabetes population appears to be just in men. Nevertheless, we did not observe a relationship of free fatty acids with coronary artery calcification, presence or severity, in either sex. Then again, we did not observe the expected difference in free fatty acids by fasting status in men, although it

was observed in women with no difference in ambient glucose levels either by sex or fasting status.

The divergent relationship of our adiposity measures, whether visceral abdominal fat, subcutaneous abdominal fat, body mass index, or waist circumference, with coronary artery calcification may help to explain our failure to show a relationship of either body mass index or waist circumference with the 18-year incidence of coronary artery disease, whether fatal or non-fatal (Appendix C). In contrast, both of these adiposity indices were associated with overall mortality and appeared to provide independent information. It appears that abdominal adiposity and body mass index, although providing independent information, are better predictors of all-cause mortality than of coronary artery disease mortality or non-fatal coronary artery disease, an interesting finding given that coronary artery disease is posited to be in the biological pathway between obesity and mortality.

We found that after adjustment for waist circumference, any adverse obesity relationship with mortality disappeared and an inversely linear relationship between body mass index and mortality emerged. This was true whether looking at baseline body mass index, average body mass index, or body mass index closer to the time of event. In contrast, the relationship between waist circumference and mortality remained positive in baseline and time-varying models. In the updated means model, the relationship between waist circumference and mortality changed from a U-shaped relationship to a positively linear relationship after adjustment for body mass index. It appears that abdominal adiposity accounted for any adverse effect of a higher BMI and any protective effect of a lower BMI, while lower lean body mass and/or peripheral adiposity accounted for any adverse effect of low abdominal fat. So here we see divergent effects of abdominal adipose tissue and lean/peripheral adipose tissue on mortality. Given that weight loss

shows a stronger association with lean body mass than fat mass in general and the very strong inverse association between weight change and mortality in our population, our finding of an inverse relationship between body mass index and mortality once accounting for abdominal fat is not surprising. More difficult to explain is the relationship of abdominal fat with mortality after accounting for body mass index since we did not find waist circumference to account for the 18-year incidence of coronary artery disease. Closer examination of the pulse pressure data revealed that although free fatty acids were associated with pulse pressure at low levels of subcutaneous adiposity in men and at high levels of subcutaneous adiposity in women, in both men and women, pulse pressure was associated with visceral abdominal fat. Estimated insulin resistance was strongly associated with pulse pressure in both sexes ($r=0.37$, $p=0.002$ in men; $r=0.41$, $p=0.0004$ in women, after age-adjustment) and after adjustment for estimated insulin resistance, the relationship of free fatty acids with pulse pressure was even stronger in women ($r=0.30$, $p=0.01$), with no effect on men ($r=0.05$, $p=0.71$). Thus it is possible that insulin resistance in men and both insulin resistance and free fatty acids in women may account for the relationship of abdominal adiposity with mortality. The increased stress imposed by complications of diabetes, as well as any acute illness, on the individual with type 1 diabetes may cause both an increase in catecholamines and a rise in free fatty acids and subsequently in insulin resistance.

Our finding of divergent relationships of adiposity for the presence versus the severity of coronary artery calcification suggested that two different processes were operant in the calcification process in type 1 diabetes. One is the atherosclerotic process of the intimal arterial wall, the other the advanced glycation end products (AGEs) driven calcification of the medial arterial wall. Our positive association of adiposity with both the presence and progression of

coronary artery calcification (158), but it's inverse, albeit non-significant association with the severity of coronary artery calcification lends support to this. We have recently shown a cross-sectional association of AGEs with coronary artery calcification and overt nephropathy, but not coronary artery disease (159). We were also able to demonstrate a relationship with distal-symmetrical polyneuropathy, but not lower arterial extremity disease. Since both overt nephropathy and coronary artery calcification show a strong relationship with coronary artery disease (160) and likewise distal-symmetrical polyneuropathy shows a strong association with lower extremity arterial disease, it is possible that any large vessel disease not accounted for by AGEs may be largely due to adiposity driven atherosclerosis. Finally, poorer glycemic control was predictive of mortality in all three of the biological risk factors time models and, in the prediction of weight change, it was associated with both weight gain and weight loss. With its constant struggle between catabolism and anabolism, lipolysis/proteolysis and lipogenesis/proteogenesis, and chronically elevated free fatty acid levels, the single most important mediator of weight change in type 1 diabetes is not any of is long-term complications, but the disease itself and duration of disease is the single most important risk factor for mortality.

8.1 LIMITATIONS

A major limitation of this study was that at a mean of 19 years duration, our population, although young, was already long duration cohort at baseline. Furthermore, with a mean age of eight years at diabetes diagnosis, effects of age could not be truly separated from those of the disease itself. The non-linear relationship between BMI and mortality observed in the general

population has been reported by some to be mainly in the elderly and that in the middle-aged or young this relationship tends to be linear. With a maximum age of 48 years at baseline, we were not able to investigate this, nevertheless, even in this young cohort the U-shaped association of baseline BMI with mortality was observed. It could be argued that type 1 diabetes is an accelerated aging disease in which diabetes complications, termed chronic diseases in the general population, occur at a very early age and life expectancy is reduced by several decades, and in this population with only an average of eight years separating birth and diagnosis of type 1 diabetes, age appears to be synonymous with the disease itself. This seems to be supported by our finding of a very strong association between surrogate measures of AGEs and the severity of coronary artery calcification (159), where an inverse relationship with adiposity was observed (VAT/calcification paper). Although both coronary artery calcification and AGEs are associated with age, AGEs increase naturally with age, and are elevated further in type 1 diabetes perhaps as a result of their formation being accelerated with chronic hyperglycemia and oxidative stress (161). Indeed, AGEs have been found to be associated with almost all long-term complications of diabetes.

Our long-duration cohort with its very strong association between age and diabetes duration also made it difficult to stratify by "pre-existing disease". It has been argued that in estimating the association of BMI with mortality, pre-existing illness should be excluded, however, it should be noted that in this population, type 1 diabetes itself is probably the most important pre-existing disease. At study baseline, nearly half of this population had a pre-existing long-term complication of type 1 diabetes. However, the increased mortality among the lean was not due to "pre-existing disease", i.e. complications of diabetes, as exclusion of this

subgroup did not alter the shape of the association. But this same observation has been repeatedly shown in the general population (162, 163).

In addition, after approximately twenty years of follow-up in which nearly one-fourth of the population has died, our long-duration cohort is now a survival cohort, and substantial survival bias in our results may be operant. While it is possible that due to the cross-sectional nature of the study design of the investigation of adiposity with coronary artery calcification, the inverse association observed between adiposity and severity of calcification resulting from left truncation of the more corpulent 16-18-year follow-up exam pre-deceased, due to the very nature of type 1 diabetes it appears more likely that those with increased adiposity near the end of our follow-up period represent those better able to fight the catabolic process of the disease. This seems to be supported by the very strong relationship we observed between weight change in mortality, and that in analyses that excluded the pediatric population. Furthermore, although a net weight gain was observed in the majority of this population at last follow-up, perhaps more importantly net weight gain, far exceeded weight loss very likely due to the competing risk of mortality. And while we tried to control for follow-up time, perhaps this too, like age, could not be truly controlled for.

Another limitation of this study is that baseline measurements of visceral and subcutaneous adiposity, coronary artery calcification, and free fatty acids were not available. These measurements were not made available until the 16-year follow-up exam, with the exception of coronary artery calcification which was first made available at the 12-year follow-up exam. In addition to the bias that this may present in the interpretation of our results as at the 16-18-year follow-up when these variables were investigated this was a survival cohort, only

cross-sectional associations coronary artery calcification with abdominal fat and free fatty acids could be determined and as such reverse causality cannot be ruled out.

Given the importance of insulin and glucose homeostasis on weight change and free fatty acids, the lack of data on mean amplitude of glucose excursions is another limitation of this study. Similarly, although hyperinsulinemic euglycemic clamp data was available on a sub-sample of our population, it would have been useful to have hyperinsulinemic hypoglycemic clamp data as well since free fatty acids provide a substantial part of the counterregulatory response to hypoglycemia (164, 165) and elevated free fatty acids from antecedent hypoglycemia also induce insulin resistance as a defense mechanism against subsequent hypoglycemia (166). However, as this was a long duration cohort even at baseline it may have been unethical to perform this type of clamp study as hypoglycemia and the free fatty acid response to hypoglycemia are hypothesized to trigger dangerous ischemia, arrhythmias, and even myocardial infarctions in those with an already compromised vasculature, even if subclinical, and is one of the hypothesized causes of dead (167) in bed syndrome and sudden death too frequently observed in type 1 diabetes (168).

Finally, this was a 98% Caucasian (2% African American) population and as such is limited in its generalizability to non-White type 1 diabetes populations. Although the prevalence of type 1 diabetes was low among African Americans relative to Caucasian Americans in the diagnosis years of EDC eligibility, it was not that low. Type 1 diabetes is one of the leading chronic diseases in African American children and African Americans account for approximately 6% of type 1 diabetes cases in the United States overall. To the extent that race has any impact on the effects of or on adiposity in type 1 diabetes above and beyond the type 1 diabetes disease process itself, our results are limited in their generalizability. However, considering the inverse

association of BMI with three-year mortality in the New Jersey 725 Cohort (169), an exclusively African American type 1 diabetes cohort, this does not appear to be the case, at least not for African Americans. Perhaps of greater concern is the overgeneralization that may be caused by the under-representation in type 1 diabetes studies of racial/ethnic groups at increased risk of type 2 diabetes relative to non-Whites. Indeed, overgeneralization of type 2 to type 1 diabetes, whether in White or non-White populations, is perhaps the biggest public health concern that adiposity poses for type 1 diabetes.

8.2 PUBLIC HEALTH AND CLINICAL IMPLICATIONS

Caution should be taken not to generalize risk factors for adverse outcomes in the general population to outcomes within diseased populations. Considering the complex relationship of adiposity in type 1 diabetes, the general lack of a relationship with long-term complications, the protective effect of weight gain on mortality and the wide BMI range associated with minimal mortality, risk factor management should focus on risk factors shown to be associated with mortality in type 1 diabetes. Although obesity itself should be avoided, it should not be at the expense of improved metabolic control and may be better focused on abdominal adiposity and risk factors correlated with obesity, such as increased blood pressure and an adverse lipid profile. Accounting for these appears to abolish any increased risk associated with obesity with mortality in type 1 diabetes. Although overweight and obesity are increasing in type 1 diabetes, the clinician should bear in mind that type 1 diabetes is the pre-existing disease. With an average disease duration of 33 years and age at death of 43 years, mortality is still occurring several

decades earlier than in the general population. Considering the years of life lost, the protective effect of weight gain may have a strong public health impact.

Future directions should focus on strategies/product development to maximize glucose control while minimizing obesity and without substantially negatively influencing quality of life. This may also be a better way of reducing chronically elevated free fatty acid levels as direct pharmacological lowering of free fatty acids may have adverse outcomes in this population strongly dependent on them as an alternative fuel source and substrate for glucose counterregulation. Finally these data raise the possibility that weight gain reflecting lean body mass and general health, may also be a positive benefit if peripheral in location. This is an area that merits further research.

APPENDIX A: Supplementary Paper for Specific Aim 3

FREE FATTY ACIDS ARE ASSOCIATED WITH PULSE PRESSURE IN WOMEN, BUT NOT MEN, WITH TYPE 1 DIABETES

Baqiyyah Conway[1], Rhobert Evans[1], Linda Fried[2], Sheryl Kelsey[1], Daniel Edmundowicz[2], Trevor Orchard[1]

Manuscript in Preparation

[1]University of Pittsburgh, Department of Epidemiology, Graduate School of Public Health

[2]University of Pittsburgh, School of Medicine

A.1 ABSTRACT

Background: Cardiovascular disease is the leading cause of death in type 1 diabetes. Pulse pressure, a measure of arterial stiffness, is also elevated in type 1 diabetes, and associated with arrhythmias, ischemic and sudden cardiac death. Free fatty acids, elevated in type 1 diabetes, females, and abdominal obesity, have associations. We therefore examined whether free fatty acids were associated with pulse pressure in an adult population (n=150) of childhood onset type 1 diabetes and whether any such association appeared to be mediated by abdominal adiposity and gender. We also investigated the relationship of free fatty acids with coronary artery calcification.

Methods: Mean age and diabetes duration were 42 and 33 yrs, respectively when CAC, visceral adiposity (VAT), and subcutaneous abdominal adiposity (SAT) were determined by electron beam tomography. Free fatty acids were determined by in vitro colorimetry. Pulse pressure was calculated as the systolic blood pressure minus diastolic blood pressure. Free fatty acids were log-transformed before analyses and all analyses were controlled for serum albumin.

Results: Free fatty acids were associated with pulse pressure in women (r=24, p=0.04), but not in men (r=0.07, p=0.55). An interaction for the prediction of pulse pressure was noted between free fatty acids and both VAT (p=0.03) and SAT (p=0.008) in women, but only a marginal interaction with SAT (p=0.09) and no interaction for VAT with free fatty acids observed (p=0.40) in men. In multivariable linear regression analysis allowing for serum albumin, age, height, heart rate, albumin excretion rate, HbA1c, HDLc, free fatty acids, SAT, and the interaction between free fatty acids and SAT, the interaction between free fatty acids and SAT remained significantly associated with pulse pressure in women (p=0.03), but not men (p=0.32). Free fatty acids showed no association with log-transformed coronary artery calcification.

Conclusion: Free fatty acids are associated with in women, but not men, with type 1 diabetes. This finding might help to explain the lost of the gender difference in cardiovascular disease in type 1 diabetes.

A.2 INTRODUCTION

Free fatty acids (FFAs) are known to be elevated in type 1 diabetes and obesity, particularly abdominal obesity (1, 2), and to be associated with insulin resistance (3). Insulin resistance in type 2 diabetes and in the general population is associated with a markedly increased risk of coronary artery disease (4, 5). However, T1D is a disease characterized by an abnormality in fuel utilization. Both intermittent insulin deficiency/absence and excess are characteristic of this disease, and both contribute to excess free fatty acid production; therefore, the adiposity FFA/relationship observed in the general population may be very different in T1D.

Coronary artery calcification is a subclinical marker of coronary artery disease. In the Epidemiology of Diabetes Complications (EDC) Study, inverse and non-existent relationships were observed between the severity of coronary artery calcification and abdominal fat (6). In type 1 diabetes it is uncertain to what extent calcification of the coronary arteries is due to atherosclerosis of the intima layer of the arterial wall (which may in part relate to obesity and other cardiovascular risk factors) or calcification of the medial layer, partially renal driven. Another subclinical measure of cardiovascular disease is arterial stiffness. All these measures are likely strongly interrelated, for example Sutton-Tyrrell (7) observed a direct relationship between visceral abdominal fat with arterial stiffness in a non-diabetes population; however, little is known about this association in type 1 diabetes. As FFAs have been postulated to be a mediator of insulin resistance and are predictive of ischemic heart disease (8), cardiac arrhythmias (9,10) and sudden cardiac death (11) in the general population, the elevated levels of free fatty acids observed in type 1 diabetes may also help to explain the greatly increased risk of CAD in this population.

The purpose of this study was to assess the association of free fatty acids with coronary artery calcification and arterial stiffness (pulse pressure) in an adult population of childhood onset type 1 diabetes and to determine whether any such association appeared to be mediated by body fat. As abdominal body fat and free fatty acids are known to vary by gender, these analyses are investigated sex-specifically. To our knowledge, this has not been investigated in type 1 diabetes.

A.3 METHODS

The EDC study is an ongoing study examining the long term complications of T1D in 658 individuals diagnosed before the age of 17 years with T1D at Children's Hospital of Pittsburgh between 1950 and 1980. This current report is based on a subset (n=150) of this population who underwent electron beam tomography (EBT) VAT and subcutaneous abdominal fat (SAT) via EBT scanning as part of the Insulin Resistance Study, a substudy of the 16-year follow-up.

Fasting blood samples were assayed for lipids, lipoproteins, and hemoglobin A1c. High-density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure, a modification of the Lipid Research Clinics method (12). Cholesterol was measured enzymatically (13). FFA were measured using the colorimetric method (Wako Pure Chemical Industries, Ltd). Only fasting samples were used. Urinary albumin was determined immuno-nephelometrically (14).

CAC was measured using EBT (Imatron C-150, Imatron, South San Francisco, CA). Threshold calcium determination was set using a density of 130 Hounsfield units in a minimum of 2 contiguous sections of the heart. Scans were triggered by ECG signals at 80% of the R-R interval. The entire epicardial system was scanned. CAC volume scores were calculated based on isotropic interpolation (15). Direct measurements of abdominal adiposity (visceral and subcutaneous abdominal adipose tissue surface area) were also taken by EBT scanning. Scans of abdominal adipose tissue were taken between the fourth and fifth lumbar regions, which were located by counting from the first vertebra below the ribs. Two 10 mm thick scans were taken during suspended respiration. The images were then analyzed using commercially available software for all pixels corresponding to fat density in Hounsfield units in the appropriate anatomical distribution (subcutaneous or visceral). Height was measured using a stadiometer.

Blood pressure was measured by a random-zero sphygmomanometer according to a standardized protocol (16) after a 5-minute rest period. Blood pressure levels were analyzed, using the mean of the second and third readings. Brachial pulse pressure was calculated (systolic blood pressure-diastolic blood pressure).

The student's *t* test was used to compare characteristics of study participants by fasting status. Further analyses were limited to participants providing fasting blood samples. Pearson correlations were used to assess the relationship between FFA, brachial pulse pressure, and coronary artery calcification, adiposity indices, height, heart rate, glycemia indices, and albumin excretion rate. FFA, VAT, SAT, BMI, coronary artery calcification, and albumin excretion rate were log-transformed before analyses. Multiple linear regression analyses with backward elimination was used to determine independent predictors of pulse pressure and coronary artery calcification. All analyses with FFA were adjusted for serum albumin as FFA travel in serum

bound to albumin. FFA and serum albumin were forced into all multivariable models. Analyses were conducted using SAS version 9.1.3 (Cary, North Carolina). All procedures were approved by the Institutional Review Board of the University of Pittsburgh.

