ADIPONECTIN AND RISK FACTORS FOR CARDIOVASCULAR DISEASE AND
TYPE 2 DIABETES IN
BLACK AND WHITE YOUTH

Ann Neighbours Jessup

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Approved by:

Joanne S. Harrell

Shrikant I. Bangdiwala

Catherine E. Champagne

Robert G. McMurray

Anne Skelly

Pamela J. Rowsey

Abstract

Ann Neighbours Jessup

Adiponectin and Risk Factors for Cardiovascular Disease and Type 2 Diabetes in Black and

White Youth

(Under the direction or Joanne S. Harrell, RN, PhD, FAAN, FAHA)

This study was done to examine the relationships among adiponectin and multiple

risk factors for CVD and T2D in a large, racially diverse sample of youth, and to determine if

relationships were moderated by gender, race and overweight status. The sample consisted

of 1215 Black and White children and adolescents, aged 7-18 years, selected from 2211

subjects who participated in the Cardiovascular Health in Children III Study. Subjects who

were selected had frozen serum samples available for analysis, and had complete data on the

following variables: race, age, gender, pubertal stage, cardiovascular fitness, high density

lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride,

insulin, glucose, systolic and diastolic blood pressure, body mass index (BMI), waist

circumference, and sum of skinfolds. SAS 9.1.3 was used for all statistical analyses.

Generalized Estimating Equations were used to account for the increased correlation among

subjects within school clusters.

Adiponectin means were lower in Black subjects and in male subjects overall, and

specifically lower in Black males than in other race/gender groups. Adiponectin means were

also lower in subjects with a family history of diabetes, but there were no differences in

adiponectin means by Tanner stage or fitness level. BMI z-score, waist circumference and sum of skinfolds were each inversely related to adiponectin, but BMI z-score and waist circumference provided the best models for prediction of adiponectin when compared by the Quasi-Likelihood in Independence Model Criterion (QIC). Adiponectin was positively associated with HDL-C and inversely associated with insulin and systolic blood pressure in multivariate regression with other risk factors, but the relationships with insulin and systolic blood pressure were dependent on one or more measures of adiposity. There was an interaction between HDL-C and gender, in that the relationship between adiponectin and HDL-C was stronger in female than in males. Insulin was only related to adiponectin in female subjects.

In summary, BMI z-score and waist circumference are strongly related to adiponectin. Race, gender and HDL-C are consistently related to adiponectin, independent of adiposity measures, but insulin and family history of diabetes are also important variables to consider in the study of adiponectin.

Dedication

Dedicated in Memory of my father, James G. Neighbours and in honor of my mother,

Catherine P. Neighbours.

Acknowledgements

I could have never accomplished this by myself, and I owe a world of gratitude to many people. I thank my daughter Kate for her love and support, and for all the joy she has given me. I am blessed to have such a wonderful daughter. I also want to thank my mother Catherine, for her never-ending love and support over my whole lifetime, and most especially during the last 6 years. I couldn't have done this without her help. And I thank them both for listening to my complaints and enduring all my grouchy moods. I am also grateful for my brother Don and my sister-in-law Dorothea, for all their prayers and support. I am truly blessed – I love you all!

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are truly possible with Him, and I am truly grateful for all He has done. I have been richly

blessed.

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CHAPTER 1

INTRODUCTION

Significance of the Problem

Being overweight or at risk for overweight during youth is a prevalent problem in the

United States, and one that is on the increase in all age groups, from preschoolers to

adolescents (Ogden et al., 2006). Overweight in children is defined as a body mass index

(BMI) greater than or equal to the 95th percentile for age and gender, and being at risk for

overweight is defined as a BMI greater than or equal to the 85th percentile, but less than the

95th percentile (Centers for Disease Control, 2003). Approximately 17% of children and

adolescents aged 2-19 years in the United States are overweight, and another 16.5% are at

risk of overweight according to research with the National Health and Nutrition Examination

(NHANES) data (Ogden et al., 2006); taken together, this means that approximately 34% of

American children and adolescents have a BMI that is of concern in relation to their health.

Overweight has been shown to track from childhood into adulthood, especially if

present in older children (Clarke & Lauer, 1993; Whitaker, Wright, Pepe, Seidel, & Dietz,

1997), and is associated with a multitude of physiological and psychological health risks for

children and adolescents (Castro-Rodriguez, Holberg, Morgan, Wright, & Martinez, 2001;

Davison & Birch, 2001; Fagot-Campagna et al., 2000; Must & Strauss, 1999; Sinha et al.,

2002; Strauss, 2000; Young-Hyman, Schlundt, Herman, De Luca, & Counts, 2001).

Children and adolescents who are overweight also face increased risk of future chronic disease. For example, Steinberger, Moran, Hong, Jacobs and Sinaiko (Steinberger, Moran, Hong, Jacobs, & Sinaiko, 2001) found that elevated BMI's in children as young as 13 years of age were associated with obesity and decreased glucose utilization as young adults, and Srinivasan, Myers and Berenson (Srinivasan, Myers, & Berenson, 2002) noted that overweight in children was linked to the metabolic syndrome in adults. Given the increasing incidence of overweight in children and adolescents and the numerous health risks of overweight during childhood and in later years, it is important that we understand the pathophysiological processes that result from increased adiposity, especially processes that are present even during youth.

Over the last 10 – 15 years, it has become increasingly apparent that adipose tissue is no longer simply a storage site for excess fat. Instead research is showing that adipose tissue is an active contributor of proteins that participate in inflammatory processes that, with chronic over-production, appear to be related to cardiovascular disease (CVD) and type 2 diabetes (T2D) (Lyon, Law, & Hsueh, 2003). Adipocytes, for example, are known to secrete substances such as interleukin-6, tumor necrosis factor alpha (TNF-α), leptin, plasminogen activation inhibitor–1, angiotensinogen, and complement factor 3 (Kershaw & Flier, 2004; Trayhurn & Beattie, 2001). Not all substances produced by adipocytes are, however, pro-inflammatory. One protein, adiponectin, appears to have anti-inflammatory properties that convey protection regarding CVD and T2D.

Adiponectin is secreted by adipocytes, but its production is paradoxically decreased in instances of obesity or overweight (Arita et al., 1999; Hu, Liang, & Spiegelman, 1996;

Scherer, Williams, Fogliano, Baldini, & Lodish, 1995). In research with adults and children, higher adiponectin levels are associated with improved insulin sensitivity (Cnop et al., 2003; Fernandez-Real, Lopez-Bermejo, Casamitjana, & Ricart, 2003; Hulthe, Hulten, & Fagerberg, 2003; Park et al., 2004) and higher high-density lipoprotein (HDL-C) (Pischon et al., 2004). In contrast, lower adiponectin levels are associated with greater levels of adiposity (Bacha, Saad, Gungor, & Arslanian, 2004; Park et al., 2004; Pischon et al., 2004; Stefan et al., 2002), waist circumference (Asayama et al., 2003; Bottner et al., 2004; Steffes et al., 2004), systolic and diastolic blood pressure (BP) (Huang et al., 2003; Iwashima et al., 2004), triglycerides (Gilardini et al., 2006; Pischon et al., 2004; Weiss et al., 2003), and insulin (Bacha et al., 2004; Baratta et al., 2004; Chu, Shen, Wu, & Lai, 2005; Fernandez-Real et al., 2003).

Although a growing number of researchers have studied adiponectin in children and adolescents, some questions remain unanswered. For example, even though racial disparities are known to exist in the prevalence of CVD and T2D, and in the prevalence of related risk factors in adults and children (Brancati, Whelton, Kuller, & Klag, 1996; Cook, Weitzman, Auinger, Nguyen, & Dietz, 2003; Freedman, Khan, Serdula, Ogden, & Dietz, 2006; Haffner et al., 1999; Jago et al., 2006; Mensah, Mokdad, Ford, Greenlund, & Croft, 2005), relatively few researchers have studied the relationships between adiponectin and risk factors for T2D and CVD in a racially diverse sample of children and adolescents. Most of the research that has been done in such samples has focused on the relationship of adiponectin to risk factors such as adiposity or insulin metabolism, and results regarding the impact of race on that relationship have been inconsistent. Moreover, very little research has been done with racially diverse samples to examine relationships between adiponectin and lipids, or blood pressure, family history or CVD or diabetes, physical fitness or activity.

Likewise, little research has been done to examine the possibility that factors such as race, gender and overweight status may influence or moderate the relationship between adiponectin and risk factors for CVD and T2D, although a few studies in adults and children seem to point to this. A moderator is a "variable that affects the direction and/or strength of the relation between an independent or predictor variable and a dependent or criterion variable." (Baron & Kenny, 1986). Research in adults has shown that the relationships between adiponectin and certain risk factors for CVD and T2D may be moderated by race (Ferris et al., 2005; Hulver, Saleh, MacDonald, Pories, & Barakat, 2004). This issue has not been examined in children and adolescents. A few research studies in children (Chu et al., 2005; Okada et al., 2005) have shown that relationships between adiponectin and lipids or insulin may be moderated by gender, showing significance in females only, or indicating that changes in adiponectin across pubertal stages are dependent on gender and overweight status alike (Woo, Dolan, Daniels, Goodman, & Martin, 2005). Likewise, a few studies in adults and children have shown that the level of overweight category may influence the relationship between adiponectin and lipids or insulin (Butte et al., 2005; Kantartzis et al., 2005; Kantartzis et al., 2006; Martin, Woo, Daniels, Goodman, & Dolan, 2005). Research to clarify the possible moderation of relationships between adiponectin and risk factors for CVD and T2D by race, gender and overweight status is needed. An increased understanding of how the relationships between adiponectin and risk factors may differ in males versus females, Black versus White, or overweight versus lean youth will be helpful in planning future research with these groups.

Purpose of the Study

This study will provide an opportunity to explore the relationships between adiponectin and multiple risk factors for CVD and T2D in a large, racially diverse sample of children and adolescents, and to determine if relationships are moderated by gender, race and overweight status. The contribution of clinically relevant measures of adiposity in youth to adiponectin levels will also be explored. This study will also be the first to provide an examination of the relationship between the different molecular weight forms of adiponectin and risk factors for CVD and T2D in children and adolescents. A conceptual framework illustrating the proposed relationships between adiponectin and the risk factors is presented in the following section.

Overview of Conceptual Model and Variables

At this point in time, there is not an existing framework in the literature that specifically links all the variables I examined in this study. Some models propose links between some of the variables, such as the one shown in figure 1 (Lutgendorf & Costanzo, 2003). This model is very broad, and encompasses the integration of pychosocial, biological (including immune mechanisms and cytokines) and behavioral factors as they relate to disease and quality of life. It does not include obesity per se, but does include associated behaviors such as diet and exercise. I am using a more focused biologically plausible framework for the relationships between adiponectin and various risk factors for T2D and CVD in children and adolescents in this study. Hill (1965), in his explanation of criteria for causality, states "What is biologically plausible depends upon the biological knowledge of the day." (p.298). Hill's statement is especially applicable today regarding the study of adiponectin and its relationship with risk factors for T2D and CVD, as knowledge in this area is rapidly expanding. Figure 2 provides an illustration of the physiological links by which