A.4 RESULTS

Twenty-nine percent of the sixteenth-year follow-up exam study participants provided non-fasting blood samples. Table A-1 shows the characteristics of the study participants by fasting status. Overall, there were no differences by fasting status, with the exception of age and FFA levels (41.7 vs. 44.5 years, p=0.02, 0.99 vs. 0.84 mmol/l, p=0.04 in fasters vs. non-fasters, respectively, data not shown). However, upon sex-specific examination, this age difference was found to be only in men. Fasting men were approximately five years younger than non-fasters (41.0 vs. 45.8, p=0.004). By contrast, there was no difference in age in women able or willing to come in for the exam fasting (42.5 vs. 43.5, p=0.56); however, fasting women, but not men, had significantly higher free fatty acid levels than non-fasters. There were no other differences by fasting status in men and women. The remaining analyses are restricted to the 150 fasting participants.

Table A-2 shows the sex-specific Pearson correlations between free fatty acids, pulse pressure, coronary artery calcification, adiposity and glycemia indices, height, heart rate, and albumin excretion rate. Free fatty acids were positively correlated with pulse pressure in women

(r=0.24, p=0.04) but not men (r=0.07, p=0.55), but showed no association with coronary artery calcification in either sex. Free fatty acids were associated with fasting glucose in both men and women, but showed no association with HbA1c, albumin excretion rate, heart rate, height, or any of the adiposity indices. Correlates of pulse pressure and coronary artery calcification are also presented in Table A-2.

Figures A-1 and A-2 show the Pearson's correlation of free fatty acids with pulse pressure by tertiles of sex-specific subcutaneous and visceral abdominal adiposity, respectively. An interaction was observed between free fatty acids and subcutaneous adiposity for pulse pressure in both men (p=0.09) and women (p=0.008), although only marginal in men. An interaction was also seen between free fatty acids and visceral abdominal adiposity in women (p=0.03), but not in men (p=0.40). Interactions between free fatty acids and adiposity were not observed for coronary artery calcification.

After multivariable linear regression analyses with backward selection, free fatty acids remained significantly associated with pulse pressure in women, but not in men. After adding the interaction term between free fatty acids and subcutaneous adiposity to the final model, the interaction term was significant in women (p=0.03), but not in men (p=0.32) (Table A-3).

Table A-4 shows the multivariable linear regression analyses, with backward selection, of free fatty acids with coronary artery calcification. Free fatty acids were not associated with coronary artery calcification in either sex.

A.5 DISCUSSION

The major finding of this study was that free fatty acids are associated with pulse pressure in women, but not coronary artery calcification in either gender in type 1 diabetes. We also observed that the relationship of free fatty acids with pulse pressure varied by level of subcutaneous and visceral abdominal adiposity. Finally we note that although abdominal fat is not associated with free fatty acids in type 1 diabetes it does appear to modify the relationship between free fatty acids and arterial stiffness in women with type 1 diabetes.

Pulse pressure, the difference between the systolic and diastolic blood pressure, is a measure of arterial distensibility, or stiffness. We found that free fatty acids were associated with an increase in brachial pulse pressure. Although little is known about the relationship of free fatty acids and arterial stiffness, Steinberg et al (17) found that free fatty acids caused endothelial dysfunction in healthy individuals. Nakayama et al (18) found that abnormal free fatty acid metabolism was associated with diastolic, but not systolic, dysfunction in individuals with essential hypertension. Free fatty acids account for a substantial proportion of the counterregulatory defense against hypoglycemia (19), a known player in endothelial dysfunction and cardiac ischemia. Acute hypoglycemia causes an increase in systolic blood pressure and a decrease in diastolic blood pressure, and therefore an increase in pulse pressure (20, 21). Although mean fasting glucose levels in our study were well out of the hypoglycemia range, preclinic nocturnal hypoglycemia and subsequent counterregulation cannot be ruled out.

The free fatty acid associated increase in pulse pressure may lead to cardiac arrhythmias, ischemia, and sudden cardiac death. In the Framingham Heart Study, pulse pressure, but not mean arterial pressure, significantly predicted atrial fribillation (22). In patients with type 2 diabetes, Paolisso et al (9) observed ventricular premature complexes to increase with increasing

free fatty acid concentration and to decrease when free fatty acids were directly lowered. The increased free fatty acids accompanying hypoglycemia counterregulation or very low to absent insulin levels in type 1 diabetes may also increase tissue ischemia. In nondiabetic men, elevated levels of free fatty acids were associated with ischemic heart disease (8). Elevated levels of free fatty acids have also been associated with sudden cardiac death (11, 23). Free fatty acid inundation of the myocardium is observed in Acute Coronary Syndromes and the better outcomes in patients in the first DIGAMI study (24, 25) randomized to insulin treatment may be due to insulin's suppression of free fatty acid release into the circulation. In the EDC population, low daily insulin dose at baseline, but not HbA1c, was independently predictive of the 18 year incidence of non-fatal coronary artery disease (Appendix C3).

In our population, the adiposity relationship of pulse pressure with free fatty acids varied by sex. In the general population as well, a sexual dimorphism exists in adiposity, particularly visceral adiposity, and insulin resistance. Visceral adiposity and insulin resistance are both higher in men; nevertheless, free fatty acids tend to be slightly increased in women (26). A sexual dimorphism in lipid metabolism is also observed (26) in the general population. Women have an increased free fatty acid response to fasting (27), while fasting glucose levels tend to be lower. Hojlund et al (28) observed during 72 hours of fasting mean plasma free fatty acids were higher in women while mean glucose levels were lower in women throughout the duration of the fast. After approximately 36 hours of fasting, mean glucose levels were approximately 3.5 mmol/l (~63mg/dl) in women, while they remained at approximately 4 mmol/l (~72gm/dl) or above in men. Similar findings were noted by Soeters et al (29). A sex difference in the counterregulatory response to hypoglycemia also exists both in the non-diabetic population (30; 31, 32) and in type 1 diabetes (33). Women have a reduced sympathetic nervous system

response to hypoglycemia (34). This decreased epinephrine, norepinephine, growth hormone, and subsequent endogenous glucose production response to declining glucose levels would be expected to produce a greater frequency of hypoglycemia in women with type 1 diabetes. However, men experience a greater blunting of the autonomic nervous system counterregulatory responses to hypoglycemia following antecedent hypoglycemia (34). Although the tightened glycemic control achieved in the intensive arm of the Diabetes Control and Complications Trial (DCCT) came at the expense of a three-fold increase in severe hypoglycemic events, there was no difference in the prevalence of hypoglycemia between men and women (35). Enhanced free fatty acid response to hypoglycemia in women (27) may account for the resistance women exhibit to the blunting effects of antecedent hypoglycemia. Nevertheless, this may come at the expense of the increased stiffness observed in women with type 1 diabetes (36, 37) and may partially account for the loss of the gender difference in coronary artery disease in type 1 diabetes.

Free fatty acids themselves, although showing a relationship with pulse pressure, failed to show a relationship with coronary artery calcification. We have previously suggested that the coronary artery calcification in type 1 diabetes might not be the obesity/lipid driven atherosclerosis, but advanced glycation end products (AGEs) driven, as we failed to show an association of adiposity with the severity of coronary artery calcification in the EDC population (with the exception of an inverse relationship if subcutaneous abdominal fat in women) (6). These findings suggest two divergent pathways to coronary artery disease unrelated to obesity driven insulin resistance in type 1 diabetes. Nevertheless, as we have previously shown BMI to predict progression of calcification (38), the obesity driven atherosclerotic pathway cannot be completely ruled out.

In conclusion, free fatty acids predict arterial stiffness, but not coronary artery calcification in type 1 diabetes. Although neither coronary artery calcification nor arterial stiffness appear to be mediated by obesity driven insulin resistance, arterial stiffness does appear to mediate in part by free fatty acid driven insulin resistance. As both low insulin dose and hypoglycemia increase the free fatty acid flux in type 1 diabetes, these findings may help to explain the inconsistent, and generally null, findings of a relationship of HbA1c and coronary artery disease in type 1 diabetes (39). Given the recent failure clinical trials to show a cardiovascular benefit, and in one study, an adverse association, of intensive glycemic control in diabetes (40), the results of our study have important clinical implications. Both hypoglycemia and hyperglycemia need to be monitored, not just HbA1c, in order to avoid elevated free fatty acid flux to the myocardium and kidney and the subsequent myocardial damage.

Acknowledgements

The authors would like to thank Beth Hauth for her help in assaying the free fatty acids.

A.6 CITED WORKS

1. Koutsari C, Jensen M. Free fatty acid metabolism and human obesity. *J Lipid Res* 2006; 47: 1643-1650.

2. Steinberg H, Gumbiner B. Pathophysiology of obesity and metabolic response to weight loss. In: Gumbiner, B (ed). Obesity. American College of Physicians: Philadelphia, 2001, pp 50-66.

3. Boden G. Gluconeogenesis and Glycogenolysis in Health and Diabetes. Journal of Investigative Medicine 2004; 52:375-378.

4. Grundy S, Cleeman J, Daniels S, Donato K, Eckel R, Franklin B, Gordon D, Krauss R, Savage R, Smith S, Spertus J, Costa F. Diagnosis and Management of the Metabolic Syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005; 13:322-327.

5. Alberti K, Shaw P, the IDF Epidemiology Task Force Consensus Group. The metabolic syndrome-a new worldwide definition. The Lancet 2005; 366:1059-1062.

6. Conway B, Miller R, Costacou T, Fried L, Kelsey S, Evans R, Edmundowicz D, Orchard T. Double-edged relationship between adiposity and coronary artery calcification in type 1 diabetes. *Diabetes Vasc Dis Res* 2007; 4:332-339.

7. Sutton-Tyrrell K, Newman A, Simonsick EM, Havlik R, Pahor M, Lakatta E, Spurgeon H, Vaitkevicius P. Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. *Hypertension* 2001; 38:429-433.

8. Pirro M, Mauriege P, Tchernof A, Cantin B, Dagenais G, Despres J, Lamarche B. Plasma free fatty acid levels and the risk of ischemic heart disease in men: prospective results from the Quebec Cardiovascular Study. *Atherosclerosis* 2002: 377-384.

9. Paolisso G, Gualdiero P, Manzella D, Rizzo M, Tagliamonte M, Gambardella A, Verza M, Gentile S, Varriccho M, D'Onofrio F. Association of fasting plasma free fatty acid concentration and frequency of ventricular premature complexes in nonischemic non-insulin dependent diabetic patients. *Am J Cardiol* 1997; 80:932-937.

10. Kurien V, Yates P, Oliver M. The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion. *Eur J Clin Invest* 1971; 1: 225-241.

11. Pilz S, Scharnag H, Tiran B, Wellnits B, Seelhorst U, Boehm B, Marz W. Elevated plasma free fatty acids predict sudden cardiac death: a 6.85-year follow-up of 3315 patients after coronary angiography. *European Heart Journal* 2007; 28: 2763-2769.

12. National Institute of Health, Department of Health, Education and Welfare. (1978) Lipid Research Clinics Program. Washington, D.C.: U.S. Govt. Printing Office 1975 (NIH pub no. 75-628).

13. Allain C, Poon L, Chan C, Richmond W, Fu P. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20(4):470-5.

14. Ellis D, Buffone G. A new approach to the evaluation of proteinuric states. Clin Chem 1977; 23: 666-670.

15. Callister T, Cooil B, Raya S, Lippolis N, Russo D, Raggi P. Coronary artery disease: improved reproducibility of calcium scoring with an electron-beam CT volumetric method. *Radiology* 1998; 208:807-814.

16. Borhani N, Kass E, Langford H, Paynr G, Remington R, Stamler J. The hypertension detection and follow-up program. *Prev Med* 1976; 5:207-215.

17. Steinberg H, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron A. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *The Journal of Clinical Investigation* 1998; 100: 1230-1239.

18. Nakayama H, Morozumi T, Nanto S, Shimonagata T, Ohara T, Takano Y, Kotani J, Watanabe T, Fujita M, Nisho M, Kusuka H, Hori M, Nagata S. Abnormal myocardial free fatty acid utilization deteriorates with morphological changes in the hypertensive heart. *Jpn Circ J* 2001; 65:783-787.

19. Fanelli C, Calderone S, Epifano L, De Vincenzo A, Modarelti F, Pampanelli S, Perriello G, De Feo P, Brunetti P, Gerich J, Bolli G. Demonstration of a Critical Role for Free Fatty Acids in Mediating Counterregulatory Stimulation of Gluconeogenesis and Suppression of Glucose Utilization in Humans. *J Clin Invest* 1993; 92:1617-1622.

20. Sommerfield A, Wilkinson I, Webb D, Frier B. Vessel wall stiffness in type 1 diabetes and the central hemodynamic effects of acute hypoglycemia. *Am J Physiol Endocrinol Metab* 2007; E1274-E1279.

21. Wright R, Firer B. Vascular disease and diabetes: is hypoglycaemia an aggravating factor? *Diabetes/Metabolism Research and Reviews* 2008; 24: 353-363.

22. Mitchell G, Vasan R, Keyes M, Parise H, Wang T, Larson M, D'Agostino R, Kannel W, Levy D, Benjamin E. Pulse Pressure and Risk of New-Onset Atrial Fibrillation. *JAMA* 2007; 297:709-715.

23. Jouven X, Charles M, Desnos M, Ducimetiere P. Circulating Nonesterified Fatty Acid Level as a Predictive Reisk Factor for Sudden Death in the Population. *Circulation* 2001; 104:756-761.

24. Malmberg K, Rydén L, Efendic S, Herlitz J, Nicol P, Waldenström A, Wedel H, Welin L. Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. J Am Coll Cardiol. 1995; 26(1):57-65.

25. Bhadriraju S, Ray KK, DeFranco AC, Barber K, Bhadriraju P, Murphy SA, Morrow DA, McCabe CH, Gibson CM, Cannon CP, Braunwald E. Association between blood glucose and long-term mortality in patients with acute coronary syndromes in the OPUS-TIMI 16 trial. *Am J Cardiol* 2006;97(11):1573-7.

26. Mittendorfer B. Sexual dimorphism in human lipid metabolism. *J Nutr*. 2005; 135:681-6.

27. Mittendorfer B, Horowitz JF, Klein S. Gender differences in lipid and glucose kinetics during short-term fasting. *Am J Physiol Endocrinol Metab*. 2001; 281(6):E1333-9.

28. Hojlund K, Wildner-Christensen M, Eshoj O, Skjarbaek C, Holst J, Koldkjaer O, Jensen D, Beck-Nielsen H. Reference intervals for glucose, β-cell polypeptides, and counterregulatory factors during prolonged fasting. *Am J Physiol Endocrinol Metab* 2001; 280: E50-E58.

29. Soeters M, Sauerwein H, Groener J, Aerts J, Ackermans M, Glatz J, Fliers E, Serlie M. Gender-Related Differences in the Metabolic Response to Fasting. *J Clin Endocrinol Metab* 92: 3646-3652.

30. Amiel S, Maran A, Powne J, Umpleby A, MacDonald I. Gender differences in counterregulation to hypoglycemia. *Diabetologia* 1993; 36: 460-464.

31. Davis S, Cherrington A, Goldstein R, Jacobs J, Price L. Effects of insulin on the counterregulatory response to equivalent hypoglycemia in normal females. *Am J Physiol Endocrinol Metab* 1993; E680-E689.

32. Diamond M, Jones T, Caprio S, Hallerman L, Meredith-Diamond M, Addabbo M, Tamborlane W, Sherwin R. Gender influences counterregulatory hormone response to hypoglycemia. *Metabolism* 1993; 42:1568-1572.

33. Davis S N, Fowler S, Costa F. Hypoglycemic counterregulatory responses differ between men and women with type 1 diabetes. Diabetes 2000; 49:65-72.

34. Davis S N, Shavers C, Costa F. Gender-related differences in counterregulatory responses to antecedent hypoglycemia in normal humans. J Clin Endocrinol Metab 2000; 85:2148-57.

35. Diabetes Control and Complications Trial Research Group. Epidemiology of Severe Hypoglycemia in the Diabetes Control and Complications Trial. 1991. *Am J Med* 90:450-457.

36. Ahlgren A, Astrand H, Sundkvist G, Lanne. Increased aortic stiffness is persistant in type 1 diabetic women: a follow-up study. *Diabetologia* 2005; 48: 780-783.

37. Ahlgren A, Lanne T, Wollmer P, Sonesson B, Hansen F, Sundkvist G. Increased arterial stiffness in women, but not men, with IDDM. *Diabetologia* 1995; 38:1082-1089.

38. Costacou T, Edmundowicz D, Prince C, Conway B, Orchard T. Progression of Coronary Artery Calcium in Type 1 Diabetes. *Am J Cardiol* 2007; 100:1543-7.

39. Orchard T, Costacou T, Kretowski A, Nesto R. Type 1 Diabetes and Coronary Artery Disease. *Diabetes Care* 2006: 29:2528-2538.