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Figure 1

Biopsychosocial Model
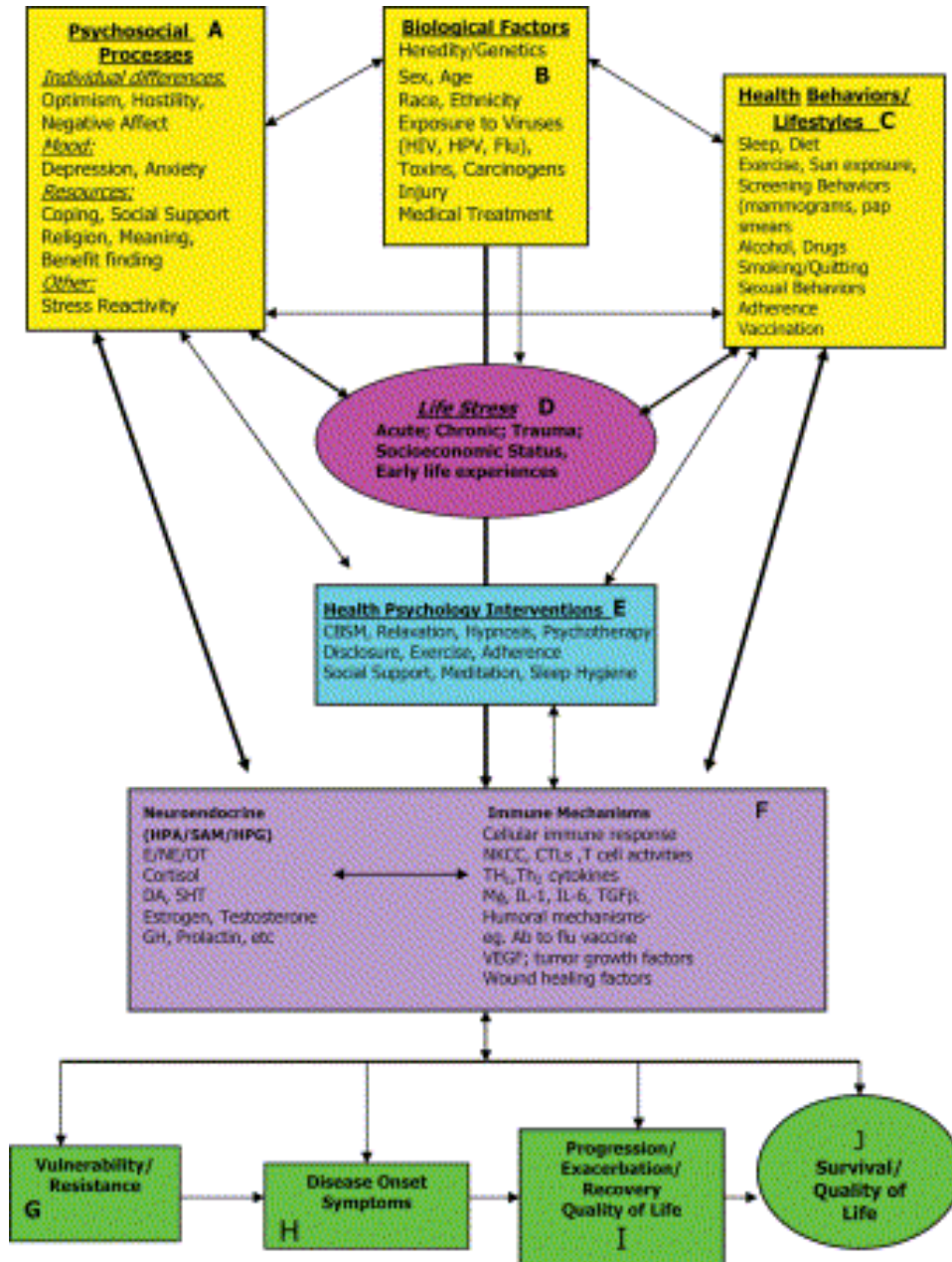
Taken from:
Lugendorf, S.K. & Costanzo, E.S. (2003). Psychoneuroimmunology and health
psychology: an integrative model. Brain, Behavior and Immunity, 17, 225-232.

Figure 2

Actions of Adiponectin

Adiponectin

↑ Nitric Oxide

↓ NF-κB actions

↓ action of adhesion molecules (VCAM-1 & ICAM-1)

↓ formation of foam cells

↓ actions of matrix metalloproteinase-1

Muscle Tissue

Liver

↑ Vasodilation

↑ uptake of glucose

↑ fatty acid oxidation

↓ gluconeogenesis

↓ Blood Pressure

↑ glucose utilization

↓ triglyceride

Decreased Risk for Type 2 Diabetes and Cardiovascular Disease
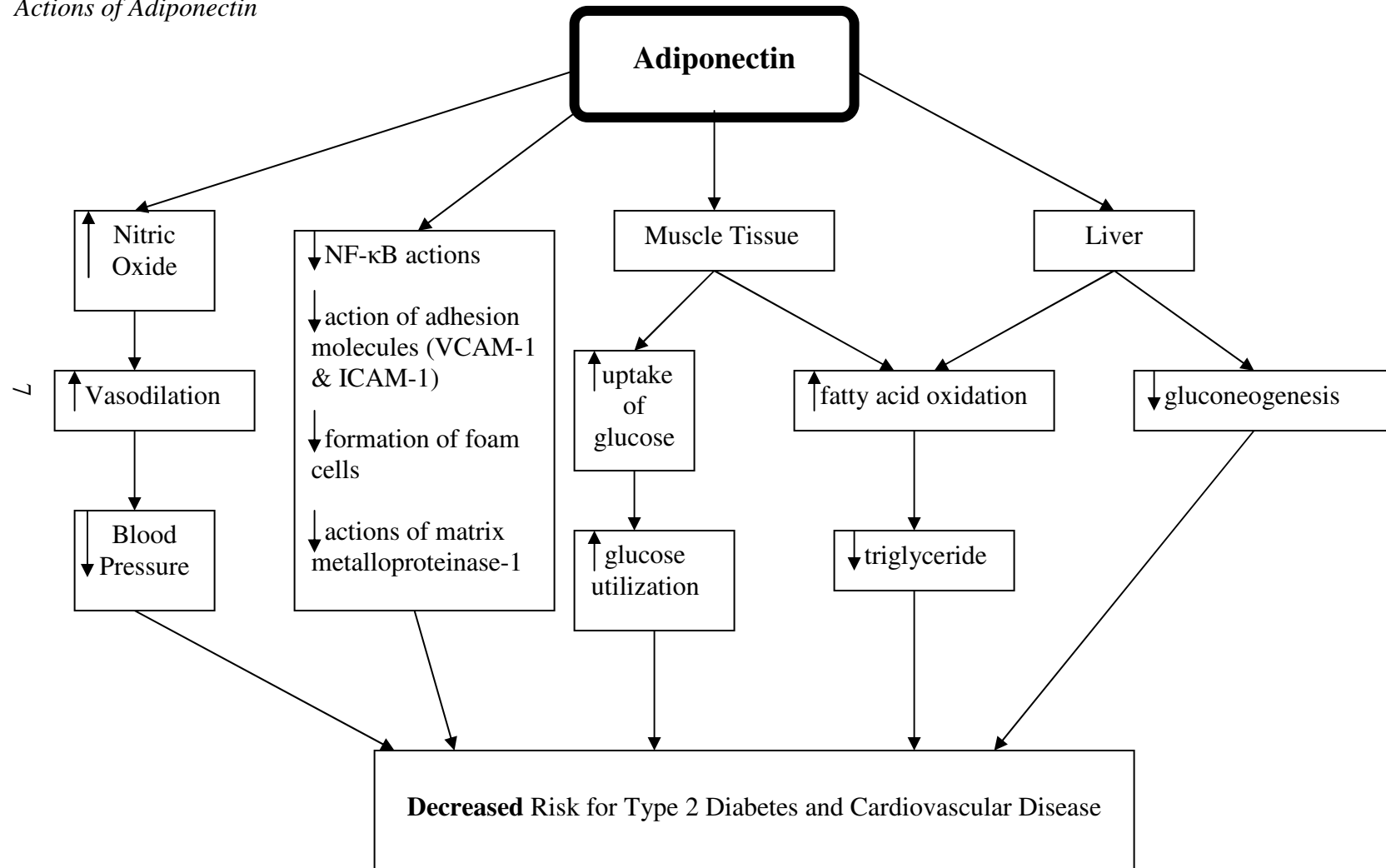
Figure 3

Basic Conceptual Framework Linking Adiponectin and Variables for Dissertation

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Pubertal Stage	Race Gender Overweight Status by BMI	Insulin
Family History		Glucose
Age		LDL Cholesterol
Physical Fitness	Adiponectin	HDL Cholesterol
BMI		Triglycerides
Waist Circumference		Blood Pressure
Sum of Skinfolds		
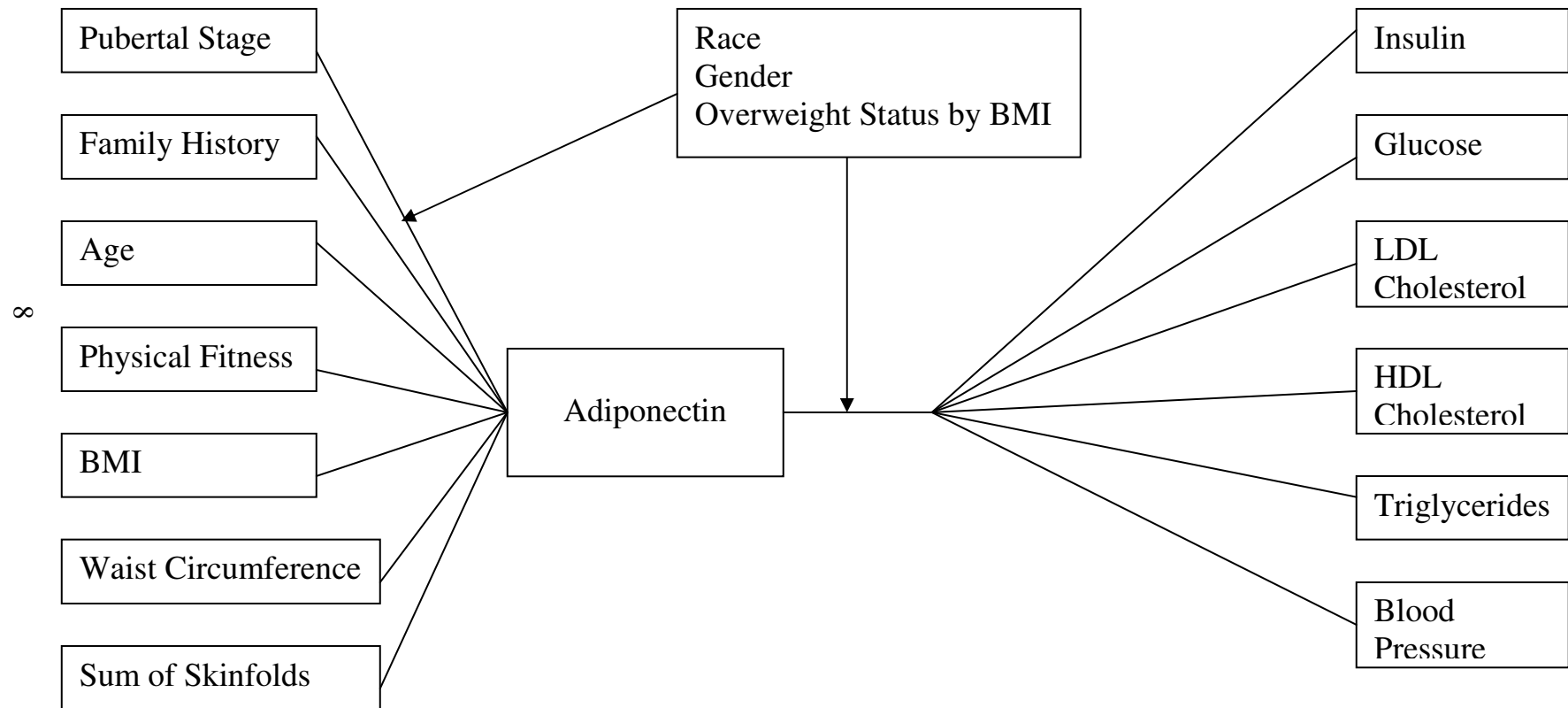
adiponectin is thought to affect risk factors associated with CVD and T2D. A conceptual

framework for the current study, based on information from research with adults and children,

may be found in figure 3.

The model in figure 3 illustrates links between adiponectin and variables that reflect

risk factors for CVD and T2D. Measures of obesity such as body mass index (BMI), waist

circumference and sum of skinfolds, demographic variables such as gender, race, age, and

pubertal status are depicted as being related to adiponectin in the framework. In addition,

cardiovascular fitness, as measured by VO_2 level is included in the model as possibly being

associated with adiponectin. Variables that reflect other risk factors for CVD and T2D such

as insulin, glucose, total cholesterol, HDL-C, LDL-C, triglyceride and blood pressure, are

depicted as being related to adiponectin. Lastly, overweight status, race and gender are

depicted as being possible moderators of the relationship between adiponectin and risk factors

for T2D and CVD. The race and gender variables each reflect two subject groups, Black and

White, and male and female, respectively. The overweight status variable reflects three

subject groups, as defined by the Centers for Disease Control (CDC, 2003) guidelines, of

normal weight, at risk for overweight and overweight. The choice of each variable and its

placement in the model in relation to adiponectin can be supported by previous research that

has indicated associations in adults or youth. These relationships will be discussed in further

detail in Chapter two.

Research Aims and Questions

This study seeks to explore the relationships among adiponectin and variables

reflecting demographic factors and risk factors for CVD and T2D in Black and White youth.

Data regarding these variables were collected from a large, racially diverse sample of children

and adolescents in the 4th and 5th cohorts of the Cardiovascular Health in Children III study (CHIC III). This data will be used in analyses to answer the research questions associated with the following research aims.

Aim 1. To examine the differences in mean adiponectin levels by demographic factors (gender, race, age, family history) and pubertal stage.

Question 1a
    Do adiponectin levels differ by the demographic factors of gender, race (non-Hispanic Black and White), age group (> 13 years, and ≥ 13 years), and family history of CVD or diabetes?

Question 1b
    Does adiponectin differ by pubertal stages I-V, controlling for age, gender or race, and does this relationship differ by race, gender or overweight status?

Aim 2. To determine which of three different measures of adiposity is the best predictor of adiponectin levels.

Question 2a
    Of BMI, waist circumference and sum of skinfolds, which measure of adiposity is the best predictor of adiponectin levels, when controlling for demographic factors and pubertal stage?

Aim 3. To examine the relationship between adiponectin and risk factors for CVD and T2D, and to determine if the relationship is moderated by race, gender or overweight status.

Question 3a
    Do mean adiponectin levels differ by risk levels for the following risk factors for CVD and T2D, when controlling for demographic factors: HDL-C, glucose, insulin, total cholesterol, LDL-C, triglycerides, systolic or diastolic BP, and level of fitness?

Question 3b
    What is the relationship of adiponectin to the following risk factors for CVD and T2D (HDL-C, LDL-C, triglyceride, total cholesterol, glucose, insulin, BP and fitness level)

when operationalized as continuous variables, when controlling for demographic factors

or measures of adiposity?

Question 3c

Do the relationships of adiponectin to the risk factors in question3b differ by race (non-

Hispanic Black or White), gender or overweight status?

CHAPTER 2

REVIEW OF LITERATURE

Results of research over the last 10 to 15 years show cells within adipose tissue to be active contributors of proteins that participate in inflammatory processes. With chronic over-production, these proteins appear to be related to CVD and T2D (Lyon et al., 2003). In contrast, one protein called adiponectin that is secreted by adipocytes appears to have anti-inflammatory properties that convey protective properties regarding CVD and T2D. Adiponectin is secreted by adipocytes, but its production is paradoxically decreased in instances of obesity or overweight (Arita et al., 1999; Hu et al., 1996; Scherer et al., 1995). High adiponectin levels are related to lower levels of many of the risk factors related to CVD and T2D (Asayama et al., 2003; Bacha et al., 2004; Bottner et al., 2004; Chu et al., 2005; Cnop et al., 2003; Gilardini et al., 2006; Huang et al., 2003; Pischon et al., 2004). This literature review will describe current knowledge of the connections between the inflammatory process and the effects of increased adipose tissue in relation to CVD and T2D, as well as the structure and anti-inflammatory functions of adiponectin. The relationship between adiponectin and risk factors for CVD and T2D in children and adolescents will also be discussed.

Inflammation

Introduction to the Inflammatory Process

Knowledge of the effects of a chronic inflammatory process on illnesses such as cardiovascular disease (CVD) and type 2 diabetes (T2D) has greatly increased over the last 30 to 40 years (Pickup & Crook, 1998; Ross, 1993). In contrast, the process of acute inflammation as a response to injury or insult has been studied for 2000 or more years. In the first century AD a physician by the name of Celsius described four signs of inflammation: redness, swelling, heat and pain; the fifth sign (loss of function) was added later that same century by Galen (Cone, 2001). The inflammatory process leading to these signs generally results in destruction of an organism that poses an injury or threat to the body. The process continues with removal of the offending organism, along with damaged or necrotic cells, by phagocytosis, and ends with tissue repair.

The body has three lines of defense against the injury or threat caused by foreign substances or microorganisms(Delves & Roitt, 2000). The skin provides a barrier as the first level, and if that barrier fails, the innate and adaptive immune systems interact to respond to the threat to homeostasis. The innate immune system responds quickly to recognition of certain pathogens with cells designed for phagocytosis, secretion of inflammatory substances such as cytokines and leukotrienes, acute phase proteins and complement, and with natural killer cells that are designed to target and kill the offending pathogens. Pattern recognition allows the innate immune system to function swiftly, without need of memory, and to respond in the same manner each time it is presented with a threat. Macrophage and dendritic cells, equipped with cell surface receptors, recognize certain pathogen-associated

molecular patterns of foreign and potentially toxic organisms and proceed to activate the innate and adaptive immune response (Medzhitov & Janeway, 2000).

The activity of cytokines such as IL-1, IL-6 and TNF- α, that are involved in the innate immune response to an acute injury, results in the release of chemo attractant substances that attract other immune cells, thereby perpetuating the cycle of inflammation. These inflammatory cytokines "operate both as a cascade and as a network in stimulating the production of acute-phase proteins." (Gabay & Kushner, 1999, p.449). Many acute phase proteins are complement components, that is proteins that are involved in the destruction of bacteria and preparation for its subsequent phagocytosis. Others are involved in coagulation and fibrinolysis. Some proteins, such as C-reactive protein (CRP), plasminogen activation inhibitor-1 and serum amyloid A, are elevated during the inflammatory process, and other proteins such as transferrin are decreased. The acute phase response also includes systemic signs and symptoms such as fever and fatigue or biological changes such as anemia or decreased lipolysis. Gabay and Kushner (1999) refer to the process of the acute phase response as a "complex, highly orchestrated process" and note that some of the molecules involved in the acute phase have pro-inflammatory and destructive actions towards foreign organisms, whereas others are involved in anti-inflammatory and healing actions. Fibrinogen is an example of an acute phase protein that is necessary for wound healing and resolution.

During activation of the acute inflammatory response, complement and chemo attractant molecules signal other immune system cells such as neutrophils. In addition, part of the complement system triggers the release of histamine by mast cells, causing increased vascular permeability at the site of insult or injury. The neutrophils are attracted to the vascular endothelium by cellular adhesion molecules, and roll along the surface of the

endothelium until stopping and becoming fixed to the vessel wall (Steeber, Venturi, & Tedder, 2005). The cells then pass through the permeable vessel wall and move along a chemotactic gradient to the infection site where offending cells are phagocytosed. More inflammatory mediators and chemoattractants are secreted and the process continues until the foreign micro-organisms are destroyed, then a process of tissue healing and resolution begins. Although the inflammatory process is intended to be a benefit to the body overall, there are times when the process results in harmful consequences; examples would be autoimmune diseases like lupus or rheumatoid arthritis, or chronic disease such as CVD or T2D.

Cardiovascular Disease and Inflammation

The pathogenesis of CVD is now believed to be an inflammatory process (Berg & Scherer, 2005; Ross, 1999). A statement by the American Heart Association and CDC notes that, "Virtually every step in atherogenesis is believed to involve cytokines, other bioactive molecules, and cells characteristic of inflammation" (Pearson et al., 2003, p.500). Libby and Aikawa (2002) describe the change in thinking of atherosclerosis as being solely due to a blockage of arteries to an inflammatory process, as "a shift from hydraulics to biology" (p.1257). An example of this shift is the recent recommendation by the American Heart Association for use of the acute phase protein CRP as a clinical screening tool for CVD (Pearson et al., 2003).

The initial fatty streak lesions of atherosclerosis, commonly found in adolescents and young adults, begin when monocytes, t-lymphocytes and platelets are attracted to an area of insult to the artery wall, consistent with the body's usual acute inflammatory response to injury (Li & Glass, 2002; McGill et al., 2000). Monocytes transform into macrophages and

"contribute to the local inflammatory responses through the production of cytokines, free oxygen radicals, proteases and complement factors" (Linton & Fazio, 2003, p.S35). Macrophages are known to release cytokines such as Il-1 and TNF- α (Nathan, 1987). These cytokines and chemokines recruit even more monocytes to the area, extending the cycle of inflammation.

The scavenger receptors found on macrophages take in esterified cholesterol, causing the macrophages to transform into foam cells that become larger and larger as they incorporate the cholesterol. This accumulation of foam cells becomes the fatty streak lesion. As the atherosclerotic lesion grows over time, a necrotic core of cells forms and is covered by a fibrin cap. This fibrin cap is normally a protective mechanism, serving as a barrier between the inner necrotic core and the circulation. In some lesions, however, the fibrin cap is thin and weak. Under the influence of matrix metalloproteinases (Newby, 2005), the weakened cap can break down and rupture, allowing release of the contents into the circulation. At this point, platelets are recruited and a thrombus is formed, which may lead to infarction and subsequent tissue damage (Li & Glass, 2002). In summary, the inflammatory process, designed to fight insults related to infection and injury, can have "unintended consequences" in the cardiovascular system (Lodish et al., 2004, p.754).

Increased levels of inflammatory markers have been associated with increased incidence of CVD in several large prospective studies with men and women. (Ridker, Cushman, Stampfer, Tracy, & Hennekens, 1997) studied the risk of future myocardial infarction (MI) and its relation to baseline levels of high sensitivity C-reactive protein (hs-CRP) in a large group of men who participated in the Physicians' Health Study. They found that men who were in the highest quartile of hs-CRP measurement were 2.9 times as likely to

have an MI as men who were in the lowest quartile. The relative risk (RR) was only slightly

lower, at 2.6, when the model was adjusted for BMI, diabetes, history of hypertension, and

family history of premature coronary artery disease. This group of men with the highest

levels of hs-CRP was also 1.9 times as likely to have a stroke. The relationship of hs-CRP

and risk of MI has also been studied in a large epidemiological sample of healthy post-

menopausal women (Ridker, Hennekens, Buring, & Rifai, 2000). Women who were in the

highest quartile of hs-CRP were 4.4 times as likely to have an MI as those in the lowest

quartile. This RR dropped to 1.5, but was still significant, when the model was adjusted for

the other CVD risk factors mentioned above and other markers of inflammation and

dyslipidemia. The risk of having an MI was also greater for the group of women whose

LDL-C was less than 130 if they were in the highest versus the lowest quartile for hs-CRP

(RR=3.1).

Studies have also been conducted on data obtained from subjects who participated in

the Physicians' Health Study to examine the relationship between MI and other inflammatory

markers. (Ridker, Rifai, Stampfer, & Hennekens, 2000) found an increased RR (2.3) for MI

in men who were in the highest versus the lowest quartile for interleukin-6 levels. Subjects

were matched for age and smoking habits, and the models were adjusted for other CVD risk

factors. Lastly, (Ma, Hennekens, Ridker, & Stampfer, 1999) noted that men who were in the

highest decile for fibrinogen levels were 2.09 times as likely to have an MI; the RR was 2.02

when adjusted for other CVD risk factors.

Type 2 Diabetes and Inflammation

Researchers also believe that inflammation contributes to insulin resistance and T2D

(Pickup, 2004). A well known pro-inflammatory cytokine, tumor necrosis-alpha (TNF- α), is

thought to participate in the pathophysiology of T2D by interfering with insulin signaling (Rask-Madsen et al., 2003). In the process of normal signaling, insulin binds to an insulin receptor at the cell's surface. A complicated signaling pathway is triggered, beginning with phosphorylation of tyrosine kinase at the receptor and ending with movement of the glucose transporter GLUT- 4 to the cell surface where it facilitates the entry of glucose into the cell. When there is a pathological process in place, TNF- α activates serine phosphorylation of insulin receptor substrate-1 (IRS-1) rather than tyrosine, which leads to a blunting of normal insulin signaling (Hotamisligil, 2003; Shepherd & Kahn, 1999).

Nuclear factor-kappa B (NF-κB), a potent nuclear transcription factor that up-regulates many pro-inflammatory cytokines, has also been found to influence insulin resistance (Shoelson, Lee, & Yuan, 2003). Moreover, research has shown that intervention with medications typically used for anti-inflammatory effect can decrease the effects of NF-κB on insulin resistance (Yuan et al., 2001). Yuan and associates found that administration of high doses of salicylates to obese, insulin resistant rodents led to a decrease in the activity of IkB kinase beta, the substance responsible for activation of NF-κB. As a result, insulin resistance was reduced. Reductions in blood glucose and lipids with administration of high dose aspirin have also been demonstrated in recent research with human subjects with type 2 diabetes, but the authors expressed concerns about the side effects of large doses of aspirin (Hundal et al., 2002). Research continues at the Joslin Diabetes Center with administration of salsalates, drugs that are similar to aspirin but have fewer side effects (retrieved 3/03/2006 from: http://www.joslin.org/1148_2696.asp). Administration of such drugs to people with diabetes has resulted in a decrease in blood glucose.