40. Taubes G. Diabetes. Paradoxical effects of tightly controlled blood sugar. *Science* 2008; 322:365-367.

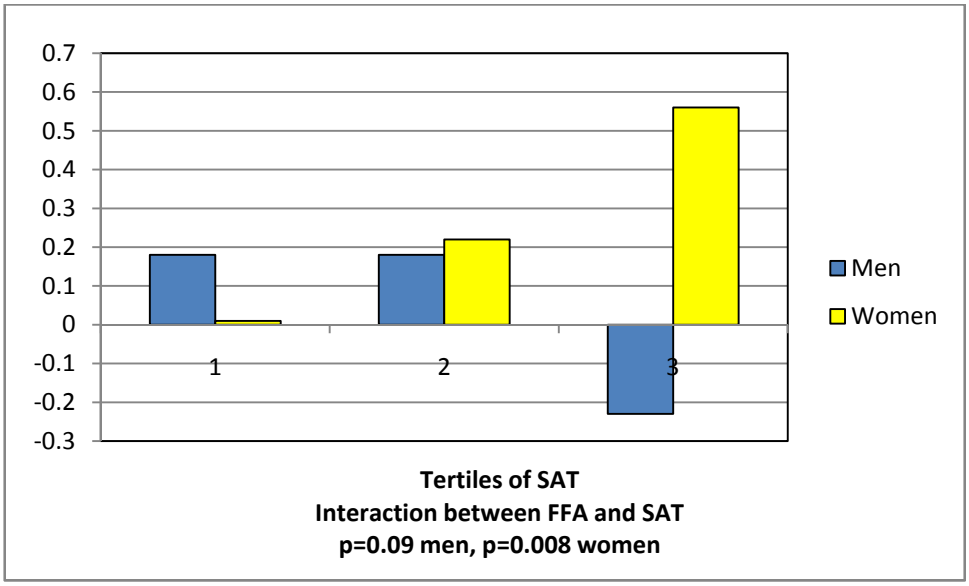

Figure A-1. Association of free fatty acids (FFA) with pulse pressure by tertiles of subcutaneous abdominal adiposity (SAT).

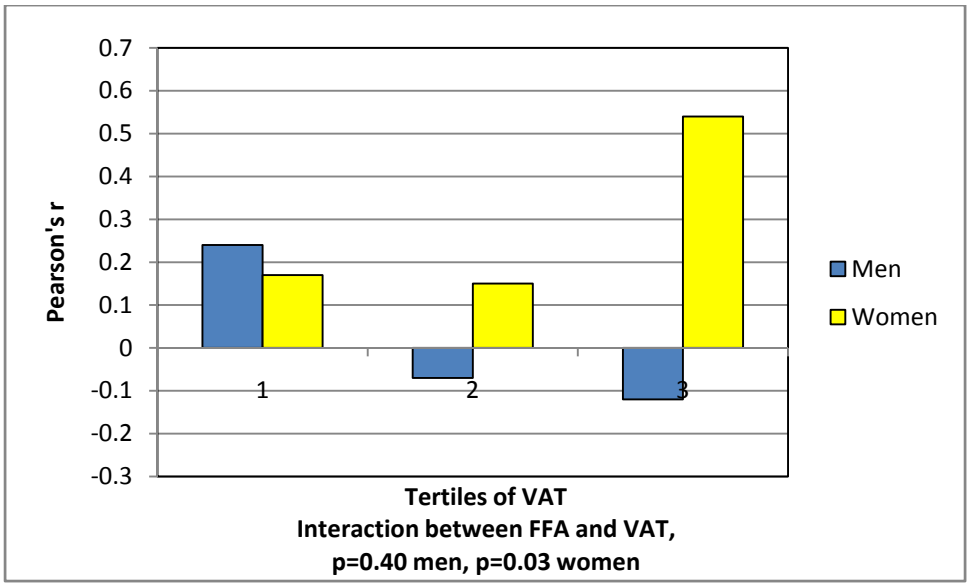

Figure A-2. Association of free fatty acids (FFA) with pulse pressure by tertiles of visceral abdominal adiposity (VAT).

Table A-1. Characteristics of Study Participants by Fasting Status at the 16th Year Follow-up Exam, mean (SD).

	Men (n=101)		Women (n=109)	
	Fasting	Non-fasting	Fasting	Non-fasting
Age, years	41.2 (6.9)	46.0 (7.4)‡	42.5 (8.0)	44.4 (8.8)
VAT*, cm²	113.8 (66.5)	136.6 (67.8)	75.2 (52.4)	75.2 (43.9)
SAT*, cm²	239.1 (387.5)	203.1 (76.2)	255.1 (138.6)	300.5 (434.9)
BMI*, kg/m²	26.8 (3.9)	26.8 (3.4)	26.1 (26.2)	25.6 (25.5)
FFA*, mmol/l	0.95 (0.49)	0.90 (0.42)	1.02 (0.48)	0.82 (0.47)†
HbA1c, %	7.9 (1.4)	8.2 (1.6)	7.9 (1.3)	7.3 (1.2)†
Glucose, mg/dl	156.2 (84.5)	187.7 (96.8)	158.1 (83.4)	162.3 (81.9)
Dose, U/kg/dy	0.67 (0.61)	0.68 (0.23)	0.57 (0.18)	0.57 (0.20)
AER, μg/min	205.3 (693.7)	91.0 (179.6)	99.0 (397.5)	119.4 (420.1)
Height, meters	174.6 (6.19)	174.9 (7.42)	162.5 (6.8)	161.1 (8.3)
Heart rate, beats/min	73.1 (11.5)	75.8 (9.6)	76.6 (11.7)	76.5 (13.3)
CAC score*	176.0 (374.8)	286.5 (523.1)	207.0 (498.1)	146.2 (244.3)
Pulse Pressure, mm HG	50.7 (14.7)	54.2 (15.0)	50.3 (14.2)	52.9 (14.4)

*Natural logarithmically transformed before analyses †$p<0.05$ ‡$p<0.01$

VAT=visceral abdominal adiposity, SAT=subcutaneous abdominal adiposity,

BMI=body mass index, FFA=free fatty acids, AER=albumin excretion rate, CAC=coronary artery calcification

Table A-2. Association of Free Fatty Acids (FFA) with Pulse Pressure (PP), Coronary Artery Calcification (CAC), Adiposity Indices, and Glycemia, Pearson's r (p-value).

	Men (n=75)			Women (n=76)		
	NEFA	PP	CAC	NEFA	PP	CAC
Pulse Pressure, mmHg	0.07			0.24 (0.04)		
CAC score*	0.12 (0.32)	0.32 (0.005)		0.06 (0.66)	0.40 (0.0003)	
VAT*, cm²	0.15 (0.22)	0.40 (0.0005)	0.37 (0.001)	0.10 (0.38)	0.20 (0.09)	0.37 (0.001)
SAT*, cm²	0.17 (0.15)	0.26 (0.03)	0.22 (0.06)	0.06 (0.63)	0.14 (0.23)	0.22 (0.06)
BMI*, kg/m²	0.19 (0.10)	0.17 (0.13)	0.20 (0.09)	0.10 (0.41)	0.17 (0.13)	0.19 (0.09)
HbA1c, %	0.14 (0.24)	-0.01 (0.92)	-0.05 (0.68)	0.02 (0.89)	0.19 (0.10)	-0.02 (0.90)
Insulin dose, U/kg/dy**	0.24 (0.07)	0.24 (0.07)	0.08 (0.54)	-0.14 (0.35)	-0.39 (0.006)	-0.26 (0.08)
Glucose, mg/dl	0.33 (0.005)	0.05 (0.66)	-0.07 (0.57)	0.49 (<0.0001)	0.23 (0.05)	0.16 (0.17)
Triglycerides, mg/dl*	0.39 (0.0007)	0.15 (0.19)	0.32 (0.006)	0.04 (0.74)	0.13 (0.26)	0.18 (0.13)
HDLc, mg/dl	-0.02 (0.92)	-0.03 (0.81)	0.21 (0.07)	0.09 (0.42)	0.16 (0.17)	0.05 (0.65)
eGDR, mg/kg/min	-0.17 (0.17)	-0.38 (0.002)	-0.15 (0.21)	0.10 (0.40)	-0.41 (0.0004)	-0.13 (0.28)
AER, µg/min *	0.14 (0.24)	0.29 (0.01)	0.17 (0.16)	-0.11 (0.34)	0.35 (0.002)	0.18 (0.11)
Height, meters	0.07 (0.73)	0.32 (0.006)	0.22 (0.05)	0.01 (0.90)	0.13 (0.25)	-0.06 (0.60)
Heart rate, beats/min	0.13 (0.27)	0.04 (0.75	-0.15 (0.20)	-0.11 (0.37)	0.22 (0.05)	0.0008 (0.99)

Analyses with NEFA are controlled for serum albumin HDLc=high density lipoprotein cholesterol AER=albumin excretion rate

**n=55 for men and 47 for women

Table A-3. Multivariable Adjusted Association of Free Fatty Acids (FFA) with Pulse Pressure in Type 1 Diabetes.

	Males (n=70)	**Females (n=74)**
	B ± SE (p-value)	**B ± SE (p-value)**
Interaction between FFA *and SAT*	-5.49 ± 5.63 (0.33)	8.80 ± 4.15 (0.04)
Serum albumin, g/dl	-0.48 ± 2.97 (0.87)	-4.16 ± 2.33 (0.08)
Age, years	0.93 ± 0.25 (0.0004)	0.64 ± 0.18 (0.005)
Height, meters	NS	0.41 ± 0.19 (0.04)
Albumin excretion rate, µg/min*	1.51 ± 0.74 (0.05)	2.54 ± 0.73 (0.0009)
Model R-square	0.32	0.45

*Natural logarithmically transformed before analysis NS=not selected

FFA=free fatty acids SAT=subcutaneous abdominal adiposity

Backward selection model controlled for free fatty acids and subcutaneous abdominal adiposity and also allowed for heart rate, HbA1c, and high density lipoprotein cholesterol

Table A-4. Multivariable Adjusted Association of Free Fatty Acids with Coronary Artery Calcification in Type 1 Diabetes.

	Males (n=70)	**Females (n=74)**
	B ± SE (p-value)	**B ± SE (p-value)**
Free fatty Acids (mmol/l)	0.43 ± 0.48 (0.37)	-0.54 ± 0.52 (0.30)
Serum albumin, g/dl	-0.65 ± 0.49 (0.19)	-0.25 ± 0.41 (0.54)
Age, years	0.22 ± 0.04 (<0.0001)	0.23 ± 0.04 (<0.0001)
High density lipoprotein cholesterol, mg/dl	-0.07 ± 0.02 (0.002)	NS
Model R-square	0.36	0.45

*Natural logarithmically transformed before analysis NS=not selected

Backward selection model also allowed for HbA1c and high density lipoprotein cholesterol log-transformed subcutaneous adiposity, log-transformed albumin excretion rate, HbA1c, daily insulin dose/kg of body weight, heart rate, and a history of unconsciousness due to hypoglycemia. Results did not vary in log-transformed visceral adiposity was used in place of log-transformed subcutaneous adiposity.

APPENDIX B: UPDATED PAPER 3

The following version of paper 3 differs marginally from the published version due to updates in the data and corresponds to the data set turned in with this dissertation.

B.1 ABSTRACT

Background: Coronary artery disease (CAD), a leading cause of death in type 1 diabetes (T1D), often occurs two or more decades earlier in this population. Although CAD generally increases with adiposity, this association is unclear in T1D. We thus examined the associations of adiposity with Coronary Artery Calcium (CAC-a subclinical marker of CAD) in 315 individuals **with T1D.**

Methods: Mean age and diabetes duration were 42 and 35 yrs, respectively at the time of assessment of CAC, visceral adiposity (VAT) and subcutaneous adiposity (SAT) by electron beam tomography and when BMI and waist circumference (WC) were measured. Chi-square frequencies and generalized linear models were used to compare the presence of any CAC and age-adjusted mean CAC total score, respectively, by tertiles of adiposity.

Results: There was a positive relationship between the presence of CAC and tertiles of VAT, SAT, BMI, and WC in both genders (p trend<0.05). The presence of CAC was also positively

associated with all four adiposity measures modeled continuously in both men ($p<0.05$) and women ($p<0.05$). However, the degree of CAC was not associated with any adiposity measure, with the exception of SAT in women. Women in the lowest tertile of SAT had more CAC than those in the second tertile ($p<0.016$).

Conclusion: Adiposity was positively associated with the presence of CAC, but the relationship with its severity tended to be inverse or reverse J-shaped. This double-edged association, which appears to be more pronounced in women, emphasizes the complex relationship between adiposity and cardiovascular risk in diabetes.

B.2 INTRODUCTION

Coronary artery disease (CAD) is the leading cause of death in type 1 diabetes (1) and often occurs two or more decades earlier than in the general population. Although the risk of CAD tends to increase with increasing BMI in the general population, this association in type 1 diabetes is unclear. Coronary artery calcification (CAC) is a subclinical marker of coronary vascular disease (2) and has been shown to be predictive of future clinical cardiac events (3). The few studies that have investigated CAC in T1D are inconsistent in terms of the relationship between CAC and adiposity. All five studies investigated the association of BMI with CAC. Both Dabelea et al (4) and Colhoun et al (5) reported a positive association between BMI and the prevalence of CAC; in contrast, in the Epidemiology of Diabetes Complications Study (6), the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study (7), and in Starkman et al (8), no association was found between BMI and CAC prevalence. Olson et al (6) and Cleary et al (7) also failed to show an association with CAC severity. Additionally, in a subgroup of the CACTI population reported on by Dabelea et al (4), Snell-Bergeon et al (9) failed to find a difference in BMI when investigating progression of CAC.

Markers of visceral adiposity, thought by many to independently relate to cardiovascular disease risk, have also been studied. Unlike BMI, the association of waist-to hip ratio (WHR) and/or waist circumference (WC) with CAC has been more consistent. With the exception of

Colhoun et al (5), who failed to show an association in men, and Starkman et al (8), who did not investigate WHR /WC, all of the studies found CAC to be positively associated with WHR/WC. Additionally, Dabelea et al (4) using a direct measurement of visceral obesity, also found intraabdominal fat to be positively associated with the prevalence of CAC, although this was not investigated sex-specifically. They also found men to be at higher risk for CAC. As sex differences in adiposity also exist, even for BMI, and not all of the above mentioned studies looked at adiposity sex-specifically (4, 7, 8), sex specific analyses are warranted. Furthermore, none of the studies investigated subcutaneous abdominal fat (SAT). This is important as SAT has also been suggested to be a major contributor of free fatty acids into both the portal and systemic circulation and thus insulin resistance (10, 11, 12, 13).

Given the above conflicts in the literature and the evidence that being overweight and obese is rising in type 1 diabetes (T1D) (14), concern and further evaluation of the association of adiposity with CAD in this population already at increased risk is warranted. This study therefore sought to determine the following: a) which measure of adiposity best identifies CAC (testing the hypothesis that measures of central obesity will be more strongly associated with CAC), b) whether any associations of adiposity with CAC vary by sex and c) whether any associations differ for the prevalence as opposed to the severity of CAC. Four different indices of body fat, i.e. BMI, WC, VAT, and SAT were investigated.

B.3 METHODS

Subjects

The EDC study is an ongoing cohort study examining the long term complications of T1D in 658 individuals diagnosed before the age of 17 years with T1D at Children's Hospital of Pittsburgh between 1950 and 1980. This current report is based on a subset (n=316) who underwent electron beam tomography (EBT) for CAC between 2000 and 2007. These participants were also scanned for VAT and SAT via EBT scanning.

Clinical Evaluation Procedures

Before attending the clinic, participants completed a questionnaire concerning demographic information, lifestyle, and medical history. An ever smoker was defined as having smoked at least 100 cigarettes in a lifetime. Participants were weighed in light clothing on a balance beam scale. Height was measured using a wall-mounted stadiometer. BMI was calculated as the weight in kilograms divided by the square of the height in meters. Two waist measurements were taken by a standard medical measuring tape, measuring from the mid-point of the iliac crest and the lower costal margin in the mid-axillary line. The average was used for data analysis.

Fasting blood samples were assayed for lipids, lipoproteins, and glycosylated hemoglobin (HbA1c). High-density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure, a modification of the Lipid Research Clinics method (15). Cholesterol and triglycerides were measured enzymatically. Glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) formula (16). Sitting blood pressures were measured according to the Hypertension Detection and Follow-up Program protocol (17)

using a random zero sphygmomanometer. The mean of the second and third readings was used. Estimated glucose disposal rate (eGDR) was calculated using the equation: eGDR=24.395-12.971 (waist-to-hip ratio)-3.388 [hypertension status (140/90 mm Hg or on hypertension medication)]-0.601 (HbA1c). This formula was derived from a substudy of 24 EDC participants (12 men and 12 women drawn from low, middle, and high age-specific tertiles of insulin resistance risk factors) who underwent euglycemic clamp studies (18).

CAC was measured using EBT (Imatron C-150, Imatron, South San Francisco, CA). Threshold calcium determination was set using a density of 130 Hounsfield units in a minimum of 2 contiguous sections of the heart. Scans were triggered by ECG signals at 80% of the R-R interval. The entire epicardial system was scanned. CAC volume scores were calculated based on isotropic interpolation (19). Direct measurements of abdominal adiposity (visceral and subcutaneous abdominal adipose tissue surface area) were also taken by EBT scanning. Scans of abdominal adipose tissue were taken between the fourth and fifth lumbar regions, which were located by counting from the first vertebra below the ribs. Two 10 mm thick scans were taken during suspended respiration. The images were then analyzed using commercially available software for all pixels corresponding to fat density in Hounsfield units in the appropriate anatomical distribution (subcutaneous or visceral).

Statistical analyses

Pearson's correlations were used to assess the association between each of the four adiposity measures. Two stage analyses were performed given the large number of subjects with no calcification and the resulting non-normal distribution. The first analysis evaluated the

presence/absence of CAC. The second analysis evaluated the degree of CAC by volume scores in individuals with any CAC. This approach also allows assessment of the third objective, i.e. whether relationships were different for prevalence versus severity.

Differences between groups with and without CAC were evaluated using the Student's *t* test for continuous variables and $\chi 2$ for dichotomous variables. Logistic regression analysis was used to determine the association of adiposity with the presence of CAC. Spearman's correlations were used to determine how well the different adiposity measures correlated with CAC volume scores, given the presence of any CAC. Generalized linear models (GLMs), which are fairly robust in analyses of non-normally distributed data, were used to compare CAC volume scores (CACs) across tertiles of the four adiposity measures.

In order determine whether any adiposity associations with CAC vary by sex, BMI, WC, VAT, and SAT were examined by sex-specific tertiles; sex interactions were also explored. Analyses were performed on the entire cohort and within only those positive for calcification. All non-normally distributed variables were transformed using an appropriate transformation or were tested nonparametrically with the Kruskal Wallis test. One was added to all values of CAC before log-transformation. All odds ratios and parameter coefficients are reported as per one standard deviation change in the continuous variables. Akaike's Information Criterion (AIC) and Pearson's r were used to determine which adiposity measurement best accounted for the prevalence and severity of CAC, respectively. The criterion for statistical significance was $P < 0.05$. Analyses were conducted using SAS version 9.1 (Cary, North Carolina).