Descriptive epidemiological studies have shown that other inflammatory markers such as the pro-inflammatory cytokine Interleukin-6 (IL-6) and C-reactive protein (CRP) also predict T2D (Duncan et al., 2003; Pradhan, Manson, Rifai, Buring, & Ridker, 2001). Duncan et al. (2003) found that subjects in the highest quartile of IL-6 had a 65% greater chance of developing T2D than subjects in the lowest quartile even after adjusting for BMI, family history of T2D, glucose, insulin, age, sex and ethnicity. (Pradhan et al., 2001) concluded that the relative risks of developing T2D were 2.9 and 4.4 for subjects in the highest quartiles of IL-6 and CRP, respectively, even when controlling for BMI.

Adipose Tissue, Inflammation and Obesity

Although the pathways leading from inflammation to CVD and T2D have not been fully delineated, it is now believed that connections exist between obesity and the inflammatory processes that lead to atherosclerosis and CVD, as well as to the insulin resistance that contributes to both T2D and CVD (Trayhurn & Beattie, 2001). Obesity, a common risk factor for both diseases, may play a role because of the numerous cytokines secreted by excess adipose tissue.

Adipose Tissue

There are two known types of adipose tissue, brown and White. Brown adipose tissue is found primarily in neonates, and is so named because of the appearance of the numerous mitochondria found within its cells when stained (Frayn, 2003). The uncoupling process that takes place within the mitochondria generates heat for young infants. This process for heat production is not as important to homeostasis at later ages, and White

adipose tissue is the primary type of adipose tissue found in adults (Frayn, 2003). This focus

for this paper is adiponectin, a protein produced by cells within White adipose tissue.

White adipose tissue comprises more than just adipocytes, or fat cells. The tissue also

contains lymph nodes, blood vessels, nerves and stromal vascular cells that include pre-

adipocytes awaiting differentiation to mature cells. (Ailhaud, Grimaldi, & Negrel, 1992;

Hausman, DiGirolamo, Bartness, Hausman, & Martin, 2001). In turn, the functions of

adipose tissue in relation to metabolism are integrated with the circulatory and nervous

systems (Frayn, Karpe, Fielding, Macdonald, & Coppack, 2003). For example, delivery of

hormones that regulate metabolism and substrates for enzyme action is dependent on

adequate blood flow through adipose tissue, and signals from the autonomic nervous system

trigger the lipolysis that releases fatty acids for energy use. The main component of adipose

tissue, in regard to production of adiponectin is the adipocyte.

Adipocytes begin their lives as adipocyte precursors derived from stem cells, then

progress through differentiation from pre-adipocytes to mature adipocytes under the

influence of transcription factors such as peroxisome proliferators-activated receptor-gamma

(PPARγ) and CCAAT/enhancer binding protein- α (C/EBPα) (Gregoire, 2001). Over the

human life span adipocytes may increase in size (hypertrophy) and or number (hyperplasia)

(Hausman et al., 2001). Hypertrophy occurs in situations of positive energy balance, ie, from

taking in more calories than are used. During such times and during normal storage for

future energy needs, triglycerides from very low density lipoprotein (VLDL) particles are

transferred to adipocytes for storage (Frayn, 2003). This process is facilitated by the action

of lipoprotein lipase, an enzyme that breaks down the triglyceride to allow it to enter the

adipocytes. The action of lipoprotein lipase is triggered by insulin, which is increased after

eating. When fatty acids are needed for energy, their release from adipocytes is facilitated by another enzyme, known as hormone sensitive lipase (Frayn, 2003).

Mechanisms that lead to adipocyte hyperplasia, however, are not fully understood. Some substances, such as insulin and corticosteroids, are known to increase differentiation of adipocytes or hyperplasia, and others such as TNF-alpha decrease it (Prins & O'Rahilly, 1997). Some authors suggest adipocyte hyperplasia follows attainment of a "critical size" (Hausman et al., 2001 p. 248) in neighboring adipocytes. They propose that the large adipocytes secrete substances that induce differentiation of pre-adipocytes to adipocytes, resulting in hyperplasia. Along the same line of thought, Levine, Jensen, Eberhardt and O'Brien (1998) found that expression of macrophage colony-stimulating factor (MCSF) was upregulated in adipocytes of subjects who gained weight by overfeeding, and also noted that injection of human MCSF into rabbit adipose tissue stimulated adipocyte hyperplasia.

Obesity and Inflammation

White adipose tissue has been described in recent years as a "secretory" or "endocrine" organ (Trayhurn & Beattie, 2001 p. 329); that is, adipose tissue produces various proteins that have endocrine or in some cases, autocrine or paracrine functions.   Adipose tissue is no longer considered to be simply a depot for storage of fat. Proteins and cytokines secreted by White adipose tissue are referred to as adipocytokines, a reflection of their function and site of origin (Fasshauer & Paschke, 2003; Nemet et al., 2003; Ouchi, Kihara, Funahashi, Matsuzawa, & Walsh, 2003). Examples of these adipocytokines are interleukin-6 (IL-6), TNF- α, adiponectin, leptin, resistin, plasminogen activation inhibitor–1, angiotensinogen, complement 3, and serum amyloid A (Kershaw & Flier, 2004; Trayhurn & Beattie, 2001). Most of these proteins are normal components of the innate immune

response, but in conditions of increased adiposity are thought to contribute to a chronic "pro-inflammatory milieu" (Lyon et al., 2003) that may lead to endothelial dysfunction and insulin resistance, and eventually to T2D and CVD.

For example, Vozarova, Weyer, Hanson Tataranni, Bogardus and Pratley (2001)(2001) noted that IL-6 levels were higher in adult subjects with greater body fat. In another study, men with higher BMIs had higher fibrinogen levels and White blood cell counts, a further indication of the link of obesity with inflammation (Church et al., 2002). CRP and White blood cells are higher in overweight children as well (Visser, Bouter, McQuillan, Wener, & Harris, 2001). In addition, Berbeoglu (2001) found that TNF alpha levels were greater in a group of obese children when compared to controls, and Nemet, et al. (2003) noted positive correlations between TNF- α and IL-6, and BMI, as well as with body fat as measured by DEXA.

Early research focused mostly on secretion of adipocytokines by adipocytes, but more recent research suggests that many of these proteins are secreted by the non-fat cells in adipose tissue, such as the stromal vascular cells or connective tissue. For instance, Fain, Bahouth and Madan (2005) found that only 5% of TNF- α was secreted by adipocytes separated from adipose tissue that was removed from obese women who were hospitalized for gastric bypass surgery. The remaining 95% came from stromal vascular cells and "matrix" (p.616), a mixture of connective tissue, pre-adipocytes and blood vessels. Similarly, more IL-6, PAI-1, and resistin (Fain, Cheema, Bahouth, & Lloyd Hiler, 2003) was secreted from matrix tissue than from adipocytes. Leptin was secreted primarily from adipocytes. The authors stated that no correction was done for adipocytes that may have been broken in the process of separation from matrix tissue, but the research is still intriguing.

In addition to adipocytes and other cell types, recent research with mice and with children has indicated that macrophages are present in adipose tissue, with an increased number of macrophages in obese subjects (Sbarbati et al., 2006; Weisberg et al., 2003; H. Xu et al., 2003); this may account for some of the increase in inflammation with obesity. One research group (Charriere et al., 2003) demonstrated that pre-adipocytes actually took on phagocytic properties when injected into the peritoneal cavity of mice. In recent research with children, Sbarbati et al (2006), found evidence of "inflammatory lesions" (p.221) in samples of adipose tissue taken from 19 obese children. Microscopic evaluation of the tissue showed the presence of lesions the authors described as "microgranuloma surrounding the debris of adipocytes" (p.221). These lesions were accompanied by macrophages and involved approximately 6.8% of the total tissue. No such lesions were noted in tissue collected from 5 normal weight control children. Although the sample was small, and the control group appeared to be slightly younger (authors did not mention if age was significantly different between groups), the results are interesting, as macrophages are known to signal the release of cytokines.

Although many adipocytokines are pro-inflammatory, some have opposite, or anti-inflammatory actions. Adiponectin is an adipocytokine with anti-inflammatory properties, and it appears that higher levels protect against atherosclerosis and insulin resistance (Furukawa et al., 2004; Ouchi et al., 2001; Ouchi et al., 2003; Ukkola & Santaniemi, 2002).

Adiponectin

Introduction

Adiponectin is a protein produced primarily by adipocytes, but its production is decreased in obese or overweight subjects (Arita et al., 1999; Hu et al., 1996; Scherer et al., 1995). Research over the last 10 years indicates that adiponectin functions in an anti-inflammatory capacity, decreasing risk for T2D and CVD (Trujillo & Scherer, 2005). Receptors for adiponectin have been located in skeletal muscle cells, pancreatic β-cells, and vascular smooth muscle and endothelial cells (Chinetti, Zawadski, Fruchart, & Staels, 2004; Civitarese et al., 2004; Kharroubi, Rasschaert, Eizirik, & Cnop, 2003). Adiponectin is positively correlated with protective factors such as HDL-C, and inversely related to many of the negative risk factors related to CVD and T2D such as increased adiposity, triglycerides, blood pressure and insulin levels in adults and youth (Asayama et al., 2003; Bacha et al., 2004; Bottner et al., 2004; Chu et al., 2005; Cnop et al., 2003; Gilardini et al., 2006; Huang et al., 2003; Pischon et al., 2004).

Structure of Adiponectin and its Receptors

Multimeric Structure of Adiponectin

Adiponectin was discovered approximately ten years ago by four separate research groups, and was assigned a different name by each of the four groups. Scherer, Williams, Fogliano, Baldini and Lodish (1995) were the first to describe the "adipocyte complement-related protein of 30 kDa (Acrp30)" (p.26746). Three other groups soon followed with descriptions of a protein resembling complement C1q and produced by adipocytes. Names given to the newly discovered protein were AdipoQ (Hu et al., 1996), "Adipose Most

Abundant Gene Transcript 1 (apM1)" (Maeda et al., 1996 p. 286), or "gel binding protein of 28 kDa (GBP28)" (Nakano et al., 1996 p. 803). The discoveries of the four research groups were soon recognized to be a single protein, known today as adiponectin.

Adiponectin is a protein composed of 244 amino acids (Maeda et al., 1996) with a molecular mass of 30 kDa (Scherer et al., 1995). The structure of adiponectin is similar to that of collagen VIII and of C1q, a component of the classical complement pathway (Arita et al., 1999). Similar to C1q, the basic unit structure of the adiponectin monomer resembles a molecule with a globular head, and attached tail (Scherer et al., 1995). Three individual monomers join to form homotrimers which further bind together by covalent disulfide bonds to form configurations of low molecular weight (LMW) hexamers or higher molecular weight (HMW) formations of up to six trimers (Nakano et al., 1996; Pajvani et al., 2003; Scherer et al., 1995; Tsao et al., 2003). This multimeric structure is illustrated in Figure 4 (Whitehead, Richards, Hickman, Macdonald, & Prins, 2006). Adiponectin was also found to circulate in globular as well as full length forms in studies by Fruebis et al. (2001). Lastly, in addition to similarities with C1q, adiponectin also bears resemblance to the TNF- α "super family," a group of substances that have a similar structure and functions (Kishore et al., 2004). Kishore and associates postulated that the similarities to the structure of C1q and TNF- α suggest "divergence from a common precursor molecule of the innate immune system." (p.556), supporting its place as a participant in immune system functions.