Results

Adiposity and the Presence or Absence of CAC

Baseline characteristics revealed that although men (n=152) had significantly higher VAT and WC, non-HDLc, and lower HDLc than women (n=164), there was no difference in percent with CAC or median CAC levels (Table B-1). Figure 1 shows the prevalence of CAC by sex-specific tertiles of adiposity. There was a significant direct linear trend between tertile of each adiposity measure and prevalence of CAC in both sexes ($p<0.05$) (data not shown). When the measures of adiposity were analyzed as continuous variables and adjusted for age, the presence of CAC was positively associated with each adiposity indice in both sexes. Further adjustment for other clinically and/or statistically significant risk factors did not alter these associations (ORs range from 1.9 to 3.8), including menopausal status. Model comparisons suggest that BMI was marginally better at accounting for CAC prevalence in both sexes.

Correlation between the Adiposity Measures and CAC

Age-adjusted CACs showed low order positive correlations with each adiposity measure overall in both sexes, which reached statistical significance only for SAT in men (Table B-2a). However, when restricted to only those with some measurable CAC, i.e. excluding those with '0' values, correlations were surprisingly in the inverse direction, but none reached statistical significance (Table B-2b).

Adiposity and the degree of CAC

Graphical examination of tertiles of adiposity in those with calcification revealed a reverse J-shaped relationship between CACs and VAT in both men and women, for SAT in

women, and an inverse relationship between CACs and BMI and WC in both sexes (Figure B2). With the exception of SAT in men, in both sexes and for all measures, the lowest tertile of adiposity had the highest median CAC scores. In order to explore whether other confounding variables may explain this finding, other risk factors were examined by tertile of SAT, where this observation was most striking. No excess of major risk factors were identified in the lowest tertile; however, age, diabetes duration, and smoking were higher in both men and women, albeit nonsignificantly while eGFR was significantly lower and overt nephropathy was higher in women (Table B-3).

Generalized linear modeling revealed that given the presence of any CAC, there was no significant association of age-adjusted CAC with any of the four adiposity measures, with the exception of SAT in women. Women in the lowest tertile of SAT had more CAC than those in the second tertile ($p<0.016$). After adjustment for age, glomerular filtration rate, having ever smoked, and a history of a renal transplant, being in the lowest tertile of SAT remained significantly associated with CAC in women (Table B-4) and after further adjustment for menopausal status which was not a strong independent predictor ($p=0.96$). Model comparisons show R^2 ranging from 39 to 45%, suggesting that all four models generally explain variance in CAC to a similar degree.

B.4 DISCUSSION

In this cross-sectional study in which we investigated the association of adiposity with CAC in T1D several important findings are of note. First, we demonstrated that the four different

measures of adiposity investigated are similarly associated with CAC, both within and between sexes. We also showed that the direction of the associations differed when looking at the presence of CAC as opposed to the degree of CAC, i.e. a lower level of adiposity was associated with higher CACs.

Contrary to expectations, central adiposity measures, e.g. VAT and WC, were not better able to identify CAC than the other body morphology parameters. A major hypothesized mechanism by which adiposity is associated with CAD is via increased lipolysis of metabolically active VAT with its consequent release of inflammatory cytokines into the systemic circulation and excess free fatty acids into the portal vein (20). Cytokines such as Il-6 and CRP are associated with atherosclerosis while increased free fatty acid flux to the liver will increase triglyceride and LDLc and small dense LDLc synthesis and are postulated to be in the causal pathway of insulin resistance (21, 13). The small dense LDL phenotype, associated with insulin resistance, is very atherogenic in high concentrations. Despite these characteristics of visceral adiposity and our previous reports of CAD events being related to WHR (22, 23) and small dense LDL (24), in these current analyses CAC was not preferentially linked with visceral compared to general obesity, suggesting possible differences in these measures in T1D. However, other investigators state that it is elevated SAT, which is correlated with VAT, which is primarily responsible for the elevated systemic levels of FFA associated with VAT (13). Nevertheless, most of the studies investigating CAC in type 1 diabetes have found WHR or WC to be associated with CAC (6, 7, 9), although BMI has been less consistent (4, 5, 6, 7). Biological plausibility notwithstanding, no adiposity parameter appears strikingly better than another in detecting CAC.

That BMI was marginally better able to detect the presence of CAC in both men and women may support the argument that BMI is not so much a measure of overall adiposity as it is a marker/predictor of health status (25). In investigating the relationship of obesity with CAD in type 1 diabetes, it must be borne in mind that adiposity associations observed in non-diseased populations may be very different than that observed in populations with pre-existing disease, such as type 1 diabetes. The inverse relationship of adiposity with severity of CAC in this population appears to lend support to this postulate.

That increasing adiposity was positively associated with the presence of CAC, while it was inversely associated with severity, albeit non-significantly for most parameters, seems to suggest at least two divergent disease processes. A search for confounding by adiposity tertile within those with any CAC revealed that estimated glucose disposal rate (eGDR) and blood pressure in men, and lipids and kidney function in women were significantly different for those in the lowest adiposity tertile and who had measurable CAC. As alluded to earlier, obesity correlates such as hypertension, dyslipidemia, inflammation, and insulin resistance, i.e. features of the metabolic syndrome, may be responsible for the increased CAC observed. Our marker for insulin sensitivity in this population, eGDR, was inversely associated with CAC severity and thus consistent with this hypothesis, though the correlation was not signifcant in women. Despite identifying these potential confounders, the significant SAT difference in women (Table B-4) remained significant.

The CAC detected, at least in some of the participants, may not be from atherosclerotic plaque, i.e. intimal, as is generally associated with CAD, but rather partially medial (4,5). This cannot be determined by EBCT. In a recent analysis demonstrating medial wall calcification in the EDC population (26), some six years prior to EBT scanning for CAC, a strong association

was seen between earlier medial wall calcification, determined six years earlier by ankle x-rays, and CAC, which remained in multivariable analyses unless neuropathy was included as a variable. Thus both processes may be at work in this population.

The majority of the studies looking at CAC in T1D have found age and diabetes duration to be the strongest correlates of CAC. Residual confounding due to factors related to long-term exposure to hyperglycemia, such as advanced glycated end products (AGEs), may also be apart of the pathogenesis of CAC. Long-term exposure to hyperglycemia may result in AGEs depositing into the extracellular matrix of the arterial wall. These AGES have the ability to stimulate osteoblastic differentiation, leading to vascular calcification. Sakata et al (27) reported increased CML, an AGEs, in the medial wall of the inter-thoracic artery of individuals with type 1 diabetes, while very little of this was noted in those with type 2 diabetes. Although there was no age-adjusted association between glycemic control and CAC in our population, this does not negate the possibility that long duration of hyperglycemia, i.e. diabetes, may be responsible for the more severe CAC, particularly in the older participants, who also happened to be the thinnest. However, increased levels of AGEs are also found in kidney disease.

CAC is a well known to be associated with kidney disease, possibly due to abnormal calcium and phosphorus metabolism (28). Extensive calcification is observed in those in renal failure, even in the young, and CAC is an important predictor of overall mortality in the kidney disease population. Colhoun et al (5) found AER to be associated with the presence of CAC in men with T1D, but not women. Thilo et al (29) observed no association between microalbiminuria and CAC in 71 participants with T1D with a mean age of 48 and disease duration of 26 years. In our population, kidney function was associated with CAC. We observed that GFR tended to increase and overt nephropathy tended to decrease with adiposity in

women, suggesting that the more severe CAC observed in women with less body fat might be partially explained by kidney disease. However, this association was not observed in men. Additionally, although renal transplant recipients had the highest levels of CAC, i.e. most severe CAC, they were not more likely to be in the lowest adiposity tertile. Nevertheless, where adiposity failed to be a strong predictor, renal function, as measured by GFR and renal failure were significantly related to CAC severity in this population.

Consistent with the literature in type 1 diabetes, there were no significant differences in CAC prevalence, severity (5, 6) or its association of body fat by sex. However, contrary to expectations, VAT, albeit nonsignificantly, appeared to be better at detecting CAC severity in women than in men. In the general population, men have an average of about twice as much visceral fat as premenopausal women when matched for total body fat (30). We did not observe such a large sex difference in our population. Visceral fat levels were 53% higher in males than premenopausal women in our T1D population (data not shown). This attenuation in the sex difference in VAT in T1D has been observed elsewhere. In the CACTI study, Dabelea et al (4) observed that men with T1D had lower WHRs and much lower levels of VAT, although similar BMI levels, than nondiabetic men. Women with T1D had higher waist-to-hip ratios, WCs, and BMIs, but similar levels of VAT. Dabelea noted that women with T1D "had a more android disposition of adipose tissue" while this was attenuated in men. It appears that VAT is not stored to the same extent in T1D, indicating a more functional role of VAT in T1D. Although many CAD risk factors increase with adiposity in this population, traditional CAD risk factors appear (including menopausal status) to be less operant in the pathogenesis of severe atherosclerosis, particularly in women, an observation noted elsewhere (31).

Study Limitations

A major limitation of this study is that we were unable to follow participants from an earlier time point when participants were free of CAC to determine if the adiposity measures predict the incidence or severity of CAC. In a population such as this, in which it is defined by its pre-existing disease, complications that are part of the natural history of the disease may be well underway after 16-18 years of follow-up. As CAC, VAT, and SAT were not available in this population at earlier time periods, the adiposity indices measured at the time of EBT may not reflect the adiposity level prior to the development of severe calcification. It is thus not possible to determine the exact causal pathways given the cross-sectional nature of our study. Survival bias may also be at issue in the current study. It is possible that the more obese died before current follow-up. However, being overweight is not a mortality risk factor in this population (14) so disruption of the natural obesity/CAC association by premature loss of the more obese is unlikely. It is also possible that longer exposure to kidney disease may result in weight loss or that increased body fat is merely a marker of better health.

In conclusion, we found that adiposity was related to the presence of calcification irrespective of the measure used. Age, which in this population is also a proxy for diabetes duration, remained, as in many studies, the strongest correlate for both the prevalence and severity of CAC. Although the presence of CAC increased with adiposity, more severe disease, i.e. greater CAC, was inversely associated with body fat, albeit only significantly for SAT in women. This was only partially explained by other risk factors, e.g. renal disease and age. At least two distinct disease processes (atherosclerosis and medial wall calcification) may be operant in the CAC seen in T1D, underscoring the complex relationship of obesity with CAC in T1D. This double-edged association, the association of CAC with both fat and thin, which

appears to vary by sex, further highlights that factors other than the standard risk factors are at work in the development of CAD in T1D.

B.5 CITED WORKS

1. Libby P, Nathan D, Abraham K, Brunzell J, Fradkin J, Haffner S, Hsueh W, Rewers M, Roberts B, Savage P, Skarlatos S, Wassef M, Rabadan-Diehl C. Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. *Circulation* 2005; 111:3489-3493.

2. Abedin M, Tintut Y, Demer L. Vascular Calcification: Mechanisms and Clinical Ramifications. *Arterioscler Thromb Vasc Biol* 2004; 24:1161-1170.

3. Kennedy J. Shavelle R, Wang S, Budoff M, Detrano R. Coronary Calcium and Standard Risk Factors in Symptomatic Patients Referred for Coronary Angiography. *AM Heart J* 1998; 135:696-702.

4. Dabelea D., Kinney G, Snell-Bergeon J, Hokanson J, Eckel R, Ehrlich J, Garg S, Hamman R, Rewers M. Effect of Type 1 Diabetes on the Gender Difference in Coronary Artery Calcification: a Role for Insulin Resistance? The Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study. *Diabetes* 2003; 52:2833-2839.

5. Colhoun H, Rubens M, Underwood R, Fuller J. The Effect of Type 1 Diabetes Mellitus on the Gender Difference in Coronary Artery Calcification. *J Am Coll Cardiol* 2000; 36:2160-2170.

6. Olson J, Edmundowicz D, Dorothy B, Kuller L, Orchard T. Coronary Calcium in Adults with Type 1 Diabetes: A Stronger Correlate of Clinical Coronary Artery Disease in Men Than in Women. *Diabetes* 2000; 49:1571-1578.

7. Cleary P, Orchard T, Genuth S, Wong N, Detrano R, Backlund J, Zinman B, Sun W, Lachin J, Nathan D, the DCCT/EDIC Research Group. The Effect of Intensive Glycemic Treatment on Coronary Artery Calcification in Type 1 Diabetic Participants of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study.

8. Starkman H, Cable G, Hala V, Hecht H, Donnely C. Delineation of Prevalence and Risk Factors for Early Coronary Artery Disease by Electron Beam Computed Tomography in Young Adults with Type 1 Diabetes. *Diabetes Care* 2003; 26:433-436.

9. Snell-Bergeon J, Hokanson J, Jensen L, MacKenzie T, Kinney G, Barbelea D, Eckel R, Ehrlich J, Garg S, Rewers M. Progression of Coronary Artery Calcification in Type 1 Diabetes: the importance of glycemic control. *Diabetes Care* 2003; 26:2923-2928.

10. Tanko L, Bagger Y, Alexanderson P, Larsen P, Christiansen C. Peripheral Adiposity Exhibits an Independent Dominant Aniatherogenic Effect in Elderly Women. *Circulation* 2003;107: 1626-1631.

11. Abate N, Garg A, Peshock R, Stray-Gundersen J, Grundy S. Relationships of generalized and regional adiposity to insulin sensitivity in men. *Journal of Clinical Investigation* 1995; 96:88-98.

12. Abate N, Garg A, Peshock R, Stray-Gundersen J, Adams-Huet B, Grundy S. Relationships of generalized and regional adiposity to insulin sensitivity in men with NIDDM. *Diabetes* 1996;45: 1684-1693.

13. Frayn K. Visceral fat and insulin resistance-causative or correlative? *British Journal of Nutrition* 2000; 83:S71-S77.

14. Conway B, Costacou T, Orchard T. Time Trends in Overweight and Obesity in Type 1 Diabetes and Their Association with Mortality. *Diabetes* 55 (S1): A382.

15. National Institute of Health, Department of Health, Education and Welfare. (1978) Lipid Research Clinics Program. Washington, D.C.: U.S. Govt. Printing Office 1975 (NIH pub no. 75-628).

16. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease. Am J Kidney Dis. 2006 May;47(5 Suppl 3):S11-145.

17. Borhani N, Kass E, Langford H, Payne G, Remington R, Stamler J. The Hypertension Detection and Follow-up Program. Prev Med 1976; 5:207-215.

18. Williams K, Erbey J, Becker D, Arslanian S. Can Clinical Factors Predict Insulin Resistance in Type 1 Diabetes? Diabetes 2000; 49: 626-632.

19. Callister T, Cooil B, Raya S, Lippolis N, Russo D, Raggi P. Coronary artery disease: improved reproducibility of calcium scoring with an electron-beam CT volumetric method. Radiology 1998; 208:807-814.

20. Bjorntorp P. 'Portal' adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis 1990; 10:493-496.

21. Arner P. Impact of visceral fat. International Journal of Obesity 1997; 21:S20.

22. Stuhldreher WL, Orchard TJ, Ellis D. The association of waist-hip ratio and risk factors for development of IDDM complications in an IDDM adult population. Diabetes Res Clin Pract 1992;17 :99-109.

23. Orchard T, Olson J, Erbey J, Williams K, Forrest K, Kinder L, Ellis D, Becker D. Insulin resistance-related factors, but not gycemia, predict coronary artery disease in type 1 diabetes. *Diabetes Care* 2003; 26:1374-1379.

24. Soedamah-Muthu S, Chang Y, Otvos J, Evans R, Orchard T. Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary

artery disease in Type 1 Diabetes. A prospective report from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetelogia* 2003; 46:674-682.

25. Fontaine K, Allison D. Obesity and Mortality Rates in Bray G, Bouchard C (eds). Handbook of Obesity: etiology and pathophysiology (2nd ed.). New York: Marcel Decker. 2004.

26. Costacou T, Husk N, Edmundowics D, Stolk R, Orchard T. Lower-extremity arterial calcification as a correlate of coronary artery calcification. *Metabolism* 2006; 55(12):1689-96.

27. Sakata N, Takeuchi K, Noda K, Saku K, Tachikawa Y, Tashiro T, Nagai R, Horiuchi S. Calcification of the medial layer of the internal thoracic artery in diabetic patients: relevance of gloxidation. *J Casc Res* 2003; 40:567-74.

28. Stenvinkel P, Pecoits-filho, Lindholm B. Coronary artery disease in end-stage renal disease: no longer a simple plumbing problem. *J Am Soc Nephrol* 2003; 14:1927-1939.

29. Thillo C, Standi E, Knez A, Reiser M, Steinbeck G, HAberl R, Schnell O. Coronary Calcification in Long-term Type 1 Diabetic Patients-A Study with Multi Slice Spiral Computed Tomography. *Exp Clin Endocrinol Diabetes* 2004; 112:561-565.

30. Nicklas B, Penninx B, Ryan A, Berman D, Lynch N, Dennis K. Visceral Adipose Tissue Cutoffs Associated with Metabolic Risk Factors for Coronary Heart Disease in Women. *Diabetes Care* 2003; 26:1413-1420.

31. Schurgin S, Rich S, Mazzone T. Increased Prevalence of Significant Coronary Artery Calcification in Patients with Diabetes. *Diabetes Care* 2001; 24:335-338.