Production of Adiponectin

Adiponectin is produced primarily by adipocytes (Hu et al., 1996; Scherer et al., 1995), and was found to be the most abundant protein gene transcript to be produced in adipose tissue (Maeda et al., 1996). More specifically, Korner et al. (2005) found that

Figure 4

Multimeric Structure of Adiponectin

MONOMER TRIMER HEXAMER

HMW MULTIMER

LMW (2-7) HMW (8-11)

2 3 4 5 6 7 8 9 10 11

Fraction number
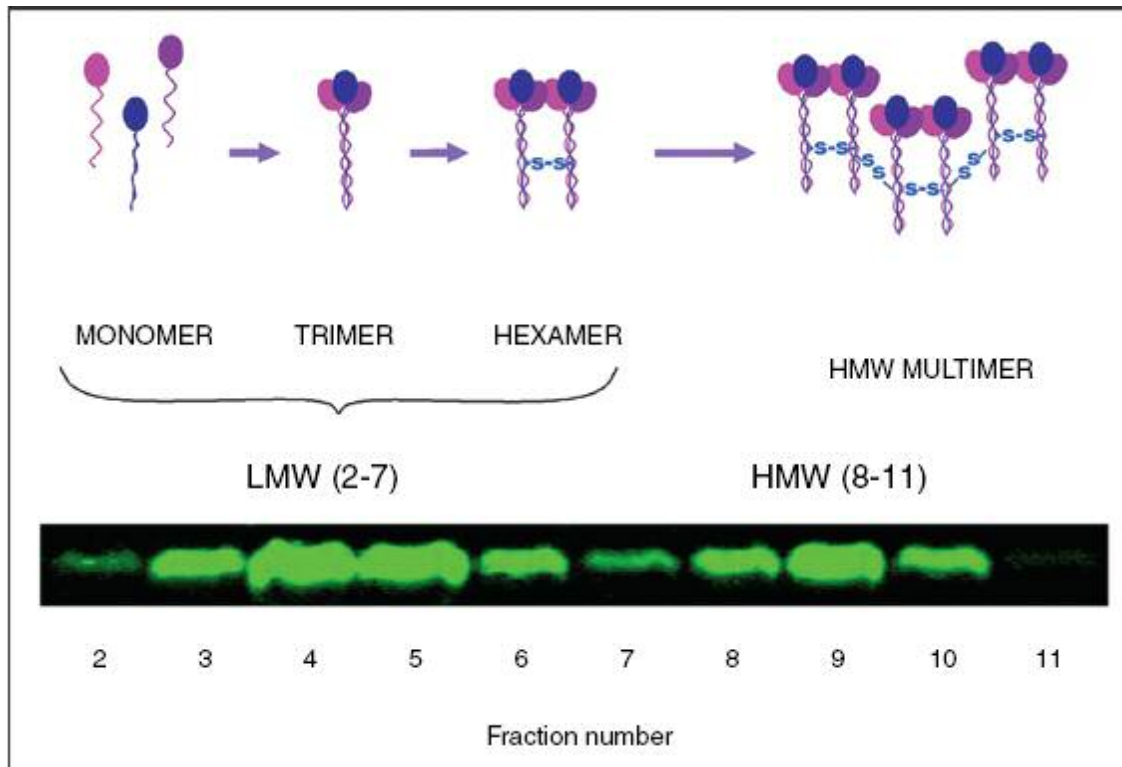
Taken from: Whitehead, J.P., Richards, A.A., Hickman, I.J., McDonald, G.A. & Prins, J.B.
(2006). Adiponectin – a key adipokine in the metabolic syndrome. Diabetes, Obesity and
Metabolism, 8, 264-280.

adiponectin was only produced by adipocytes that had begun the process of differentiation to

mature adipocytes; pre-adipocytes were not noted to produce adiponectin. Although the

early research mentioned above indicated that adiponectin was produced solely by

adipocytes, more recent research has indicated that it is also produced by other cells

(Berner et al., 2004; Katsiougiannis, Kapsogeorgou, Manoussakis, & Skopouli, 2006; Pineiro

et al., 2005).The amount produced, however, appears to be less than that produced by

adipocytes. Berner et al. (2004) found that adiponectin was produced by osteoblasts taken

from human fibulas and tibias, but at only "3% of the level found in human subcutaneous

adipose tissue" (p. 846) that was used as a positive control. Moreover, the authors noted an

increase in bone cell proliferation when osteoblasts were incubated with adiponectin. The

authors speculated that adiponectin may be related to bone growth as well as to functions

related to metabolism. Adiponectin was also found to be produced in higher amounts by

salivary gland epithelial cells of patients with Sjögrens Syndrome than in patients without the

disease (Katsiougiannis et al., 2006), and by human cardiomyocytes (Pineiro et al., 2005);

the amount produced by the different types of cells was again not equal to the amount

typically produced by adipocytes. The authors of these studies proposed that adiponectin

produced at these sites might be used in an autocrine or paracrine manner at the local area for

activities related to the immune system or cell metabolism. At this point in time it appears

that adiponectin is produced in the highest amount by adipocytes, but clearly the science

related to the overall production and function of adiponectin is a complex and changing

issue.

Adiponectin Receptors

The comprehensive research of Yamauchi et al. (2003) was the first to provide information on the receptors that interact with adiponectin. This group reported cloning two distinct receptors, referred to as Adiponectin Receptor 1 (AdipoR1) and Adiponectin Receptor 2 (AdipoR2), and described them as "integral membrane proteins" (p.766). AdipoR1 was found to be ubiquitously expressed, but was expressed to a higher degree in skeletal muscle cells; this was also noted in later research by Chen et al. (2005). AdipoR2 was expressed mostly by liver cells. Each type of receptor was shown to bind globular as well as full length adiponectin, but AdipoR1 had a higher affinity for globular adiponectin; AdipoR2 bound both globular and full length adiponectin at an intermediate level. In the study by Yamauchi et al. (2003), both AdipoR1 and AdipoR2 facilitated adiponectin actions such as activation of 5-AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor alpha (PPAR-α), and stimulation of fatty acid oxidation. Other research groups have also investigated adiponectin receptors since the original work by Yamauchi et al. (2003), and have located AdipoR1 and AdipoR2 in tissues other than skeletal muscle and liver.

Kharroubi, Rasschaert, Eizirik and Cnop (2003) located AdipoR1 and AdipoR2 receptors in human and rat pancreatic β cells, and stated the level of expression was similar to that found in liver cells. The location of adiponectin receptors in pancreatic β cells lends evidence to the probable participation of adiponectin in cellular functions related to diabetes. Civitarese et al. (2004) studied subjects of Mexican American descent with and without a family history of T2D. They found that AdipoR1 and AdipoR2 were both expressed in skeletal muscle cells, but expression of each was lower in subjects with a family history of

T2D. Levels of expression of both AdipoR1 and AdipoR2 in the study by Civitarse and colleagues were positively correlated with insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp studies ($r = .64$, $p < .01$ and $r = .47$, $p < .05$, respectively).

Receptors for adiponectin have also been located in areas that suggest involvement with vascular processes. Chinetti et al. (2004) found AdipoR1 and AdipoR2 in human aortic smooth muscle cells, and in "microvascular endothelial cells" (p. 152), as well as in atherosclerotic plaque lesions that had been removed during carotid endarterectomy. The authors went on to report that exposure of macrophage cells to PPAR-α ligands up-regulated AdipoR2 receptors and suggested that this may be one mechanism for the reduction of foam cell accumulation by adiponectin that has been noted in other studies (Furukawa et al., 2004; Ouchi et al., 2001).

Lastly, AdipoR1 and AdipoR2 may be influenced by obesity and lifestyle habits. Rasmussen et al. (2006) studied a group of obese women who were hospitalized for gastric partitioning surgery and a group of lean women who were having gynecological surgeries for benign causes. Lean subjects had a BMI less than 26, and the BMI of obese subjects was greater than 35. They found that AdipoR1 mRNA was decreased in subcutaneous adipose tissue (SAT) and omental adipose tissue (OAT) from obese subjects; AdipoR2 mRNA was decreased as well in SAT from obese subjects, but not in omental tissue. Change in lifestyle habits shows promising results related to AdipoR1, however. Rasmussen et al. (2006) also studied adiponectin receptor mRNA in a group of subjects who lost approximately 12 kilogram over eight weeks by adherence to a low calorie diet. AdipoR1 mRNA, but not AdipoR2 mRNA, increased by 81% in this subject group.

In summary, the receptors that bind with adiponectin are located in various tissues, including adipose tissue, skeletal muscle, liver, blood vessels and pancreatic β-cells, and expression is related to overweight status and lifestyle change. The actions of adiponectin related to CVD and T2D may be facilitated by the location of receptors in these cells. These actions will be described further in the sections that follow.

Adiponectin and Obesity

Paradoxically, although adiponectin is primarily produced in adipose tissue, blood levels are lower in people who are more overweight or obese. The reasons for this are unclear, but Havel (2004) suggests that the large visceral adipocytes observed in individuals with obesity do not function as well as smaller cells, possibly because the large cells produce less adiponectin as they become laden with triglycerides. Another possible reason may be the influence of IL-6 and TNF-α, pro-inflammatory cytokines that are also produced by adipocytes. IL-6 is produced in greater amounts by visceral or omental adipose tissue than by subcutaneous adipose tissue (Fried, Bunkin, & Greenberg, 1998), and TNF-α is strongly associated with measures of central adiposity such as waist-to-hip ratio and visceral adipose tissue (r=.55 and .45, respectively, p<.01) (Ziccardi et al., 2002). These cytokines and or their receptors inhibited adiponectin in in-vitro human and rodent studies (Bruun et al., 2003; Fasshauer et al., 2003).

The type of adipose tissue may also be important in the production of adiponectin. In a group of Korean women, greater amounts of visceral adipose tissue, but not subcutaneous adipose tissue, predicted lower adiponectin levels (Park et al., 2004). Central adiposity may influence adiponectin levels in children as well. Using CT scans to measure visceral adipose tissue in a small sample of children and adolescents, Bacha, Saad, Gungor and Arslanian

(2004) found that adiponectin levels were somewhat lower in subjects with more visceral adipose tissue (p=.05), even though BMI did not differ significantly from that of subjects who had less visceral adipose tissue.

Adiponectin and CVD

In-vitro studies with lab animals and human cells have shown that adiponectin has multiple actions that can affect the processes leading to CVD. For example, adiponectin prevents the apoptosis of human umbilical vein endothelial cells (Kobayashi et al., 2004), and has been shown to interrupt activation of NF-κ B in human aortic endothelial cells (Ouchi et al., 2000). These actions are helpful, because healthy endothelial cells are the first line of defense against an insult that might trigger an inflammatory process in vessel walls. In addition, a globular form of adiponectin has been shown to increase production of nitric oxide in bovine aortic endothelial cells, and to up-regulate endothelial nitric oxide synthase in human aortic endothelial cells (Hattori, Suzuki, Hattori, & Kasai, 2003). The authors suggest that these actions "explain some of the vascular protective effects" (p.1548) of adiponectin by leading to the production of nitric oxide, a potent vasodilator.

Adiponectin has also been shown to have anti-atherogenic effects that likely interfere with development of an atherosclerosis lesion. Kawanami et al. (2004) found that adiponectin inhibited the up-regulation of cellular adhesion molecules, cells that participate in the inflammatory process by facilitating the adhesion of monocytes to the artery wall. Adiponectin also interferes with the formation of foam cells from human monocyte-derived macrophages that lead to fatty streak lesions (Furukawa et al., 2004; Ouchi et al., 2001), and decreased the phagocytic activity of human macrophages in an in-vitro study by Yokota et al. (2000). The addition of a C1q receptor antibody blocked the suppression of phagocytic

activity; the authors proposed that the C1q receptor acted as a mediator of adiponectin's effect on the macrophages. Lastly, Kumada et al. (2004)(2004) have shown that adiponectin contributes to the inhibition of matrix metalloproteinase-1 (MMP-1) by up-regulating IL-10, an anti-inflammatory cytokine. MMP-1 is one of the factors responsible for breakdown of the fibrin cap that covers the atherosclerotic lesion, allowing for plaque rupture and thrombus formation.

Several investigators have examined the relationships of adiponectin to CVD in clinical studies with humans. Most research indicates that higher levels of adiponectin are associated with lower risk of CVD, although recent research suggests that findings may differ somewhat by gender. Low adiponectin is associated with increased risk for myocardial infarction and coronary artery disease, independent of other CVD risk factors, in men (Kumada et al., 2003; Pischon et al., 2004). Kumada et al. (2003) found that Japanese men in the lowest quartile of adiponectin were more likely to have coronary disease as diagnosed by angiography (RR=2.05) when compared to those in the highest quartile, even when controlling for diabetes, lipid abnormalities, smoking habits, blood pressure and BMI. In a large prospective study of male health care professionals, Pischon et al. (2004) noted that men with adiponectin levels in the highest quintile were much less likely to develop coronary heart disease (RR=.39); these authors also adjusted for common risk factors for CVD such as BMI and family history of MI. In contrast, Lawlor, Smith, Ebrahim, Thompson and Sattar (2005) found no significant decrease in risk with a "doubling of adiponectin" (p.5679) (RR=.93) in a prospective study of British women. More research is needed to explore these gender differences.

Links between adiponectin and early cardiovascular pathology have even been noted in youth (Pilz et al., 2005). These researchers found an inverse correlation between adiponectin levels and carotid intima-media thickness (r = -.34, p<0.001) in a group of normal weight and obese children and adolescents. This correlation was significant even when controlling for CVD risk factors such as BMI, HOMA-IR, cholesterol, triglyceride, blood pressure, hs-CRP, gender and age.