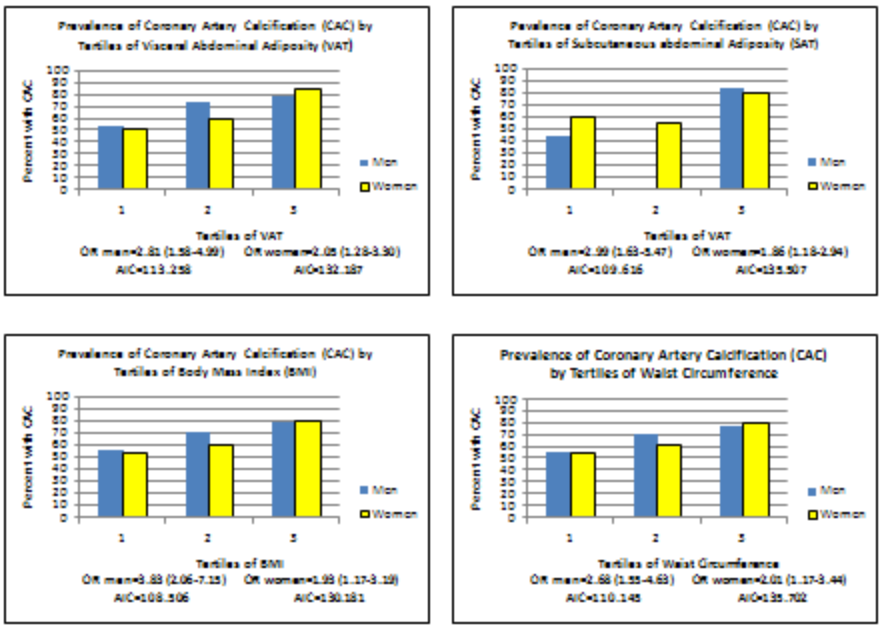

Figure B-1. The prevalence of coronary artery calcification by tertiles of adiposity.

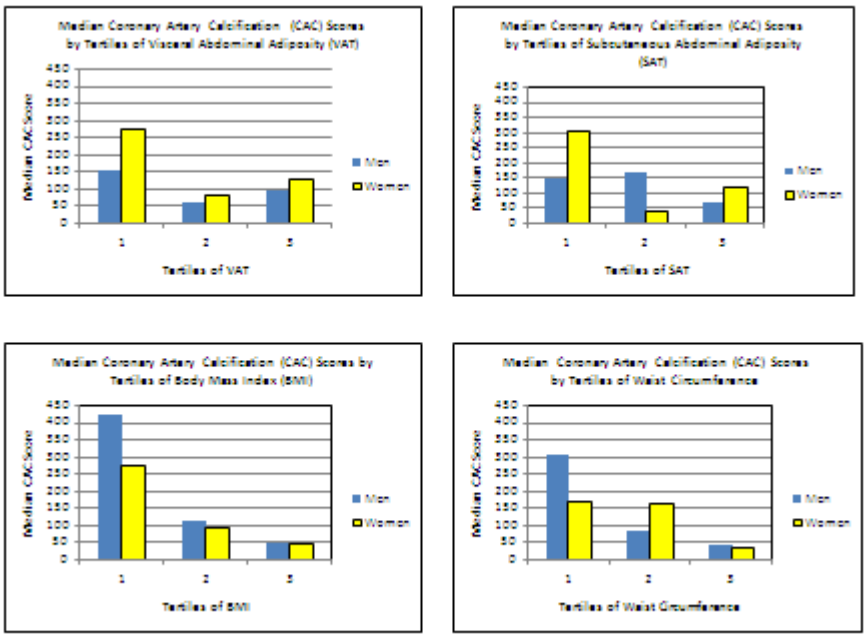

Figure B-2. Median total coronary artery calcification scores in those with some measureable calcification by tertiles of adiposity.

Table B-1. Characteristics by Gender. The Pittsburgh Epidemiology of Diabetes Complications Study.

Characteristics	Males (n=152)	Females (n=164)	p-value
Age (years)	42.8 (7.4)	43.3 (7.8)	0.55
Duration (years)	34.8 (7.3)	34.7 (7.8)	0.90
CAC+ (%)	68.2 (104)	65.2 (107)	0.54
CACs (median)*	22.1 (0-313.3)	8.8 (0-279.0)	0.34
HbA1c (%)	7.8 (1.5)	7.5 (1.3)	0.12
Hypertension (%)	36.6 (53)	26.4 (43)	0.05
Ever smoker (%)	38.6 (56)	37.1 (59)	0.79
HDL-C (mg/dl)	50.3 (12.2)	64.4 (14.6)	<0.0001
Non-HDL-C (mg/dl)	132.5 (35.9)	125.8 (28.4)	0.07
VAT (cm^2)*	121.7 (64.3)	82.3 (48.9)	<0.0001
SAT (cm^2)*	226.7 (276.4)	282.2 (232.4)	0.0006
BMI (kg/m^2)	26.8 (3.6)	26.7 (5.2)	0.88
WC (cm)	93.7 (9.6)	84.8 (11.7)	<0.0001
Menopause**		25.0 (40)	

*Natural logarithmically transformed before analysis. **n=160

Table B-2a. Age-Adjusted Correlations* of Adiposity with Calcification (R, p). 2000-2007.

	SAT	BMI	Waist Circumference	eGDR	Age	CACs
Men, n=152						
VAT**	0.65	0.66	0.77	-0.36	0.28	0.09
	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.25
SAT**		0.69	0.76	-0.27	0.11	0.20
		<0.0001	<0.0001	0.002	0.17	0.01
BMI			0.88	-0.24	-0.03	0.13
			<0.0001	0.005	0.67	0.10
Waist Circumference				-0.44	0.08	0.12
				<0.0001	0.38	0.16
eGDR					-0.11	-0.23
					0.22	0.007
Age						0.57
						<0.0001
Women, n=164						
VAT*	0.62	0.70	0.79	-0.31	0.23	0.13
	<0.0001	<0.0001	<0.0001	<0.0001	0.003	0.06
SAT**		0.75	0.73	-0.08	0.03	0.09
		<0.0001	<0.0001	0.31	0.68	0.28
BMI			0.85	-0.19	-0.03	0.12
			<0.0001	0.02	0.67	0.13
Waist Circumference				-0.34	0.08	0.14
				<0.0001	0.36	0.09
eGDR					-0.10	-0.13
					0.22	0.13
Age						0.60
						<0.0001

*Pearson for adiposity measures with each other; Spearman for correlations with CAC **Natural-logarithmically transformed

Table B-2b. Age-Adjusted Correlations* of Adiposity with Calcification (R, p) in those with Coronary Artery Calcification. 2000-2007.

Men, n=112						
	SAT	BMI	Waist Circumference	eGDR	Age	CACs
VAT**	0.50	0.65	0.76	-0.43	0.09	-0.14
	<0.0001	<0.0001	<0.0001	<0.0001	0.37	0.15
SAT**		0.64	0.69	-0.20	-0.14	-0.10
		<0.0001	<0.0001	0.05	0.14	0.32
BMI			0.84	-0.19	-0.28	-0.14
			<0.0001	0.07	0.004	0.15
Waist Circumference				-0.46	-0.23	-0.20
				<0.0001	0.02	0.05
eGDR					-0.02	-0.14
					0.83	0.19
Age						0.60
						<0.0001
Women, n=104						
VAT*	0.57	0.68	0.78	-0.34	-0.006	-0.06
	<0.0001	<0.0001	<0.0001	0.0006	0.95	0.53
SAT**		0.76	0.72	-0.04	-0.16	-0.15
		<0.0001	<0.0001	0.73	0.10	0.13
BMI			0.84	-0.16	-0.24	-0.09
			<0.0001	0.11	0.01	0.34
Waist Circumference				-0.34	-0.09	-0.08
				0.001	0.40	0.43
eGDR					-0.11	-0.06
					0.27	0.58
Age						0.55
						<0.0001

*Pearson for adiposity measures with each other; Spearman for correlations with CAC **Natural-logarithmically transformed

Table B-3. Characteristics of Participants with CAC by Tertiles of Subcutaneous Abdominal Adiposity and Gender.

Variable, mean (SD)	Tertile 1	Tertile 2	Tertile 3	p-value	Variable, mean (SD)	Tertile 1	Tertile 2	Tertile 3	p-value
Men					*Women*				
Age (yrs)	46.2 (7.2)	43.8 (6.8)	44.0 (7.4)	0.20	Age (yrs)	48.0 (5.9)	45.6 (8.3)	44.1 (6.2)	0.02
Diabetes Duration (yrs)	37.9 (7.9)	35.7 (7.1)	35.3 (6.9)	0.14	Diabetes Duration (yrs)	38.5 (6.5)	37.4 (7.8)	35.7 (7.4)	0.11
HbA1c (%)	7.5 (1.5)	7.5 (1.7)	8.0 (1.2)	0.15	HbA1c (%)	7.7 (1.4)	7.4 (1.3)	7.2 (1.2)	0.16
eGDR (mg/kg/min)	6.7 (1.9) ‡	6.4 (2.2)	4.9 (2.0)	0.0008	eGDR (mg/kg/min)	8.0 (2.5)	7.9 (1.9)	7.7 (2.2)	0.60
HDL (mg/dl)	51.5 (12.1)	48.4 (10.9)	47.6 (13.3)	0.19	HDL (mg/dl)	64.8 (16.3)	63.0 (13.1)	62.1 (14.1)	0.46
Non-HDLc (mg/dl)	125.7 (32.4)	133.7 (45.4)	137.6 (38.2)	0.21	Non-HDLc (mg/dl)	124.0 (30.9) ‡	126.0 (19.9)	140.5 (28.7)	0.01
SBP (mm/Hg)	120.8 (18.4) ‡	125.3 (16.6)	132.2 (16.1)	0.007	SBP (mm/Hg)	120.3 (16.8)	113.8 (13.3)	121.9 (16.6)	0.67
DBP (mm/Hg)	70.4 (10.2)	72.5 (9.6)	74.9 (8.0)	0.05	DBP (mm/Hg)	63.0 (7.6)	64.8 (9.0)	67.6 (9.7)	0.04
MDRD (mg/min)γ	83.6 (22.7)	83.1 (25.5)	85.1 (25.7)	0.81	MDRD (mg/min)γ	63.4 (21.9) †‡	80.5 (19.5)	79.2 (28.4)	0.01
Overt Nephropathy (%)	41.2 (14)	41.9 (13)	31.6 (12)	0.39	Overt Nephropathy (%)	48.5 (16)	24.3 (9)	25.0 (9)	0.04
Transplant recipient (%)	14.7 (5)	12.5 (4)	5.3 (2)	0.19	Transplant recipient (%)	15.2 (5)	10.8 (4)	8.1 (3)	0.35
Ever Smoker (%)	44.1 (15)	40.0 (12)	35.1 (13)	0.44	Ever Smoker (%)	45.5 (15)	35.3 (12)	35.1 (13)	0.38
CAC, median (IQR) ***	143.9 (31.8-762.4)	16.27 (16.4-582.2)	69.2 (17.5-492.6)	0.39	CAC, median (IQR) ***	304.9 (94.2-799.7)	37.6 (3.1-273.9)	118.4 (9.3-410.0)	0.009

*Significantly different from (sdf) Tertile (T) 1, †sdf T2, ‡ sdf T3 at p<0.017 **Natural logarithmically transformed before analysis

***Nonparametrically tested γTransplant recipients excluded

Table B-4. Generalized Linear Models for the Association of Visceral Adiposity (VAT), Subcutaneous Abdominal Adiposity (SAT), Body Mass Index (BMI), and Waist Circumference (WC) with Coronary Artery Calcification by Gender. The Epidemiology of Diabetes Complications Study.

	VAT	SAT	BMI	WC
Characteristics	ß± (p)	ß± (p)	ß± (p)	ß± (p)
Men				
Adiposity*				
2nd tertile	-0.50 ± 0.41 (0.23)	-0.03 ± 0.42 (0.94)	-0.29 ± 0.43 (0.50)	-0.53 ± 0.46 (0.25)
3rd tertile	-0.08 ± 0.40 (0.85)	0.13 ± 0.40 (0.75)	-0.20 ± 0.44 (0.66)	-0.59 ± 0.46 (0.21)
1st tertile	N/A	N/A	N/A	N/A
AGE	0.97 ± 0.19 (<0.0001)	0.95 ± 0.19 (<0.0001))	0.93 ± 0.19 (<0.0001)	0.86 ± 0.21 (0.0001)
MDRD	-0.20 ± 0.19 (0.29)	-0.24 ± 0.18 (0.20)	-0.26 ± 0.19 (0.17)	-0.31 ± 0.19 (0.11)
Ever Smoker	0.46 ± 0.34 (0.17)	0.50 ± 0.34 (0.14)	0.49 ± 0.35 (0.16)	0.31 ± 0.37 (0.41)
Transplant	1.59 ± 0.59 (0.008)	1.60 ± 0.59 (0.009)	1.48± 0.61 (0.02)	1.42± 0.62 (0.03)
R^2	0.42	0.41	0.41	0.41
Women				
	VAT	SAT	BMI	WC
Characteristics	ß± (p)	ß± (p)	ß± (p)	ß± (p)
Adiposity*				
2nd tertile	-0.38 ± 0.43 (0.38)	-1.32 ± 0.43 (0.003)†	-0.26 ± 0.45 (0.57)	-0.16 ± 0.43 (0.71)
3rd tertile	-0.57 ± 0.44 (0.20)	-0.54 ± 0.43 (0.21)	-0.05 ± 0.45 (0.91)	-0.55 ± 0.44 (0.21)
1st tertile	N/A	N/A	N/A	N/A
AGE	1.07 ± 0.18 (<0.0001)	1.08 ± 0.18 (<0.0001)	1.08 ± 0.19 (<0.0001)	1.08 ± 0.19 (<0.0001)
MDRD	-0.29 ± 0.20 (0.14)	-0.15 ± 0.19 (0.43)	-0.27 ± 0.20 (0.17)	-0.31 ± 0.20 (0.12)
Ever Smoker	0.66 ± 0.36 (0.07)	0.56 ± 0.34 (0.11)	0.65 ± 0.37 (0.08)	0.61 ± 0.36 (0.09)
Transplant	0.84 ± 0.59 (0.16)	0.90± 0.57 (0.11)	0.88 ± 0.60 (0.14)	0.86 ± 0.57 (0.14)
R^2	0.40	0.45	0.39	0.45

*VAT, SAT, BMI, and WC, respectively †Different (lower) from the first tertile at p<0.0167.

APPENDIX C: SUPPLEMENTARY ANALYSES

C.1 SUPPLEMENTARY ANALYSES FOR PAPER 1

Table C1-1. Time Trends in BMI in Men with Type 1 Diabetes.

			BMI		
	N (%)	<20	20-<25	25-<30	≥30
1986-1988	Overall	23 (8.3)	157 (56.5)	92 (33.1)	6 (1.8)
	Age group				
	20-<30	11 (3.4)	85 (30.6)	51 (18.4)	4 (1.4)
	30-<40	12 (4.3)	59 (21.2)	34 (12.2)	2(0.7)
	40-<50	0 (0)	13 (4.7)	7 (2.5)	0 (0)
1988-1990	Overall	10 (4.3)	129 (54.9)	90 (38.3)	6 (2.6)
	Age group				
	20-<30	1 (0.9)	66 (60.6)	39 (35.8)	3 (2.8)
	30-<40	9 (9.0)	50 (50.0)	39 (39.0)	2 (2.0)
	40-<50	0 (0)	13 (50.0)	12 (46.2)	1 (3.9)
1990-1992	Overall	8 (3.9)	107 (51.9)	81 (39.3)	10 (4.9)
	Age group				
	20-<30	1 (1.3)	43 (53.8)	32 (40.0)	4 (5.0)
	30-<40	5 (5.4)	47 (51.1)	36 (39.1)	4 (4.4)
	40-<50	2 (5.9)	17 (50.0)	13 (38.2)	2 (5.9)
1992-1994	Overall	9 (4.6)	94 (47.7)	77 (39.1)	17 (8.6)
	Age group				
	20-<30	2 (3.6)	26 (46.4)	23 (41.1)	5 (8.9)
	30-<40	4 (4.1)	49 (49.5)	38 (38.4)	8 (8.1)
	40-<50	3 (7.3)	19 (46.3)	15 (36.6)	4 (9.8)
	≥50	0 (0)	0 (0)	1 (100)	0 (0)

Table C1-1 continued

1994-1996	Overall	7 (3.3)	102 (47.4)	87 (40.5)	19 (8.8)
	Age group				
	20-<30	2 (3.9)	23 (45.1)	22 (43.1)	4 (7.8)
	30-<40	0 (0)	48 (48.0)	44 (44.0)	8 (8.0)
	40-<50	5 (9.1)	28 (50.9)	15 (27.3)	7 (12.7)
	≥50	0 (0)	3 (33.3)	6 (66.7)	0 (0)
1996-1998	Overall	9 (3.4)	126 (47.2)	108 (40.2)	24 (9.0)
	Age group				
	20-<30	3 (6.7)	26 (57.8)	13 (28.9)	3 (6.7)
	30-<40	1 (0.8)	58 (45.7)	58 (45.7)	10 (7.9)
	40-<50	4 (5.1)	37 (46.8)	30 (38.0)	8 (10.1)
	≥50	1 (6.3)	5 (31.3)	7 (43.8)	3 (18.8)
2000-2003	Overall	4 (2.4)	60 (35.7)	74 (44.1)	30 (17.9)
	Age group				
	20-<30	0 (0.0)	4 (36.4)	3 (27.3)	4 (36.4)
	30-<40	1 (1.3)	35 (45.5)	28 (36.4)	13 (16.9)
	40-<50	5 (7.7)	27 (41.5)	16 (24.6)	17 (26.2)
	≥50	5 (13.2)	15 (39.5)	10 (26.3)	8 (21.1)
2004-2007	Overall	4 (2.2)	56 (30.8)	83 (45.6)	39 (21.4)
	Age group				
	20-<30	0 (0.0)	3 (75.0)	1 (25.0)	0 (0.0)
	30-<40	1 (2.1)	13 (27.7)	22 (46.8)	11 (23.4)
	40-<50	1 (1.2)	26 (30.6)	39 (45.9)	19 (22.4)
	≥50	2 (4.4)	14 (30.4)	21 (45.7)	9 (19.6)

Table C1-2. Time Trends BMI in Women with Type 1 Diabetes.

			BMI		
	N (%)	<20	20-<25	25-<30	≥30
1986-1988	Overall	31 (12.7)	157 (58.8)	64 (24.0)	12 (4.5)
	Age group				
	20-<30	19 (13.9)	77 (56.2)	37 (27.0)	4 (2.9)
	30-<40	11 (10.4)	64 (60.4)	24 (22.6)	7 (6.6)
	40-<50	4 (16.7)	16 (66.7)	3 (12.5)	1 (4.2)
1988-1990	Overall	18 (8.6)	120 (57.1)	56 (26.7)	16 (7.6)
	Age group				
	20-<30	9 (9.7)	51 (54.8)	27 (29.0)	6 (6.5)
	30-<40	6 (6.6)	53 (58.2)	24 (26.4)	8 (8.8)
	40-<50	3 (16.7)	16 (61.5)	5 (19.2)	2 (7.7)
1990-1992	Overall	23 (11.0)	122 (58.4)	52 (24.9)	12 (5.7)
	Age group				
	20-<30	8 (9.3)	46 (53.5)	29 (33.7)	3 (3.5)
	30-<40	11 (12.9)	49 (57.7)	18 (21.2)	7 (8.2)
	40-<50	4 (10.8)	26 (70.3)	5 (13.5)	2 (5.4)
	≥50	0 (0)	1 (100)	0 (0)	0 (0)
1992-1994	Overall	19 (9.7)	99 (50.8)	60 (30.8)	17 (8.7)
	Age group				
	20-<30	4 (6.8)	28 (47.5)	23 (39.0)	4 (6.8)
	30-<40	8 (9.0)	46 (51.7)	25 (28.1)	10 (11.2)
	40-<50	7 (17.1)	20 (48.8)	12 (29.3)	2 (4.9)
	≥50	0 (0)	5 (83.3)	0 (0)	1 (16.7)

Table C1-2 continued

1994-1996	Overall	21 (9.6)	109 (49.8)	66 (30.1)	23 (10.5)
	Age group				
	20-<30	3 (6.0)	26 (52.0)	15 (30.0)	6 (12.0)
	30-<40	7 (7.1)	50 (50.5)	30 (30.3)	12 (12.1)
	40-<50	9 (14.3)	30 (47.6)	20 (31.8)	4 (6.4)
	≥50	2 (28.6)	3 (42.9)	1 (14.3)	1 (14.3)
1996-1998	Overall	31 (12.3)	116 (46.0)	73 (29)	32 (12.7)
	Age group				
	20-<30	2 (4.2)	20 (41.7)	17 (35.4)	9 (18.8)
	30-<40	9 (8.3)	55 (50.5)	31 (28.4)	14 (12.8)
	40-<50	13 (17.1)	32 (42.1)	23 (30.3)	8 (10.5)
	≥50	7 (36.8)	9 (47.4)	2 (10.5)	1 (5.3)
2000-2003	Overall	11 (5.8)	81 (42.4)	57 (29.8)	42 (22.0)
	Age group				
	20-<30	1 (0.0)	4 (50.0)	3 (37.5)	0 (0.0)
	30-<40	1 (2.5)	20 (31.8)	31 (49.2)	12 (19.1)
	40-<50	1 (1.2)	26 (35.6)	33 (45.2)	14 (19.2)
	≥50	2 (4.9)	10 (41.7)	7 (29.2)	4 (16.7)
2004-2007	Overall	9 (4.6)	68 (34.7)	75 (38.3)	44 (22.5)
	Age group				
	20-<30	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
	30-<40	0 (0.0)	22 (41.5)	18 (34.0)	13 (24.5)
	40-<50	4 (4.7)	25 (29.4)	38 (44.7)	18 (21.2)
	≥50	5 (8.8)	21 (36.8)	18 (31.6)	13 (22.8)

Table C1-3. Mixed models: Association of Baseline Age Group with Biennial BMI for up to 18 Years.

	ß±SE	P-value
Baseline Age Group		
<20 years	-0.95±1.42	0.50
20-29 years	-0.05±1.03	0.96
30-39 years	0.28±0.70	0.69
40-49 years	0.0	N/A
Baseline age	-0.04±0.05	0.39
Sex	0.18±0.27	0.50

Overall effect for age group p-value=0.26

Table C1-4. Association of Baseline Characteristics Weight Gain and Loss Adults with Type 1 Diabetes.

	Gain ≥ 5 kg/m² (n=119)	Loss ≥ 2 kg/m² (n=40)
	HR (95% CI)	HR (95% CI)
BMI (kg/m²)	1.20 (0.99-1.45)	1.50 (1.08-2.07)
Age (years)	0.95 (0.78-1.15)	1.13 (0.82-1.57)
Diabetes duration (years)	0.98 (0.81-1.18)	1.09 (0.79-1.49)
HbA1c (%)	1.34 (1.10-1.64)	1.66 (1.18-2.35)
Daily insulin dose (U/kg/dy)	1.05 (0.86-1.28)	1.12 (0.79-1.58)
Insulin injections/day*	1.06 (0.90-1.25)	1.16 (0.88-1.52)
Physical activity (kcal/week)*	0.91 (0.75-1.10)	0.69 (0.53-0.90)
Albumin excretion rate (mg/dl)*	1.13 (0.94-1.36)	1.65 (1.23-2.20)
Sex (female)	1.24 (0.86-1.78)	2.21 (1.12-4.34)
Coronary artery disease	1.02 (0.42-2.50)	2.14 (0.66-6.98)
Overt nephropathy	0.82 (0.51-1.30)	3.59 (1.89-6.81)
Proliferative Retinopathy	1.25 (0.84-1.85)	1.51 (0.77-2.99)
Symptomatic autonomic neuropathy	1.15 (0.46-2.86)	9.02 (3.68-22.12)
Intensive insulin therapy	0.83 (0.41-1.71)	2.09 (0.87-5.01)
Family history of T2D	1.10 (0.68-1.78)	0.64 (0.23-1.79)
Annual Household Income		
(<$20,000)	1.77 (1.18-2.64)	1.19 (0.59-2.37)
Some college	1.15 (0.76-1.72)	1.02 (0.51-2.02)
Current smoker	1.38 (0.91-2.11)	2.52 (1.30-4.89)
Alcohol consumption (3+gl/wk)	0.64 (0.40-1.01)	0.79 (0.38-1.67)

BMI units=kg/m² *Natural-logarithmically transformed before analysis.

All analyses, except baseline BMI, controlled for baseline BMI

Continuous variables expressed as per standard deviation change in the predictor variable

Table C1-5. Baseline Predictors of Weight Gain and Loss in Adults with Type1 Diabetes.

	≥5 kg/m² Gain	≥ 2 kg/m² Loss
	HR (95% CI)	HR (95% CI)
Sex (female)		3.57 (1.57-8.14)
HbA1c (%)		1.62 (1.13-2.32)
Overt nephropathy		3.27 (1.58-6.75)
Symptomatic autonomic neuropathy		5.80 (2.13-15.78)
Alcohol consumption (3+gl/wk)	0.53 (0.31-0.91)	
Current smoker		2.79 (1.28-6.06)
Low household income (<$20,000/yr)	1.63 (1.08-2.47)	
Baseline BMI	1.18 (0.95-1.47)	2.79 (1.28-6.06)

Weight gain Cox stepwise selection model allowed for sex, age, HbA1c, intensive insulin therapy, overt nephropathy, coronary artery disease, physical activity, alcohol consumption, and smoking. Weight gain Cox stepwise selection model allowed for sex, age, HbA1c, physical activity, coronary artery disease, overt nephropathy, symptomatic autonomic neuropathy, and smoking.

C.2 TIME TRENDS IN INTENSIVE INSULIN THERAPY AND SELF MONITORING IN TYPE 1 DIABETES

Andrea Rogers Fischl Baqiyyah Conway Trevor Orchard

Since the results of the DCCT demonstrating a delay in the development or progression of complications in type 1 diabetes (T1D) for those on intensive insulin therapy, the use of intensive insulin therapy in the general T1D population has appeared to gradually increase. In addition to multiple dose insulin therapy (MDI), defined as at least 3 injections/day and or on the insulin pump, self monitoring of blood glucose levels is also an important component of treatment. Although there appears to be a rise in MDI, it is not clear to what extent those on MDI are also self-monitoring blood glucose levels at least four times a day as in the treatment arm of the DCCT. We therefore investigated the use of intensive insulin therapy in 658 from the Pittsburgh Epidemiology of Diabetes Complications Study, an 18-year prospective study of childhood onset (age<17) T1D. Intensive self-monitoring of blood glucose (SM) was defined as ≥4 times/day. Participants were categorized according to their MDI and SM status and were followed from 1986-1988 to 2004-2007. Those in these groups were compared by HbA1c level at the latest follow-up (2004-2007). Overall MDI rose from 6.7% in 1986-1988 to 82% in 2004-2007 (Figure 8-5). SM increased from 11.9% in 1990-1992 (when this data was first collected) to 53.1 % in 2004-2007. When intensive insulin therapy was defined as both MDI and SM, MDI rose from 4.7% in 1990-1992 to 46.0% in 2004-2007. At latest follow-up (2004-2007), HbA1c differed by MDI and SM category. Those in the noMDI/noSM category had the highest mean HbA1c ($p<0.0001$) while those in the SM/MDI group had a lower mean HbA1c than those in the MDI/no SM group (Figure 8-5) ($p<0.0001$). The prevalence of both MDI and of SM has

increased substantially over time; however, the prevalence of SM has not increased to the same degree as MDI. Glycemic control is a function of both MDI and SM. It is therefore equally as important to prescribe SM in addition to MDI as part of the diabetes management. While the importance of intensive insulin therapy appears to have gotten across, more work needs to done on getting home the concomitant message of frequent self monitoring of blood glucose.

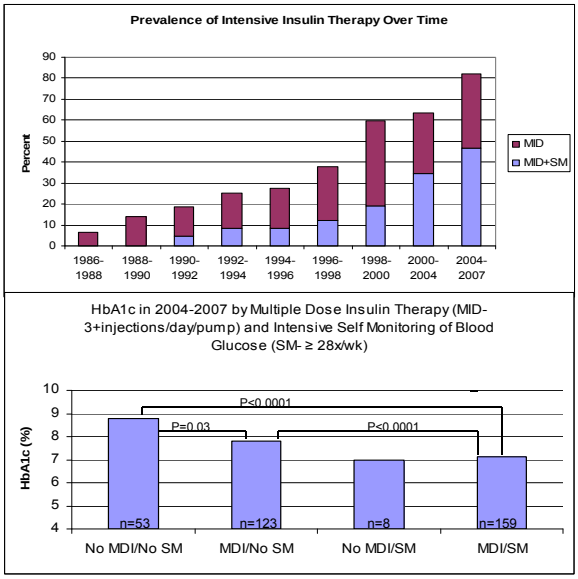

Figure C2-1. Time trends in intensive insulin therapy and self monitoring and their association with HbA1c.

C.3 INSULIN DOSE, HBA1C, AND CORONARY ARTERY DISEASE

Table C3-1. Baseline Predictors of Nonfatal Coronary Artery Disease (CAD), mean (SD) or % (n).

	Nonfatal CAD (n=109)	Non-cases (n=463)	p-value
Sex (female)	42.2 (46)	51.4 (238)	0.09
Age (years)	33.1 (6.3)	28.0 (6.6)	<0.0001
Duration (years)	24.8 (6.4)	19.2 (6.9)	<0.0001
BMI (kg/m²)	24.0 (3.3)	23.8 (3.0)	0.50
Waist (cm)	82.1 (8.7)	79.7 (8.9)	0.01
males	85.4 (6.7)	83.5 (7.9)	0.08
females	77.5 (9.2)	76.1 (8.2)	0.30
HbA1c (%)	8.9 (1.6)	8.7 (1.5)	0.25
Daily insulin dose/kg body wt	0.71 (0.21)	0.78 (0.24)	0.009
AER* (μg/min)	177.5 (13.3-935.6)	14.8 (7.6-100.6)	<0.0001
eGFR* (mg/min/1.73 m²)	98.8 (65.1-120.6)	110.4 (89.4-135.3)	<0.0001
WBC (x 10³/mm²)	7.4 (2.1)	6.5 (1.9)	<0.0001
SBP (mm Hg)	123.3 (21.8)	112.9 (14)	<0.0001
DBP (mm Hg)	79.1 (12.4)	72.3 (10.2)	<0.0001
Hypertension	39.5 (43)	12.1 (56)	<0.0001
Hypertension medication	17.9 (19)	3.0 (13)	<0.0001

Table C3-1 continued

HDLc (mg/dl)	50.6 (11.4)	54.5 (12.9)	0.005
males	46.3 (8.2)	49.4 (10.0)	0.03
females	56.4 (12.6)	59.3 (13.4)	0.18
Non-HDLc (mg/dl)	161.3 (42.0)	133.9 (40.7)	<0.0001
males	167.9 (44.3)	136.4 (41.2)	<0.0001
females	152.5 (37.3)	131.5 (40.2)	0.001
Fibrinogen (mg/dl)*	323.1 (99.8)	281.3 (85.8)	<0.0001
Beck Depression Inventory* (n=506)	7 (3-14)	5 (2-10)	0.02
History of smoking**	54.2 (58)	38.2 (172)	0.003
Physical activity* (kcal) (n=532)	924 (420-2296)	1512 (616.5-2912.0)	0.09
Overt Nephropathy (n=525)	50.9 (55)	24.9 (104)	<0.0001
Proliferative Retinopathy	65.7 (71)	25.5 (118)	<0.0001
Symptomatic Autonomic Neuropathy (n=459)	19.8 (19)	6.6 (24)	<0.0001
History of hypoglycaemia resulting in unconsciousness (%) (n=544)	58.1 (61)	41.9 (44)	0.004

AER=albumin excretion rate eGFR=estimated glomerular filtration rate WBC=white blood cell count SBP=systolic blood pressure DBP=diastolic blood pressure *Natural logarithmically transformed before analysis **Having smoked at least 100 cigarettes in a lifetime

Table C3-2. Baseline Predictors of Mortality from Fatal Coronary Artery Disease, mean (SD) or % (n).

	Fatal CAD (n=48)	Non-cases (n=544)	p-value
Sex (female)	45.8 (22)	49.5 (269)	0.63
Age (years)	33.8 (6.1)	28.7 (6.7)	<0.0001
Duration (years)	25.4 (6.3)	20.0 (7.1)	<0.0001
BMI (kg/m²)	24.3 (3.6)	23.8 (3.0)	0.27
Waist (cm)	82.9 (9.3)	80.0 (8.9)	0.03
males	86.0 (8.0)	83.8 (7.7)	0.18
females	79.1 (9.5)	76.1 (8.2)	0.09
HbA1c (%)	9.2 (1.6)	8.7 (1.5)	0.03
Daily insulin dose/kg body wt	0.77 (0.25)	0.76 (0.24)	0.91
AER* (μg/min)	509.1 (71.6-1006.9)	16.6 (7.9-153.6)	<0.0001
eGFR* (mg/min/1.73 m²)	90.4 (56.9-116.1)	109.3 (87.0-134.2)	0.002
WBC (x 10³/mm²)	8.0 (2.1)	6.6 (1.9)	<0.0001
SBP (mm Hg)	124.0 (21.9)	114.1 (15.4)	0.004
DBP (mm Hg)	78.3 (13.5)	73.3 (10.7)	0.01
Hypertension (%)	37.5 (18)	16.2 (88)	0.0002
Hypertension medication (%)	12.8 (6)	5.4 (28)	0.04
HDLc (mg/dL)	50.8 (13.1)	53.9 (12.6)	0.11
males	46.8 (9.9)	48.8 (9.7)	0.32
females	55.2 (14.9)	59.1 (13.1)	0.20

Table C3-2 continued

Non-HDLc (mg/dl)	166.1 (52.4)	137.9 (42.0)	0.0008
males	169.5 (60.8)	142.1 (42.5)	0.04
females	162.4 (42.4)	133.6 (41.2)	0.002
Fibrinogen (mg/dl)*	328.2 (84.7)	287.5 (92.1)	0.001
Beck Depression Inventory* (n=525)	8 (5-14)	6 (2-10)	0.04
History of smoking**	61.7 (29)	40.2 (213)	0.004
Physical activity* (kcal) (n=550)	1201 (574-2016)	1428 (616-2816)	0.07
Overt Nephropathy (%) (n=541)	67.4 (31)	28.1 (139)	<0.0001
Proliferative Retinopathy (%)	72.9 (35)	31.0 (168)	<0.0001
Symptomatic Autonomic Neuropathy (%) (n=468)	19.4 (7)	9.1 (39)	0.04
History of hypoglycaemia resulting in unconsciousness (%) (n=563)	44.4 (20)	46.0 (238)	0.85

AER=albumin excretion rate eGFR=estimated glomerular filtration rate WBC=white blood cell count SBP=systolic blood pressure DBP=diastolic blood pressure *Natural logarithmically transformed before analysis **Having smoked at least 100 cigarettes in a lifetime

Table C3-3. Predictors of the 18 Year Incidence of Nonfatal or Fatal Coronary Artery Disease in Type 1 Diabetes.

	Nonfatal CAD events 109 cases; 463 non-cases	CAD as the Primary Cause of Death 48 cases; 544 non-cases
Risk Factors	HR (95% CI)	HR (95% CI)
Diabetes duration (years)	2.07 (1.64-2.61)	1.95 (1.40-2.71)
HbA1c (%)		1.55 (1.14-2.10)
Daily insulin dose/kg body weight	0.75 (0.57-0.97)	
AER (μg/min)		1.89 (1.38-2.57)
eGFR (mg/min/1.73 m²)	0.73 (0.60-0.88)	
DBP (mm Hg)	1.52 (1.25-1.85)	
HDLc (mg/dl)	0.74 (0.59-0.92)	
Non-HDCc (mg/dl)	1.44 (1.20-1.73)	
WBC (x 10^3/mm²)	1.42 (1.20-1.68)	1.48 (1.16-1.88)

AER=albumin excretion rate eGFR=estimated glomerular filtration rate DBP=diastolic blood pressure SBP=systolic blood pressure HDLc=high density lipoprotein cholesterol Non-HDLc=non-high density lipoprotein cholesterol WBC=white blood cell count

Models allowed for sex, diabetes duration, insulin dose/kg body weight, HbA1c, waist circumference, (log) AER, (log) eGFR, DBP, use of hypertension medication, WBC, HDLc, non-HDLc, (log) fibrinogen, and a history of smoking.