Adiponectin and T2D

Adiponectin also appears to have protective properties in regard to T2D and insulin resistance. For example, researchers have shown that treatment of obese, hyperglycemic and hyperinsulinemic mice with recombinant adiponectin led to significant improvements in glucose and insulin levels (Yamauchi et al., 2001). Moreover, in another study by Yamauchi et al. (2002), administration of adiponectin to mice increased the activation of AMPK in skeletal muscle and liver cells, thereby increasing fatty acid oxidation and glucose uptake . The globular form of adiponectin was effective in activating AMPK in liver cells, whereas both globular and full length adiponectin were effective in muscle cells. In the same study, adiponectin was shown to decrease the expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, enzymes used by the liver during gluconeogenesis, thereby decreasing the amount of new glucose synthesized by the liver. In a separate study, Combs, Berg, Obici, Scherer and Rossetti (2001) noted a 50% decrease in the same enzymes with administration of adiponectin. In addition, human studies have demonstrated that when thiazolidenediones (TZDs), drugs that are given to increase insulin sensitivity, are administered to human subjects with diabetes there is an increase in expression of adiponectin as well as an improvement in insulin sensitivity (Yki-Jarvinen, 2004). Lastly, as

mentioned in the section on adiponectin receptors, the receptors for adiponectin are located in human and rat pancreatic β cells (Kharroubi et al., 2003), and adiponectin exhibits a protective effect against the apoptosis of β cells caused by other cytokines or free fatty acids (Rakatzi, Mueller, Ritzeler, Tennagels, & Eckel, 2004).

Adiponectin is related to development of T2D or insulin resistance in clinical studies with lab mammals and humans. In a prospective study with rhesus monkeys that progressed to type 2 diabetes, adiponectin levels decreased along with insulin sensitivity (Hotta et al., 2001). Low adiponectin is also a predictor of insulin resistance (Yamamoto, Hirose, Saito, Nishikai, & Saruta, 2004), and of T2D in human adults (Snehalatha et al., 2003; Spranger et al., 2003), and children (Cruz et al., 2004). In a longitudinal study with Pima Indians, low adiponectin was a significant predictor of lower insulin stimulated glucose disposal at follow-up, independent of baseline glucose disposal, age, sex, and percent body fat (Stefan et al., 2002). Adiponectin may also differ by type of diabetes, at least in children and adolescents (Morales et al., 2004). In the Morales study, children with T2D had lower adiponectin than children with type 1 or control children; this result was noted in all subjects, and also in children with a BMI greater than the 85th percentile. One limitation of the study however, was that subjects were not fasting when adiponectin levels were drawn.

Some studies using human cells have shown that a person's obesity status and or a diagnosis of T2D may affect the ability of adiponectin to function as well as it possibly might (Bruce, Mertz, Heigenhauser, & Dyck, 2005; Chen et al., 2005). Bruce and associates studied the effects of globular adiponectin on skeletal muscle cells taken from lean and obese volunteers. Lean subjects had a body mass index (BMI) less than 27 kg/meters[2], and the BMI of obese subjects was greater than 30 kg/meters[2]. The authors reported that globular

adiponectin stimulated glucose uptake in cells from both lean and obese subjects. However, the added beneficial effects of adiponectin on insulin stimulated glucose uptake and increased fatty acid oxidation were decreased in cells from obese participants ($p < .05$). In a similar study, Chen and colleagues found that activation of AMPK by globular adiponectin was blunted in skeletal muscle cells taken from volunteers who were either obese or obese and diabetic when compared to the level of activation in cells from lean subjects. Higher levels of globular adiponectin were required to achieve AMPK activation responses in cells from either group of obese subjects. Further research on the effects of adiponectin in relation to obesity or overweight status will help to clarify its role in T2D.

Increased production of pro-inflammatory proteins and the decreased production of anti-inflammatory proteins such as adiponectin by adipose tissue in obesity are thought to contribute to a chronic inflammatory process, potentially leading to the development of risk factors for CVD and T2D. Lifestyle changes in diet and activity behaviors can, however, result in a decrease in many risk factors for CVD and T2D, such as BMI and dyslipidemia, and may lead to an increase in adiponectin levels and a decrease in the chronic inflammatory state. Research concerning the effects of lifestyle change on adiponectin and inflammatory markers is discussed in the following section.

Effects of Lifestyle Change on Adiponectin and Inflammatory Markers

Studies in Adults

Several research studies in adults with interventions resulting in weight loss have demonstrated an improvement in adiponectin levels and inflammatory and metabolic profiles. Interventions have ranged from lifestyle changes in dietary intake and physical activity (Esposito et al., 2003; Monzillo et al., 2003), to very low calorie diets (as low as 800

cal/day) (Hotta et al., 2000), to gastric surgery or medical treatment for weight loss (Valsamakis et al., 2004; Yang et al., 2001). Findings from other studies however, seem to be contradictory, and have not demonstrated changes (Ryan, Nicklas, Berman, & Elahi, 2003; Xydakis et al., 2004).

The most frequently studied markers of inflammation in intervention studies have been TNF- α, IL-6, CRP and adiponectin. Relatively small amounts of weight loss resulted in improvements in levels of these biomarkers in most of the lifestyle studies. Monzillo et al. (2003) studied 24 adult obese diabetic and non-diabetic subjects (gender not specified) and found significant decreases in IL-6 with an average 6.9 kilogram weight loss resulting from an intervention consisting of calorie restriction and 30 minutes of supervised exercise three times a week. A significant increase in adiponectin levels was only noted in the diabetic subjects. Esposito et al. (2003) noted similar results with decreases in IL-6 and CRP and an increase in adiponectin with a five point decrease in BMI in obese women, achieved over two years. The intervention in this study included a "Mediterranean diet" and increased physical activity. Valsamakis et al. (2004) found significant decreases in CRP and increases in adiponectin with a 5.4% weight loss over six months in 20 non-diabetic female subjects treated with sibutramine. There were no significant changes in glucose or insulin, but triglycerides decreased and HDL increased.

Weight loss interventions such as surgical weight reduction or weight loss by calorie restriction have also been shown to lead to an increase in adiponectin (Hotta, et al., 2000; Yang et al, 2001). In the study by Yang and associates, a 21% decrease in weight resulting from gastric surgery was associated with a 46% increase in adiponectin ($p < .001$); significant decreases in glucose, insulin resistance and triglycerides were also noted. Calorie

restriction leading to a 12% and 10% loss of body weight in diabetic and non-diabetic

subjects, respectively, resulted in an increase in adiponectin levels by 65% in diabetic

subjects and 42% in non-diabetic subjects in the study by Hotta et al. (2000).

Not all studies have, however had favorable results in relation to adiponectin levels.

Ryan, Nicklas, Berman et al. (2003) designed a 6-month intervention of dietary change and

thrice weekly 45-minute exercise sessions for obese post-menopausal women. Although the

average weight loss was 6% of body weight, adiponectin did not change significantly; it did

increase by 40%, but variability was high and the change was not statistically significant.

The results were favorable overall, however, for a decrease in glucose, insulin and leptin. In

another study, Xydakis et al. (2004) studied a sample of 80 obese men and women who lost

approximately 7% of body weight over a 4-6 week period by ingesting 600-800 calories per

day; no significant changes were noted in adiponectin or TNF- α. The authors did note

significant decreases in glucose, insulin, leptin, CRP and triglycerides. The authors

speculated that the lack of change in adiponectin may have resulted from the extreme calorie

restriction, although Hotta et al. (2000) observed increases in adiponectin with similar, but

slightly higher calorie levels.

Interventions that promote weight loss in adults can have beneficial effects on

inflammatory and metabolic profiles, including adiponectin levels. Lifestyle changes

resulting in prevention of or decrease in a pro-inflammatory state may also be possible at

earlier ages as well. Studies in children and adolescents are showing promising results.

Studies in Children

Several small studies in children have focused on the effects of healthy lifestyle

changes on adiponectin and inflammatory markers. Some authors have documented

improvements similar to results of adult studies with weight maintenance, or with weight loss (Balagopal et al., 2005; Balagopal, George, Yarandi, Funanage, & Bayne, 2005; Reinehr, Roth, Menke, & Andler, 2004). Reinehr et al. (2004) found that a decrease in average BMI from 26 to 22.7 over 1 year resulted in a significant increase in adiponectin and decrease in insulin resistance. The children in this study who lost weight were more likely to be younger and pre-pubertal. Balagopal, George, Patton et al. (2005) enrolled adolescent subjects in a three month intervention program of dietary and behavioral counseling, and encouraged 45 minute aerobic activity sessions three times a week. Subjects in the intervention group maintained their weight over the time of the intervention, whereas the control group gained weight. In addition, body fat percent decreased and bone-free lean mass increased in the intervention group. Even with maintenance of weight instead of weight loss, there were still significant improvements in levels of CRP, fibrinogen and IL-6 in the intervention group. In a separate analysis of this intervention (Balagopal, George, Yarandi et al., 2005), the mean adiponectin increased by 34% in subjects in the intervention group. In contrast, other studies have found no change in adiponectin with weight loss or improvements in fitness, even with positive changes in insulin sensitivity (Nassis et al., 2005).

In summary, most interventions that promote weight loss or maintenance, whether by lifestyle change, medication or surgical methods, appear to improve inflammatory and metabolic profiles, and lipid levels in adults and children. Even a relatively small percent weight loss seems to have beneficial effects. An increased understanding of the relationships between adiponectin and risk factors for CVD and T2D, including adiposity and physical fitness, will be helpful in planning future interventions that can encourage lifestyle change and potentially impact inflammatory profiles that contribute to CVD and T2D.

Conceptual Framework of Relationships Among Adiponectin and Variables

in this Study

The current literature regarding the relationships between adiponectin and the variables in my conceptual framework as illustrated in figure 3 will be reviewed in the remaining sections of chapter two. Thirty-five studies regarding adiponectin in children and youth were reviewed. One of the studies is only briefly mentioned because subjects were not fasting when blood samples were drawn (Morales et al., 2004), and another was not included in the following review because complete information about recruitment and data collection methods was not given (Gil-Campos, Canete, & Gil, 2004). The article by Gil-Campos and associates was primarily a review article about adiponectin during childhood, in which the authors also included limited information on findings from a small study they had recently completed. Findings from the remaining articles regarding the relationship between adiponectin and risk factors for CVD and T2D will be addressed in the pages that follow.

Demographic Variables and Adiponectin

Several researchers have examined the relationship of adiponectin to demographic variables such as age, gender and race in studies with adults and children. Demographic variables are included in the conceptual framework for this study as they are known to be associated with adiponectin, and with risk for CVD and diabetes. Moreover, gender and race may be moderators of the relationship between adiponectin and risk factors related to carbohydrate and lipid metabolism, and vascular function.

Age

Age appears to be positively associated with adiponectin levels in the majority of studies in adults, with correlations ranging from .16 to .30 (Baratta et al., 2004; Cnop et al., 2003; Fernandez-Real et al., 2004). One study (A. J. Hanley, Connelly, Harris, & Zinman, 2003), however, found an inverse relationship with age ($r = -.23$, $p < .001$). As discussed below, the positive relationship of adiponectin to age found in most studies with adults is in contrast to the inverse relationship found in samples composed of older children and adolescents.

Studies that have examined the relationship between adiponectin and age in children and adolescents (Bottner et al., 2004; Butte et al., 2005; Cianflone, Lu, Smith, Yu, & Wang, 2005; Cruz et al., 2004; Huang et al., 2004; Punthakee et al., 2006; Singhal et al., 2005; Tsou et al., 2004) indicate that adiponectin is inversely correlated with age, i.e., it decreases as age increases. Butte et al. (2005) found that adiponectin decreased most sharply from ages 4-10 years, with an overall rate of decline of 0.5 μg/ml per year from age 4 to age 19. The relationship between adiponectin and age may vary by gender. A relationship between the two variables was noted only in males in one study (Tsou et al., 2004), and Punthakee et al. (2006) found an interaction between age and gender, in that adiponectin decreased more with age in boys than in girls, most likely due to pubertal changes.

No one has studied the relationship between adiponectin and age in a racially diverse sample, although one such study used age as a covariate in other analyses. Research is needed that explores the relationship of age and adiponectin in a racially diverse sample. Moreover, judging from results in the studies mentioned above, it will likely be necessary to

use age as a covariate in analyses of relationships between adiponectin and risk factors for CVD and T2D.

Race

Few studies have examined the relationship of adiponectin to race in adults. Weyer et al. (2001) found that adiponectin in adults was higher in Whites than in Pima Indians, independent of adiposity, but this relationship was lost when adjusting for insulin and insulin sensitivity. Hulver et al. (2004) examined adiponectin levels in obese and non-obese Black and White women, and found that adiponectin levels were higher in the group of non-obese White women than in obese White women or either group of Black women. In addition, race was shown to be a moderator of the relationship between adiponectin and several risk factors for CVD and T2D in this study. Moderators are those variables that affect "the direction and/or strength of the relation between an independent or predictor variable and a dependent or criterion variable" (Baron & Kenny, 1986). In the study by Hulver and colleagues, adiponectin was only inversely correlated with BMI, insulin, glucose and insulin resistance in White women; no significant correlations were found in Black women.