C.4

ASSOCIATION OF ADIPOSITY WITH THE 18-YEAR INCIDENCE OF COMPLICATIONS IN TYPE 1 DIABETES

Table C4-1. Association of Baseline Body Mass Index, Waist Circumference, and Hip Circumferences with the 18-year Incidence of Complications of Type 1 Diabetes.

	Coronary Artery Disease	Overt Nephropathy	Symptomatic Autonomic Neuropathy	Proliferative Retinopathy	Confirmed Distal Symmetrical Polyneuropthy	Lower Extremity Arterial Disease
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
BMI						
Underweight	1.12 (0.65-1.94)	1.37 (0.69-2.73)	1.26 (0.64-2.46)	0.96 (0.59-1.58)	1.16 (0.66-2.03)	1.13 (0.63-2.05)
Normal	Ref					
Overweight	1.50 (1.07-2.11)	0.72 (0.39-1.32)	1.13 (0.74-1.71)	1.04 (0.74-1.46)	1.07 (0.75-1.53)	1.13 (0.77-1.68)
Obese	1.42 (0.62-3.25)	0.93 (0.22-3.82)	1.56 (0.62-3.91)	1.49 (0.66-3.40)	0.87 (0.35-2.16)	1.16 (0.42-3.17)
Waist Circumference						
Quartile 1	1.19 (0.73-1.93)	0.69 (0.36-1.32)	0.62 (0.34-1.11)	0.91 (0.61-1.37)	1.07 (0.67-1.70)	1.56 (0.92-2.67)
Quartile 2	Ref					
Quartile 3	1.22 (0.75-1.97)	0.69 (0.36-1.34)	0.76 (0.43-1.32)	1.09 (0.73-1.64)	1.09 (0.69-1.74)	1.29 (0.74-2.23)
Quartile 4	2.38 (1.53-3.71)	0.85 (0.44-1.65)	1.40 (0.86-2.28)	1.23 (0.81-1.88)	1.69 (1.08-2.65)	2.12 (1.26-3.56)
Hip Circumference						
Quartile 1	1.78 (1.14-2.79)	0.86 (0.46-1.60)	0.85 (0.49-1.49)	0.93 (0.62-1.40)	0.77 (0.48-1.22)	1.57 (0.98-2.50)
Quartile 2	Ref					
Quartile 3	1.17 (0.74-1.87)	0.78 (0.43-1.44)	0.91 (0.55-1.51)	0.78 (0.52-1.18)	0.77 (0.49-1.21)	0.58 (0.33-1.03)
Quartile 4	1.47 (0.94-2.30)	0.40 (0.19-0.83)	0.92 (0.56-1.51)	0.90 (0.61-1.33)	1.14 (0.76-1.70)	1.26 (0.78-2.02)

Table C4-2. Association of Updated Mean Body Mass Index, Waist Circumference, and Hip Circumference with the 18-year Incidence of Complications of Type 1 Diabetes.

	Coronary Artery Disease	Overt Nephropathy	Symptomatic Autonomic Neuropathy	Proliferative Retinopathy	Confirmed Distal Symmetrical Polyneuropthy	Lower Extremity Arterial Disease
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
BMI						
Underweight	1.42 (0.76-2.67)	1.98 (0.88-4.48)	1.37 (0.63-3.02)	1.16 (0.64-2.12)	1.17 (0.59-2.34)	0.96 (0.42-2.23)
Normal	Ref					
Overweight	1.09 (0.78-1.52)	1.07 (0.65-1.77)	0.73 (0.48-1.11)	1.22 (0.91-1.65)	1.20 (0.86-1.67)	1.18 (0.81-1.72)
Obese	1.42 (0.83-2.40)	1.16 (0.49-2.77)	1.08 (0.57-2.04)	0.75 (0.40-1.40)	0.75 (0.40-1.40)	1.61 (0.92-2.85)
Waist Circumference						
Quartile 1	1.10 (0.67-1.80)	3.23 (1.43-7.30)	1.03 (0.55-1.92)	0.96 (0.64-1.43)	0.78 (0.48-1.27)	0.29 (0.03-2.62)
Quartile 2	Ref					
Quartile 3	1.30 (0.81-2.08)	2.63 (1.16-5.96)	1.41 (0.82-2.42)	1.20 (0.82-1.75)	1.00 (0.65-1.54)	1.01 (0.27-3.80)
Quartile 4	2.32 (1.51-3.57)	3.40 (1.54-7.55)	1.52 (0.90-2.58)	1.43 (0.97-2.10)	1.62 (1.07-2.45)	0.94 (0.20-4.33)
Hip Circumference						
Quartile 1	1.44 (0.93-2.34)	1.15 (0.60-2.22)	0.81 (0.48-1.37)	0.70 (0.47-1.04)	0.82 (0.50-1.33)	1.21 (0.72-2.04)
Quartile 2	Ref					
Quartile 3	1.04 (0.66-1.63)	1.27 (0.69-2.36)	0.67 (0.40-1.13)	0.81 (0.55-1.19)	1.35 (0.87-2.08)	1.28 (0.78-2.11)
Quartile 4	1.29 (0.83-1.99)	0.80 (0.40-1.59)	0.88 (0.54-1.43)	0.93 (0.64-1.35)	1.35 (0.88-2.08)	1.35 (0.82-2.22)

BIBLIOGRAPHY

1. Dart A, Kingwell B. Pulse pressure-a review of mechanisms and clinical relevance. J Am Coll Cardiol2001;37:975-84.
2. The effect of intensive treatment of diabets on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. NEJM1993;329:977-86.
3. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Efect of intensive therapy on the microvascular complications of type 1 diabetes. Journal of the American Medical Association2002;287:2563-9.
4. The Diabetes Control and Complications Trial Research Group. Weight gain associated with intensive insulin therapy use in the Diabetes Control and Complications Trial. Diabetes Care1988;11:567-73.
5. Purnell J, Hokanson J, Marcovina S, Steffes M, Cleary P, Brunzell J. Effect of excessive weight gain with intensive therapy in type 1 diabetes on lipid levels and blood pressure. Results from DCCT. JAMA1998;280:140-6.
6. Krolewski A, Kosinski E, Warram J, Leland O, Busick E, Asmal A, et al. Magnitude and determinants of coronary artery disease in juvenile-onset, insulin dependent diabetes mellitus. Am J Cardiol1987;59:750-5.
7. Nathan D, Cleary P, Backlund J, Genuth S, Lachin J, Orchard T, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. NEJM2005;353:2643-53.
8. Rossing P, Hougaard P, Borch-Johnsen K, Parving H. Predictors of mortality in insulin dependent diabetes: 10 year observational follow-up study. BMJ1996;313:779-84.
9. Orchard T, Olson J, Erbey J, Williams K, Forrest K, Kinder L, et al. Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes. Diabetes Care2003;26:1374-9.
10. Soedamah-Muthu S, Chaturvedi N, Toeller M, Ferriss B, Reboldi P, Michel G, et al. Risk factors for coronary artery disease in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study. Diabetes Care2004;27:530-7.
11. Shankar A, Klein R, Klein B, Moss S. Association between glycosylated hemoglobin level and cardiovascular and all-cause mortality in type 1 diabetes. Am J Epidemiol2007;166:393-402.
12. Klein B, Klein R, McBride P, Cruickshanks K, Palta M, Knudtson M, et al. Cardiovascular disease, mortality, and retinal microvascular characteristics in type 1 diabetes: Wisconsin Epidemiologic Study of Diabetic Retinopathy. Arch Intern Med2004;164:1917-24.
13. Marti A, Marcos A, Martinez J. Obesity and Immune Function Relationships. Obes Rev2001;2(131-140).

14. Chou S, Grossman M, Saffer H. An economic analysis of adult obesity: results from the behavioral risk factor surveillance system. National Burau of Economic Research Working Paper 2002;9247.
15. National Center for Health Statistics. Health United States, 2005. Hyattsville2005.
16. The International Obesity Task Force. The Global Epidemic of Obesity. [cited 2003 8/12/2003]; Available from: http://www.iotf.org/meida/obesitytable.htm.
17. O'Brien, Dixon J. The extent of the obesity problem. Am J Surg2002;184:4S-8S.
18. Corditz G. Economic costs of obesity and inactivity. Med Sci Sports Exerc1999;31:S663-S7.
19. Wolf A, Corditz G. Current estimates of the economic cost of obesity in the United States. Obes Res1998;6:173-5.
20. Schlosser E. Fast Food Nation: the dark side of the all-American meal. New York: Houghton Mifflin; 2002.
21. Conway B, Rene A. Obesity as a disease: no lightweight matter. Obesity Reviews2004;5:145-51.
22. Gross L, Li L, Ford D, Simm L. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecological assessment. American Journal of Clinical Nutrition2004;79:774-9.
23. Bray G, Nielsen S, Popkin B. Consumption of high-fructose corn syrup beverages may play a role in the epidemic of obesity. American Journal of Clinical Nutrition2004;79:537-43.
24. Elliott S, Keim N, Stern J, Teff K, Havel P. Fructose, weight gain, and the insulin resistance syndrome. American Journal of Clinical Nutrition2002;76:911-22.
25. Basciano H, Federico L, Khosrow A. Fructose, insulin resistance, and metabolic dyslipidemia. Nutrition and Metabolism2005;2(1):5-19.
26. Per Bjorntorp. Thrifty genes and human obesity. Are we chasing ghosts? Lancet2001;358:1006-8.
27. Wilkin T, Voss L. Metabolic syndrome: maladaptation to a modern world. Journal of the Royal Society of Medicine2004;97:511-20.
28. Tremblay A, Doucet E. Obesity: a disease or biological adaptation. Obesity Reviews2000;1:27-35.
29. Allison D, Fountaine K, Manson J, Stevens J, VanItallie. Annual deaths attributable to obesity in the United States. JAMA1999;282:1530-8.
30. Flegal K, Graubard B, Williamson D, Gail M. Excess deaths associated with underweight, overweight, and obesity. JAMA2005;293:1861-7.
31. Rimm E, Stampfer M, Giovannucci E, Ascherio A, Spiegelman D, Coditz G, et al. Body size and fat distribution as predictors of coronary heart disease in middle-aged and older men. Am J Epidemiol1995;141(2):1117-27.
32. Anderson J, Konz E. Obesity and disease management: effects of weight loss on comorbid conditions. Obes Res2001;9(4S):326S-34S.
33. National Center for Health Statistics. Deaths/Mortaltiy. 2005 [cited 2005 April 25]; Available from: http://www.cdc.gov/nchs/fastats/deaths.htm.
34. Mokdad A, Serdula M, Dietz W, Bowman B, Marks J, Koplan J. The Continuing Epidemic of Obesity in the United States. JAMA2000;284:1650-1.
35. Raven P. Metabolic Syndrome. Journal of Insurance Medicine2004;36:133-42.
36. Kaufman F. Diabesity: the Obesity-Diabetes Epidemic that Threatens America-and What We Must do to Stop it. New York: Bantam; 2005.

37. Astrup A, Finer N. Redefining type 2 diabetes: "Diabesity" of "Obesity Dependent Diabetes Mellitus"? Obesity Reviews2000;2000:57-9.
38. Albu J, Pi-Sunyer X. Obesity in Diabetes. In: Bray G, Bouchard C, W J, editors. Handbook of Obesity. New York: Marcel Dekker, Inc; 1998. p. 697-708.
39. Owens T. Morbid obesity: the disease and complications. Crititcal Care Nursing Quarterly2003;26:162-5.
40. Colditz G, Willet W, Rotnitzky A, Manson J. Weight as a risk factor for clinical diabetes in women. Annals of Internal Medicine1995;122(7):481-6.
41. Chan J, Rimm E, Colditz G, Stampfer M, Willet W. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care1994;17:961-9.
42. Ohlson L, Larson B, Svardsudd K, Welin L, Ericksson H, Wilhelmsen L, et al. The influence of body fat distribution on the incidence of diabetes mellitus. Diabetes1985;34:1055-8.
43. West K, Kalbfleish. Influence of nutritional factors on prevalence of diabetes. Diabetes1971;20:99-108.
44. Resnick H, Valsania P, Halter J, Lin X. Relation of weight gain and weight loss on subsequent diabetes risk in overweigh adults. J Epidemiol Community Health2000;54:596-602.
45. Boykyo E, Fujimoto W, Leonetti D, Newell-Morris L. Visceral adiposity and risk of type 2 diabetes. A prospective study among Japanese Americans. Diabetes Care2000;23:465-71.
46. Kern P, Saghizadeh M, Ong J, Bosch R, Deem R, Simsolo R. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. Journal of Clinical Investigations1995;95:2111-9.
47. Boden G. Free fatty acids, insulin resistance, and type 2 diabete mellitus. Proceedings from the Association of American Physicians1999;111:421-8.
48. Bray G. Medical Consequences of Obesity. Journal of Clinical Endocrinology and Metabolism2004;89:2583-9.
49. Eckel R, Krauss R. American Heart Association Call to Action: Obesity as a major risk factor for coronary heart disease. Circulation1998;97:2099-100.
50. Sharma A. Obesity and cardiovascular disease. Growth Hormone and IGF Research2003;13:S10-S7.
51. Kenechiah S, Evans J, Levy D, Willson P, Benjamin E, Larson M, et al. Obesity and risk of heart failure. The New England Journal of Medicine2002;347:305-13.
52. Zhou B, Wu Y, Yang J, Li Y, Zhang H, Zhao L. Overweight as an independent risk factor for cardiovascular disease in Chinese populations. Obesity Reviews2002;3:147-56.
53. Manson J, Coditz G, Stampfer M, Willet W, Rosner B, Monson R, et al. A prospective study of obesity and risk of coronary heart disease in women. The New England Journal of Medicine1990;322:882-9.
54. Rexrode K, Hennekens C, Willet W, Colditz G, Stampfer M, Rich-Edwards J, et al. A prospective study of body mass index, weight change, and risk of stroke in women. JAMA1997;227:1539-45.
55. Tishler P, Larkin E, Schluchter M, Redline S. Incidence of sleep-disordered breathing in an urban adult population: the relative importance of risk factors in the development of sleep-disordered breathing. JAMA2003;289:2230-7.
56. Baik I, Curhan G, Rimm E, Bendich A, Willett W, Fawzi W. A prospective study of age and lifestyle factors in relation to community-aquired pneumonia in U.S. men and women. Archives of Internal Medicine2000;160:3082-8.

57. Calle E, Rodriguez C, Walker-Thurmond K, Thun M. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. NEJM2003;348:1625-38.
58. Huang X, Hankinson S, Coditz G, Stampfer M, Hunter D, Manson J, et al. Dual effects of weight and weight gain on breast cancer risk. JAMA1997;278:1407-11.
59. Murphy T, Calle E, Rodriguez C, Kahn K, Thun M. Body mass index and colon cancer mortality in a large prospective study. Am J Epidemiol2000;152:847-54.
60. Grundy S, Cleeman J, Daniels S, Donato K, Eckel R, Franklin B, et al. Diagnosis and Management of the Metabolic Syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation2005;13:322-7.
61. Alberti K, Shaw P, the IDF Epidemiology Task Force Consensus Group. The metabolic syndrome-a new worldwide definition. Lancet2005;366:1059-62.
62. Raven G. Role of insulin resistance in human disease (syndrome X): an expanded definition. Annual Reviews of Medicine1993;44:121-31.
63. Raven G. The metabolic syndrome or the insulin resistanc syndrome? Different names, different concepts, and different goals. Endocrinology and Metabolism Clinics of North America2004;33:283-303.
64. WHO Expert Committe. Physical Status: the and Interpretation of Anthropometry. Geneva: WHO; 1995.
65. Wilson P. Estimating cardiovascular disease risk and the metabolic syndrome: a Framingham view. Endocrinology and Metabolism Clinics of North America2004;33:467-81.
66. Fruhbeck G, Gomex-Ambrosia J, Muruzabal F, Burrell M. The adipocyte: a model for integration and metabolic signaling in energy metabolism regulation. Am J Physiol Endocrinol Metab2001;280:E827-E47.
67. Fasshauer M, Paschke R. Regulation of adipocytokines and insulin resistance. Diabetalogia2003;46:1594-603.
68. Flier J. Obesity. In: Braunwald E, Fauci A, Isselbacher K, Kasper D, Hauser S, Longo D, et al., editors. Harrison's Principles of Internal Medicine. New York: McGraw Hill; 2003.
69. Poirier P, Alpert M. Heart Disease. In: Eckel R, editor. Obesity: Mechanism and Clinical Management. Philadelphia: Lippincott Williams & Williams; 2003. p. 181-201.
70. Tanaka S, Inoue S, Isoda F, Waseda M, Ishihara M, Yamakawa T, et al. Impaired immunity in obesity: suppressed but reversible lymphocyte responsiveness. Int J Obes Relat Metab Disord1993;17:631-6.
71. Steinberg H, Gumbiner B. Pathophysiology of obesity and metabolic response to weight loss. In: Gumbiner B, editor. Obesity. Philadelphia: American College of Physicians; 2001. p. 50-66.
72. Boden G. Gluconeogenesis and Glycogenolysis in Health and Diabetes. Journal of Investigative Medicine2004;52:375-8.
73. Hutley L, Prins J. Fat as an endocrine organ: relationship to the metabolic syndrome. American Journal of Medical Science2005;330:280-9.
74. Warne J. New perspectives on endocrine signalling: tumor necrosis factor α: a key regulator of adipose tissue mass. J Endocinol2003;177:351-5.
75. Bray G. An Atlass of Obesity and Weight Control. New York: Parthenon; 2003.
76. Ras I. Diabetes lipidus: cure through intervention in lipid metabolism and storage? [cited 2006 June 25]; Available from: http://www.d4pro.com/idm/site/diabetes_cure_through_.htm.