Relatively little research has been done to compare mean adiponectin levels in children and adolescents by Black and White racial groups, or to explore the relationship between adiponectin and risk factors for CVD and T2D in a racially diverse sample. The majority of studies have been done with White subjects (Bottner et al., 2004; Gilardini et al., 2006; Pilz et al., 2005; Punthakee et al., 2006; Singhal et al., 2005), or with subjects from various countries such as Taiwan (Chu et al., 2005; Huang et al., 2003; Huang et al., 2004; Tsou et al., 2004), Japan (Asayama et al., 2003; Ogawa et al., 2005; Okada et al., 2005) or children of Hispanic descent (Butte et al., 2005; Cruz et al., 2004; Gil-Campos et al., 2004).

Most of the studies conducted with racially diverse samples have found that mean adiponectin levels are lower in Black children and adolescents than in Whites, even when controlling for gender, puberty, BMI z-scores and HOMA scores (Woo et al., 2005) or for gender puberty, abdominal visceral or subcutaneous adipose tissue and leptin (Lee, Bacha, Gungor, & Arslanian, 2006). Pre-pubertal children in the study by Bacha, Saad, Gungor and Arslanian (2005) were similar in age, body composition and visceral adiposity, yet adiponectin levels were lower in the Black children, and Bush, Darnell, Oster, Goran and Gower (2005)(2005) found that levels were lower in Black children, independent of measures of peripheral or trunk fat. One study in a racially diverse sample indicated an interaction between race and gender, with adiponectin levels being lower in Black boys than in White boys or girls of either gender (Degawa-Yamauchi et al., 2003). This interaction was noted even though mean BMI, glucose, insulin levels were similar by gender and race.

Studies conducted with racially diverse samples have concentrated mainly on the relationship between adiponectin and measures of adiposity or insulin/insulin resistance, and results have been contradictory concerning the impact of race on the relationship between adiponectin and insulin sensitivity (Bacha et al., 2005; Bush et al., 2005; Degawa-Yaumachi, et al., 2003; Lee et al, 2006; Woo et al., 2005). These studies and others will be discussed more fully in the sections on measures of carbohydrate metabolism. In addition, the relationship between adiponectin and other risk factors for CVD or T2D has seldom been examined in racially diverse samples. For example, although lipid levels are known to differ by race (Cook et al., 2003; Jago et al., 2006), only one study has been done exploring the relationship between adiponectin and lipids in a racially diverse sample (Martin et al., 2005). Race was treated as covariate in this study; the researchers did not examine the possibility

that the relationship might be moderated by race. Moreover, no researchers have explored the relationship between adiponectin and blood pressure, physical fitness or family history in a racially diverse sample.

In summary, racial disparities are known to exist in the prevalence of CVD and T2D, and in the prevalence of related risk factors in adults and children (Brancati et al., 1996; Cook et al., 2003; Freedman et al., 2006; Haffner et al., 1999; Jago et al., 2006; Mensah et al., 2005). Race may function as a moderator of relationships between adiponectin and risk factors for CVD and T2D. However, relatively few researchers have examined the relationship between adiponectin and risk factors for CVD and T2D in racially diverse samples of children and adolescents. More research is needed to examine the relationship between adiponectin and risk factors in a large, racially diverse sample, and to determine if the relationship differs by race.

Gender

In adults, most investigators report higher adiponectin levels in females than in males (Cnop et al., 2003; Fernandez-Real et al., 2004; Kern, Di Gregorio, Lu, Rassouli, & Ranganathan, 2003)((Baratta et al., 2004; Snehalatha et al., 2003). Other researchers found no difference in mean adiponectin levels in male and female subjects (Xydakis et al., 2004). Almost all the researchers who have investigated adiponectin in child and adolescent samples have compared mean adiponectin levels by gender, and results have been inconsistent. About half of the studies with youth have indicated that adiponectin is higher in girls (Butte et al., 2005; Chu et al., 2005; Gilardini et al., 2006; Huang et al., 2004; Kim et al., 2006; Nemet et al., 2003; Punthakee et al., 2006), even when adjusting for puberty (Singhal et al., 2005; Woo et al., 2005). No research has shown adiponectin to be higher in boys.

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In contrast, other researchers have found no difference in adiponectin levels by gender (Asayama et al., 2003; Bacha et al., 2004; Bottner et al., 2004; Bush et al., 2005; Cianflone et al., 2005; Lee et al., 2006; Okada et al., 2005; Pilz et al., 2005; Stefan et al., 2002). Most of these groups did not control for pubertal stage, however. Two studies were done with samples that were likely pre-pubertal, or in early stages of puberty. Children in the study by Cianflone et al. (2005) were of ages 2-6 years, and therefore most likely pre-pubertal. Subjects in the study by Stefan et al. (2002) were 5 and 10 years old, and probably pre-pubertal or in early pubertal stages. Other studies in older children did not measure pubertal stage (Asayama et al., 2003; Okada et al., 2005), or did not control for it (Bacha et al., 2004; Bottner et al., 2004; Bush et al., 2005). In the study by Pilz et al. (2005), however, adiponectin was higher in female subjects through all stages of puberty. Lastly, only a few of the studies that compared adiponectin levels by gender were done in racially diverse samples (Woo et al., 2005; Lee et al., 2006; Bush et al., 2005), and results were conflicting as noted above.

Perhaps most intriguing, the relationships between adiponectin and certain risk factors for CVD and T2D may be moderated by gender, or interactions between gender and age or overweight status may exist. For example, Okada et al. (2005) found that adiponectin was significantly related to lipids, such as triglycerides, HDL-C and LDL-C, only in female subjects. This finding was independent of BMI and fat mass measures, although sum of skinfolds was higher in girls. Woo et al. (2005) found that adiposity affected adiponectin levels differently in girls and boys. For example, during puberty, adiponectin levels decreased in both lean and overweight boys while levels only decreased in overweight girls; levels remained stable in lean girls. Lastly, Chu et al. (2005) found that although adiponectin

was only related to insulin in female subjects, independent of BMI, and a 'yes/no' measure of puberty.

In summary, evidence for differences in mean adiponectin levels by gender in children and adolescents is contradictory and few studies have been done in racially diverse samples. Moreover, gender may be a moderator of the relationship between adiponectin and risk factors for CVD and T2D. Further research will be helpful to more fully clarify the inconsistent relationship found in the literature between gender and mean adiponectin, and to examine the possible moderating effect of gender on the relationships between adiponectin and risk factors for CVD and T2D.

Measures of Obesity and Adiponectin

As shown in the conceptual framework in figure 3, and discussed earlier in the literature review, obesity is a key variable for study in relation to adiponectin. Research in adults indicates that low adiponectin is associated with greater obesity or adiposity, as measured by various methods such as BMI or waist circumference (Cnop et al., 2003; Hulthe et al., 2003; Park et al., 2004; Pischon et al., 2004). Adiponectin levels are also decreased in children who are overweight or obese (Asayama et al., 2003; Bacha et al., 2004; Stefan et al., 2002).

The relationship between adiponectin and three clinically relevant measures that reflect of different aspects of increased adiposity, the sum of tricep and subscapular skinfolds, BMI and waist circumference, are examined in this dissertation. Measurement of the tricep and subscapular skin folds gives the researcher information about subcutaneous obesity in the regional areas from which the measurements are taken (Wells & Fewtrell, 2006). Skinfold measurements are frequently summed and used in equations to predict total

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body fatness (Slaughter et al., 1988). BMI, a ratio of weight in kilograms to height in meters squared, gives the researcher information about whether the subject's weight is appropriate for his or her height, and is an indication of overall overweight status. Waist circumference provides an indication of central adiposity, a type of adiposity associated with the other risk factors for CVD and T2D (Cook et al., 2003). The anthropometric measures of skinfolds, BMI and waist circumference are used frequently in research on obesity, especially in large trials, because of the increased expense of gold standard methods for estimating adiposity such as dual x-ray absorptiometry (DXA) or air displacement plethysmography.

Both BMI and waist circumference are recognized as valid measures of obesity when compared to gold standards in children and adolescents, although BMI does not account for differences in non-fat mass such as muscle or bone, especially as children progress from childhood through adolescence (Mei et al., 2002; Neovius, Linne, & Rossner, 2005; Wang, 2004). BMI and waist circumference have both been shown to be effective predictors of metabolic and cardiovascular risk (Katzmarzyk et al., 2004). It will be important to include these two measures, as well as sum of skinfolds, in my model in order to investigate the relationships of adiponectin with obesity in the most comprehensive manner.

Body Mass Index

BMI, BMI percentiles and BMI z-scores

There is great variation in the research literature related to adiponectin, regarding the definitions of obesity or overweight in childhood. Definitions and terms referring to weight category frequently vary from the three widely accepted CDC weight classification guidelines of: normal weight (<85th percentile for age and gender), at risk for overweight ($\geq$85[th] to <95[th] percentile), and overweight ($\geq$ 95th percentile) (CDC, 2003). A child's

overweight may also be measured by BMI percentile or BMI z-score, adjusted for gender and

age. Guidelines for appropriate BMI percentiles and z-scores for age and gender are

available on the CDC website, found at

http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/clinical_charts.htm, and

http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/zscore/zscore.htm, respectively. These

methods take into account the changes in BMI that occur with development and growth.

  Most authors who have studied the relationship between BMI and adiponectin in

children have used two types of subject groups when comparing mean adiponectin levels,

with one group usually defined as having a normal weight and the other as being obese or

overweight. Some authors refer to their higher weight category group as "overweight" (Butte

et al., 2005; Chu et al., 2005; Vikram, Misra, Pandey, Dwivedi, & Luthra, 2004), and others

use the term obese (Asayama et al. 2003; Bottner et al., 2004; Gilardini et al., 2006).

Definitions for these terms also vary in the literature. Examples of definitions for obesity

include a BMI greater than the 97th percentile for age and gender (Gilardini et al., 2006), or

greater than the 95th percentile (Weiss et al., 2005).   Overweight subjects might be defined

as those with a BMI greater than or equal to the 85th percentile for age and gender (Chu et al.,

2005), or those with a BMI greater than the 95th percentile who also have a fat mass

measurement by DEXA of greater than the 85th percentile (Butte et al., 2005). Other authors

use BMI z-scores to differentiate between weight groups (Bottner et al., 2004; Weiss et al.,

2004), but no one has compared adiponectin means by the three groups defined by the CDC

guidelines.

BMI and Adiponectin

Adiponectin is positively correlated with weight in newborns (Sivan et al., 2003). Virtually all of the studies in older children and adolescents, however, have shown adiponectin to be lower in subjects who are overweight or obese than those with a normal weight, or to be inversely correlated with BMI or BMI z-score. Inverse correlations between adiponectin and BMI have been found in lean and obese children alike (Bottner et al., 2004). Correlations overall have ranged from r = -.20 (Tsou et al., 2004) to r = -.70 (Bacha et al., 2004). Stefan et al., (2002) reported a longitudinal negative correlation between adiponectin and obesity in Pima Indian children. As BMI and body fat increased over time, adiponectin levels decreased. There was only one study in the literature that did not find a correlation between adiponectin and BMI z-score (Singhal et al., 2005). This group of 294 adolescents was relatively lean overall, with a mean BMI of 21.3 +/- 3.8, although a range of 14-41 was reported; 73% of the sample had a BMI < 25. Clearly, BMI and/or BMI z-score must be considered when studying the relationships between adiponectin and risk factors for CVD and T2D.

Lastly, overweight status may have a moderating effect on the relationship between adiponectin and other risk factors for CVD and T2D in children, and in adults. Risk factors such as HDL-C, triglycerides or insulin were more strongly associated with adiponectin in children when subjects were overweight (Martin, et al., 2005; Butte et al., 2005). In a recent study, Kantartzis et al. (2006) found that adiponectin was associated with HDL-C more strongly in adults with higher body fat percent, and triglycerides were only associated with adiponectin in the higher body fat group. These results were noted even though the division between lean and obese groups was 26% body fat for males and 36% body fat for females,

both relatively high percentages. These effects of overweight status on the relationship between adiponectin and risk factors for CVD and T2D will be discussed more fully in the following sections related to the different risk factors.