77. Henry H, Mudaliar S. Obesity and type II diabetes mellitus. In: Eckel R, editor. Obesity: Mechanisms and Clinical Management. Philadelphia: Lippincott Williams & Williams; 2003. p. 229-72.
78. Das U. Is obesity an inflammatory condition? Nutrition2001;17:953-66.
79. Wallace M, McMahon A, Packard C, Kelly A, Shepherd J, Gaw A, et al. Plasma Leptin and Risk of Cardiovascular Disease in the West of Scotland Coronary Prevention Study (WOSCOPS). Circulation2001;104:3052-6.
80. Dawson S, Henney A. The status of PAI-1 as a risk factor for arterial and thrombotic disease: a review. Atherosclerosis1992;95:105-17.
81. Sturk T, Hauner A. Obesity and impaired fribronolysis: a role of adipose production of plasminogen activator inhibitor-1. International Journal of Obesity2004;28:1357-64.
82. Alessi M, Lijnen R, Bastelica D, Juhan-Vahue I. Adipose tissue and atherothrombosis. Pathophysiology of Haemostasis and Thrombosis2003/2004;33:290-7.
83. Hauner H. The new concept of adipose tissue funtion. Physiology and Behavior2004;53:653-8.
84. Juhan-Vague I, Alessi M, Mavri A, Morange P. Plasminogen activator inhibitor-1, inflammation, obesity, and insulin resistance in vascular disease. Journal of Thrombosis and Haemostasis2003;1:1575-9.
85. Epstein F. Plasminogen-activator inhibitor type-1 and coronary artery disease. NEJM2000;342:1792-801.
86. Berg A, Scherer P. Adipose tissue, inflammation, and cardiovascular risk. Circulation2005;96:939-49.
87. Willerson J, Ridker P. Inflammation as a cardiovascular risk factor. Circulation2004;109:ii2-ii10.
88. Greenberg A, Obin M. Obesity and the role of adipose tissure in inflammation and metabolism. The American Journal of Clinical Nutrition2006;83:461S-5S.
89. Perseghin G, Lattuada G, Danna M, Sereni L, Maffi P, De Cobelli F, et al. Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. American Journal of Physiology, Endocrinology, and Metabolism2003;285:E1174-E81.
90. DeBlock C, De Leeuw I, Van Gaal L. Impact of overweight on chronic microvascular complications in type 1 diabetic patients. Diabetes Care2005;28:1649-55.
91. Costacou T, Zgibor J, Evans R, Otvos J, Lopes-Virella M, Tracy R, et al. The prospective association between adiponectin and coronary artery disease among individuals with type 1 diabetes. The Pittsburgh Epidemiology of Diabetes Complications Study. Diabetologia2005;48:41-8.
92. Schram M, Chatruvedi N, Schalkwijk, Fuller J, Stehouer C, EURODIAB Prospective Complications Study. Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes-the EURODIAB Prospective Complications Study Diabetologia2005;48:370-8.
93. Stephens J, Vidal-Puig A. An update on visfatin/pre-B cell colony-enhancing factor, an ubiquitously expressed illusive cytokine that is regulated in obesity. Current Opinion in Lipidology2006;17:128-31.
94. CDC. National Diabetes Fact Sheet. [cited 2006 July 10]; Available from: http://www.cdc.gov/diabetes/pubs/estimates05.htm#top.

95. Stene L, Joner G, The Norwegian Childhood Diabetes Study Group. Use of cod liver oil during the first year of lifeis associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. Lancet2003;78:1128-34.
96. LaPorte R, Matsushima M, Chang Y. Prevalence and Incidence of Insulin-dependent Diabetes. In: Harris M, Bennett P, Boyko E, Cowie C, Dorman J, Everhart J, et al., editors. Diabetes in America: National Institutes of Health. National Diabetes and Digestive and Kidney Diseases NIH p1465ublication no. 95-1468; 1995.
97. International Federation of Diabetes. Prevalence. [cited 2002 November 27]; Available from: www.idf.org/index.ofm?node=264.
98. Besser G, Bodansky H, Cudworth A. Clinical Diabetes. Philadelphia: Lippencott; 1988.
99. Rewers M, Norris J, Dabelea D. Epidemiology of type 1 diabetes. In: Eisenbarth G, editor. Type 1 Diabetes: Molecular, Cellular, and Clinical Immunology. New York: Kluwer Academic/Pleum Publishers; 2004.
100. Medical Management of Type1 Diabetes. Fifth ed. Kaufman Fe, editor. Alexandria: American Diabetes Association; 2008.
101. Waernbaum I, Blohme G, Ostman J, Sundkvist G, Eriksson J, Arnqvist H, et al. Excess mortality in incident cases of diabetes mellitus aged 15 to 34 years at diagnosis: a population-base study (DISS) in Sweden. Diabetologia2006;49:653-9.
102. Soedamah-Muthu S, FullerJ, Mulnier H, Raleigh V, Lawrenson R, Colhoun H. All-cause mortality rates in type 1 diabetes mellitus compared with a non-diabetic population from the UK general practice research database, 1992-1999. Diabetologia2006;49:660-6.
103. McAlpine R, Morris A, Emslie-Smith A, James P, Evans J. The annual incidence of diabetic complications in a population of patients with Type 1 and Type 2 diabetes. Diabetes Medicine2005;22:348-52.
104. Pambianco G, Costacou T, Ellis D, Becker D, Klein R, Orchard T. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study Experience. Diabetes2006;55:1463-9.
105. Zhang L, Krzentowski G, Albert A, Lefebvre P. Risk of developing retinopathy in the Diabetes Control and Complications Trial type 1 diabetic patients with good or poor metabolic control. Diabetes Care2001;24:1275-9.
106. Dorchy H, Claes C, Verougstraete C. Risk factors of developing proliferative retinopathy in type 1 diabetic patients: role of BMI. Diabetes Care2002;25:798-9.
107. Stuhldreher W, Becker D, Drash A, Ellis D, Kuller L, Wolfson S, et al. The association of waist/hip ratio with diabetes complications in an adult IDDM population. J Clin Epidemiol1994;47:447-56.
108. Williams K, Becker D, Erbey J, Orchard T. Improved glycemic control reduces the impact of weight gain on cardiovascular risk factors in type 1 diabetes. Diabetes Care1999;22:1091-9.
109. Sibley S, Palmer J, Hirsch I, Brunzell J. Visceral obesity, hepatic lipase activity, and dyslipidemia in type 1 diabetes. Journal of Clinical Endocrinology and Metabolism2003;88:3379-84.
110. Allison D, Wright C. Body morphology differentially predicts coronary artery calcification. International Journal of Obesity2004;28:396-401.
111. Olson J, Edmundowicz D, Becker D, Kuller L, Orchard T. Coronary calcium in adults with type 1 diabetes: a stronger correlate of clinical coronary artery disease in men than in women. Diabetes2000;49:1571-8.

112. Stuhldreher W, Orchard T, Ellis D. The association of waist-hip ratio and risk factors for development of IDDM complications in an IDDM adult population. Diabetes Research and Clinical Practice1992;17:99-109.
113. Olson J, Erbey J, Forrest K, Williams K, Becker D, Orchard T. Glycemia (or, in women, estimated glucose disposal rate) predict lower extremity arterial disease in type 1 diabetes. Metabolism2002;51:248-54.
114. Lloyd C, Kuller L, Ellis D, Becker D, Wing R, Orchard T. Coronary artery disease in IDDM: gender differences in risk factors but not risk. Arterioscler Thromb Vasc Biol1996;16:720-6.
115. Jarrett R, Shipley M. Mortality and associated risk factors in diabetics. Acta Endocrinologica1985;272:21-6.
116. Moy C, Songer T, LaPorte R, Dorman J, Kriska A, Orchard T, et al. Insulin-dependent diabetes mellitus, physical activity, and death. American Journal of Epidemiology1993;137:74-81.
117. National Institute of Health DoH, Education, and Welfare,. Lipid Research Clinics Program. Washington, DC: U.S. Govt Printing Office (NIH pub no. 75-628); 1978.
118. Allain C, Poon L, Chan C, Richmond W, Fu P. Enzymatic determination of total serum cholesterol. Clin Chem1974;20:470-5.
119. Borhani N, Kass E, Langford H, Payne G, Remington R, Stamler J. The hypertension detection and follow-up program. Prev Med1976;5:207-15.
120. Diabetes Control and Complications Trial Research Group. The effect of intensive diabetes therapy on measures of autonomic nervous function in the Diabetes Control and Complications Trial (DCCT). Diabetologia1998;41:416-23.
121. Feldman E SM, Thomas P, Brown M, Canal N, Green D,. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. Diabetes Care1994;17:1281-9.
122. Eknoyan G. Adolphe Quetelet (1796-1874)--the average man and indices of obesity. Nephrol Dial Transplant2008;23:47-51.
123. Keyes A, Fidanza F, Karvonen M, Kimura N, Taylor H. Indices or relative weight and adiposity. J Chronic Dis1972;25:329-43.
124. Allison D, Zhu S, LPlankey M, Faith M, Heo M. Differential associations of body mass index with all-cause mortality among men in the first and second National Health and Nutrition Examination Surveys (NHANES I and NHANES II) follow-up studies. International Journal of Obesity2002;26:410-6.
125. Manson J, Willet W, Stampfer M, Colditz G, Hunter D, Hankinson S, et al. Body weight and mortality among women. NEJM1995;333:677-85.
126. Lee E, Garrinkel L. Variations in moratlity by body weight among 750,000 men and women. J Chronic Dis1979;32:563-76.
127. Menotti A, Descovich, Lanti M, Spagnolo A, Dormi A, Seccareccia F. Indexes of obesity and all-cause mortality in Italian epidemiological data. Prev Med1993;22:293-303.
128. Nicoletti I, Mariantonietta C, Morando G, Benazzi C, Prati D, Morani G, et al. Impact of body mass index on short-term outcome after acute myocardial infarction: Does excess body weight have a paradoxical protective role? International Journal of Cardiology2006;107:395-9.
129. Lavie C, Osman A, Milani R, Mehra M. Body composition and prognosis in chronic systolic heart failure: the obesity paradox? Am J Card Imaging2003;91:891-4.

130. Kalantar-Zadeh K, Horwich T, Oreopoulos A, Kovesdy C, Younessi H, Anker S, et al. Risk factor paradox in wasting diseases. Current Opinion in Clinical Nutrition and Metabolic Care2007;10:433-42.
131. Wang Y, Beydoun M. The Obesity Epidemic inthe United States-Gender, Age, Socioeconomic, Racial/Ethnic, and Geographic Characteristics: A Systematic Review and Meta-Regression Analysis. Epidemiol Rev2007;29:6-28.
132. Ogden C, Carroll M, Curtin L, McDowell M, Tabak C, Flegal K. The prevalence of overweight and obesity in the United States, 1999-2004. JAMA2006;295:1549-55.
133. Flegal K, Carroll M, Ogden C, Johnson C. Prevalence and trends in obesity among US adults, 1999-2000. JAMA2002;288:1723-7.
134. Mokdad A, serdula M, Dietz W, Bowman B, Marks J, Koplan J. The spread of the obesity epidemic in the United States, 1991-1998. JAMA1999;282:1519-22.
135. Conway B, Fried L, Orchard T. Hemoglobin and Overt Nephropathy Complications in Type 1 Diabetes. Ann Epidemiol2008;18:147-55.
136. Raju T. A mysterious something: The discovery of insulin and the 1923 Nobel Prize for Frederick G. Banting (1891-1941) and John J.R. Macleod (1876-1935). Acta Pediatrica2006;95:1155-6.
137. Rosenfeld L. Insulin: discovery and controversy. Clin Chem2002;48:2270-88.
138. Bloomberg Z. Type 1 Diabetes and Glucose Monitoring. Diabetes Care2007;30:2965-71.
139. Bruttomesso D, Crazzolara D, Maran A, Costa S, Dal Pos M, Girelli A, et al. In Type 1 diabetic patients with good glycemic control, blood glucose variability is lower during continuous subcutaneous insulin infusion than during multiple daily injections with insulin glargine. Diabet Med25;25:326-32.
140. Arias P, Kerner W, Zier H, Navascues I, Pfeiffer E. Incidence of hypoglycemic episodes in diabetic patients under continuous subcutaneous insulin and intensified conventional insulin treatment: assessment by means of semiambulatory 24-hour continuous blood glucose monitoring. Diabetes Care1985;8:134-40.
141. Kaufman F, editor. Medical Management of Type 1 Diabetes. Fifth ed. Alexandria: American Diabetes Association; 2008.
142. Wright R, Frier B. Vascular disease and diabetes: is hypoglycemia an aggravating factor? Diab Met Res Rev2008;24:353-63.
143. Ho J, Cannaday J, Barlow C, Mitchell T, Cooper K, FitzGerald S. Relation of the number of metabolic syndrome risk factors with all-cause and cardiovascular mortality. Am J Cardiol2008;102:689-92.
144. Russell-Jones D, Khan R. Insulin-associated weight gain-causes, effects and coping strategies. Diabetes Obes Metab2007;9:799-812.
145. Carlson M, Campbell P. Intensive insulin therapy and weight gain in IDDM. Diabetes1993;42:1700-7.
146. Tamborlane W, Ahern J. Implications and results of the Diabetes Control and Complications Trial. Pediatr Clin North Am1997;44:285-300.
147. Otto-Buczkowska E, Mazur-Dworzecka U, Dworzecki T. Role of amylin in glocose homeostasis and its perspective use in diabetes management. 652008;Przegl Lek:135-9.
148. Edelman S, Cabellero L. Amylin replacement therapy in patients with type 1 diabetes. Diabetes Educ2006;32:119S-27S.
149. Kurien V, Yates P, Oliver M. The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion. Eur J Clin Invest1971;1:225-41.

150. Paolisso G, Gualdiero P, Manzella D, Rizzo M, Tagliamonte M, Gambardella A, et al. Association of fasting plasma free fatty acid concentration and frequency of ventricular premature complexes in nonischemic non-insulin dependent diabetic patients. Am J Cardiol1997;80:932-7.
151. Pirro M, Mauriege P, Tchernof A, Cantin B, Dagenais G, Despres J, et al. Plasma free fatty acid levels and risk of ischemic heart disease in men: prospective results from the Quebec Cardiovascular Study. Atherosclerosis2002;160:377-84.
152. Pilz S, Scharnag H, Tiran B, Wellnits B, Seelhorst U, Boehm B, et al. Elevated plasma free fatty acids predict sudden cardiac death: a 6.85-year follow-up of 3315 patients after coronary angiography. European Heart Journal2007;28:2763-9.
153. Mitchell G, Vasan R, Keyes M, Parise H, Wang T, Larson M, et al. Pulse Pressure and Risk of New-Onset Atrial Fibrillation. JAMA2007;297:709-15.
154. Safar M, Smulyan H. Coronary ischemic disease, arterial stiffness, and pulse pressure. American Journal of Hypertension2004;17:724-6.
155. Mittendorfer B. Sexual dimorphism in human lipid metabolism. J Nurtr2005;135:681-6.
156. Mittendorfer B, Horowits J, Klein S. Gender differences in lipid and gluocose kinetics during short-term fasting. Am J Physiol Endocirnol Metab2001;281:E1333-E9.
157. Soeters M, Sauerwein H, Groener J, Aerts J, Ackermans M, Glatz J, et al. Gender-related Differences in the Metabolic Response to Fasting. J Clin Endocrinol Metab2007;92:3646-52.
158. Costacou T, Prince C, Conway B, Orchard T. Progression of coronary artery calcification in type 1 diabetes. Am J Cardiol2007;100:1543-7.
159. Conway B, Wang J, Ediger M, Orchard T. Skin Fluorescence and Type 1 Diabetes Complications: A New Marker of Complication Risk. Diabetes2008;57:A287.
160. Orchard T, Costacou T, Kretowski A, Nesto A. Type 1 Diabetes and Coronary Artery Disease. Diabetes Care2006;29(11):2528-38.
161. Forbes J, Soldatos G, Thomas M. Below the radar: advanced glycation end products that detour "around the side". Is HbA1c not an accurate enough predictor of long term progression and glycemic control in diabetes? Clin Biochem Rev2005;26:123-34.
162. The BMI in Diverse Population Collaborative. Effect of Smoking on the Body Mass Index-Mortality Relationship: Emperical Evidence from 15 Studies. Am J Epidemiol1999;150.
163. Troiano R, Frongillo E, Sobal A, Levitsky D. The relationship of body weight and mortality: a quantitative analysis of combined information from existing studies. Int J Obes Relat Metab Disord1996;20:63-75.
164. Fanelli C, Calderone S, Epifano L, De Vincenzo A, Modarelli F, S. P, et al. Demonstration of a Critical Role for Free Fatty Acids in Mediating Counterregulatory Stimulation of Gluconeogenesis and Suppression of Glucose Utilization in Humans. J Clin Invest1993;92:1617-22.
165. Galassetti P, Neill A, Tate D, Ertl A, Wasserman D, Davis S. Sexual Dimorphism in Counterregulatory Responses to Hypoglycemia after Antecedent Exercise. J Clin Endocrinol Metab2001;86:3516-24.
166. Davis S, Tate D. Effects of Morning Hypoglycemia on Neuroendocrine and Metabolic Responses to Subsequent Afternoon Hypoglycemia in Normal Man. J Clin Endocrinol Metab2001;86:2043-50.
167. Campbell I. Dead in bed syndrome: a new manifestation of nocturnal hypoglycemia? Diabet Med1991;8:3-4.

168. McNally P, Lawrence I, Panerai R, Weston P, Thurston H. Sudden death in type 1 diabetes. Diabetes Obes Metab1999;1:151-8.
169. Roy M, Rendas-Baum R, Skurnick J. Mortality in African Americans with Type 1 Diabetes: he New Jersey 725. Diabet Med2006;23:698-706.