Waist Circumference and Adiponectin

Waist circumference is another clinically relevant measure that has been found to be associated with adiponectin. Waist circumference encompasses two types of abdominal adipose tissue, visceral and subcutaneous. Increased visceral adipose tissue has been found to be predictive of lower adiponectin levels in adult men and women (Cote et al., 2005; Park et al., 2004). Central adiposity may influence adiponectin levels in children as well. Using CT scan technology to measure visceral adipose tissue at the L_4-L_5 level in a small sample of 26 obese children, Bacha et al. (2004) found that adiponectin levels were lower in children who had greater visceral adipose tissue, even though their BMI, fat mass and subcutaneous abdominal adipose tissue did not differ significantly from that of children who had less visceral adipose tissue (p=.05). Asayama et al. (2005) found a strong correlation (r = -.53, p<.001) between adiponectin and visceral adipose tissue in 53 Japanese children that was still significant even when controlling for percent overweight, percent body fat, age and gender. Weiss et al. (2003), however, did not detect a significant correlation between adiponectin and either subcutaneous or visceral adipose tissue, although adiponectin was correlated with total body fat (r = .48, p < .05); the sample in this study was comprised of only 18 children.

Most studies with children have shown that adiponectin is inversely related to waist circumference itself, with correlations ranging from r = -.38 (Bottner et al., 2004; Kim et al., 2006) to r = -.56 (Asayama et al., 2005). Singhal et al. (2005) found that adiponectin decreased by .9% with every unit increase in waist measurement. Inverse correlations have

been found in both normal weight and overweight children (Bottner et al., 2004). Vikram et al. (2004) were the only researchers to find no association between adiponectin and waist circumference, although they did note a correlation between adiponectin and BMI in their sample of Asian Indian males. Little research has been done regarding adiponectin and waist circumference in racially diverse samples of youth (Martin et al., 2005; Woo et al., 2005), although waist circumference measures are different for Black and White youth, especially at the higher percentiles for waist measures (Fernandez, Redden, Pietrobelli, & Allison, 2004). The studies by Woo et al. (2005) and Martin et al. (2005) were done with the same sample of youth.

Sum of Skinfolds and Adiponectin

Very few studies have examined the relationship between adiponectin and skinfold measures, and results have been contradictory. Sums of four skinfolds (triceps, biceps, sub-scapular and iliac) were not correlated with adiponectin in Asian Indian male adolescents (Vikram et al., 2004) or in a sample of 294 male and females from the United Kingdom (Singhal et al., 2005). In contrast, studies that measured sums of two skinfolds (triceps and sub-scapular) have found inverse correlations, primarily in female subjects. Okada et al. (2005) found an inverse correlation ($r = -.17$) in females only; no correlation was noted in male subjects. Punthakee et al. (2006) found adiponectin to be inversely correlated with sub-scapular skinfold measures in both genders, but only in females with triceps skinfolds. The only study done in a racially diverse study (Degawa-Yamauchi et al., 2003) found that the sum of tricep and sub-scapular skinfolds in their sample of male and female Black and White youth was independently related to adiponectin in a regression equation with race, gender and a race by gender interaction term; this relationship was no longer significant, however,

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when BMI or BMI percentile were added to the equation. Further research is needed to clarify the relationship between adiponectin and measures of skinfolds. Gender will be important to consider when exploring this relationship since previous research has found differences by gender.

Studies That Have Compared the Relationships Between Adiponectin and Different Measures of Obesity in the Same Study

As described in the previous sections, most studies in the literature have been concerned with the relationship between adiponectin and BMI, and some studies have examined the relationship with waist circumference or skinfolds. Relatively few researchers have, however, evaluated the relationship between adiponectin and two or more of the three clinically relevant measures of adiposity in the same study, and only two research groups have investigated this type of relationship in a racially diverse sample of youth (Degawa-Yamauchi et al., 2003; Woo et al., 2005). Two studies have assessed the relationship of all three measures of adiposity with adiponectin in the same study (Singhal et al., 2005; Vikram et al., 2004). Results were inconsistent in that Vikram et al. noted a significant relationship between adiponectin and only BMI, while Singhal et al. only detected a relationship with waist circumference. The sample for the study by Vikram and associates was comprised of only male adolescents, while Singhal and colleagues included adolescents of each gender in their sample. Neither group found a significant relationship between adiponectin and sum of skinfolds; the specific skinfold measures used were the triceps, biceps, sub-scapular and supra-iliac skinfolds.

The four studies that only looked at BMI and waist circumference also produced contradictory results (Bottner, et al., 2004; Huang et al., 2004; Kim et al., 2006; Woo et al.,

2005). Bottner et al. (2004) studied a group of lean male adolescents and found adiponectin to be significantly associated only with a standardized BMI score, and not with waist. It was unclear if the standardized scores were adjusted for age and gender. Woo et al. (2005) found that waist and BMI z-scores were both significantly related to adiponectin, but the model using BMI z-scores produced the lowest Bayesian Information Criteria (BIC) score, and was therefore the best fitting model. Huang et al. (2004) found only logarithmically transformed (log) waist circumference to be associated with adiponectin in regression with log BMI, age and gender. Kim et al. (2006) noted inverse correlations between adiponectin and both BMI and waist circumference, but they did not compare to see which measure was independently associated with adiponectin.

The only study to compare the relationships between adiponectin and BMI or sum of skinfolds found that only BMI percentile was independently related to adiponectin, in a regression that included the sum of tricep and sub-scapular skinfolds, race, gender, and a race by gender interaction term (Degawa-Yamauchi et al., 2003). As mentioned in a previous section, Okada et al. (2005) found inverse correlations between sum of triceps and sub-scapular skinfolds and BMI in females, but only with BMI in male subjects. These authors did not compare to determine if contributions to adiponectin levels were stronger from BMI or skinfold measures.

In summary, results of studies regarding the strength of the contribution of BMI, waist circumference and sum of skinfolds have been conflicting and little has been done with racially diverse samples. The three clinically relevant measures of adiposity are compared in this dissertation, to determine which contributes the most variance to adiponectin in this sample of Black and White youth.

Measures Related to Carbohydrate Metabolism and Adiponectin

Adiponectin appears to be closely related to the metabolic processes regulating insulin and glucose. For example, in research with mice, Yamauchi et al. (2002) found that administration of adiponectin facilitated the activation of 5- AMP-activated protein kinase (AMPK), thereby increasing fatty acid oxidation and glucose uptake in muscle cells. Adiponectin is positively correlated with non-oxidative glucose disposal in adults and obese children (Weiss et al., 2005; Yokoyama et al., 2006). Researchers have also demonstrated improvements in the glucose and insulin levels of hyperglycemic and hyperinsulinemic mice with administration of recombinant adiponectin led to (Yamauchi et al., 2001). I therefore plan to investigate the relationship of adiponectin to fasting insulin and glucose levels.

Insulin and Adiponectin

In adult humans, decreased adiponectin levels predict insulin resistance, that is, higher insulin levels and decreased insulin sensitivity (Baratta et al., 2004; Fernandez-Real et al., 2003; A. J. Hanley et al., 2003). This relationship with adiponectin in adults may be independent of adiposity. For example, in one study where subjects were matched for age and BMI, adiponectin levels were lower in insulin resistant than in insulin sensitive subjects (Xydakis et al., 2004).

Many researchers have examined the relationship between adiponectin and insulin, and measures of insulin resistance or insulin sensitivity in children. Almost all of the studies that studied adiponectin and insulin **sensitivity** in youth to date have shown a positive relationship between the two. Correlations ranged from r = .38 (Bush et al., 2005) to r = .61 (Lee et al., 2006); p values were less than 0.01 for either r value. One study, however, found no relationship with insulin sensitivity, but did note an inverse correlation with insulin

secreted during the first ten minutes of a hyperglycemic clamp study (r = -.47, p=0.001) (Bacha et al., (2005).

The majority of researchers who have studied the relationship between adiponectin and insulin or insulin **resistance** have found inverse relationships. Correlations between adiponectin and insulin have ranged from r = -.14 (Chu et al., 2005) to r = -.66 (Bacha et al., 2004). Correlations between adiponectin and insulin resistance as measured by HOMA-IR have ranged from r = -.18 (Tsou et al., 2004) to r = -.43 (Bottner et al., 2004) for insulin resistance. Degawa-Yamauchi et al. (2003) found no bivariate correlations between adiponectin and insulin or with HOMA-IR scores in their sample group of 86 Black and Causasian youth, although they did note an inverse relationship between adiponectin and HOMA-IR when in regression with race, gender and a race by gender interaction term.

Effects of Gender, Measures of Adiposity and Race on the Relationship Between Adiponectin and Insulin

Gender

The relationship between adiponectin and insulin or insulin resistance may be moderated to some extent by gender (Chu et al., 2005; Singhal et al., 2005; Tsou et al., 2004). Chu et al. (2005) found adiponectin to be inversely correlated with insulin in both boys and girls in bivariate analyses, but only in girls when analyzed in multiple regression with other covariates, including BMI and a yes/no measure of puberty. Singhal et al. (2005) noted an inverse correlation between adiponectin and fasting insulin or HOMA-IR in subjects overall, but only in females when the analysis was run by gender group. Tsou et al. (2004) noted inverse correlations of adiponectin with insulin in females overall and at ages 11-14

years, but only at ages 15-18 in males. This finding may have been due to differences in timing of puberty onset for males and females.

Measures of Adiposity

Overweight status may also moderate the relationship between adiponectin and insulin, but studies have been few and results are inconsistent. Martin et al. (2005) noted an inverse association between adiponectin and insulin in a regression equation that included puberty, age, gender and race. This relationship was only noted, however, in the "non-lean" (p. 4256) subject group (p < .0001). Non-lean was defined as having a BMI greater than the 85th percentile. Butte et al. (2005) remarked that the correlation between adiponectin and insulin, as well as other risk factors for CVD and T2D, in their sample of Hispanic children and adolescents "was especially striking at the higher levels of adiposity" (p. 4174). In contrast, other research groups have found an inverse correlation between adiponectin and insulin or HOMA-IR even when analysis was restricted to lean subjects whose BMI was less than the 85th percentile (Singhal, 2005).

Studies that have examined the relationship of insulin, insulin resistance or insulin sensitivity to adiponectin when controlling for measures of adiposity such as BMI, waist circumference or sum of skinfolds have also shown contradictory results, depending on the actual measure studied. Only one study controlled for sum of skinfolds when examining the relationship between insulin and adiponectin (Vikram et al., 2004). These authors found that only the sum of skinfolds, and the ratio of adiponectin to sum of skinfolds were significantly related to insulin or HOMA-IR, in regression with BMI, waist circumference, percent body fat or adiponectin. The relationship was inverse, in that subjects with higher adiponectin levels and lower sum of skinfolds had lower insulin or HOMA-IR scores.

The two studies that controlled for waist circumference found similar results (Singhal et al. 2005; Vikram et al., 2004). The relationship between adiponectin and insulin or HOMA-IR was found to be independent of waist measurement. In a similar vein, the relationships between insulin or insulin sensitivity and adiponectin were independent of visceral abdominal adipose tissue in the studies that controlled for its effect (Lee et al., 2006; Asayama et al., 2003). Lee et al. (2006) found that the relationship of adiponectin to insulin sensitivity was independent of Tanner stage, gender, race and visceral adipose tissue. Furthermore, within the groups of children and adolescents with low, moderate and high visceral adipose tissue, the subjects with higher levels adiponectin levels were more insulin sensitive (p<.05). Taken together, these studies support the need to consider waist circumference measures when studying the relationships between adiponectin and insulin or insulin sensitivity.

Results of studies that examined whether the relationship of adiponectin and insulin, HOMA-IR or insulin sensitivity was independent of BMI or related measures such as BMI z-score or percentile were more variable. Six of ten studies showed an independent relationship. Insulin was inversely associated with adiponectin, independent of BMI or BMI z-score, in two studies with Hispanic children and adolescents (Butte et al., 2005; Cruz et al., 2004), and of BMI for the female subjects only in a large sample of Taiwanese adolescents (Chu et al., 2005); Chu and associates also adjusted for puberty in their analyses. Pilz et al. (2005) found that HOMA-IR was inversely related to adiponectin, independent of BMI in their sample of children and adolescents. Measures of insulin sensitivity were positively correlated with adiponectin, independent of BMI in a study of 135 obese children and adolescents (Bottner et al., 2004), and in a small group of young adolescents (Bacha et al.,

2004). Results in the study by Bottner et al. (2004) were also independent of pubertal level in a group of obese children and adolescents. Bacha et al. (2004) found that BMI and adiponectin explained 73% of the variance in peripheral insulin sensitivity.

In contrast, the relationship between adiponectin and insulin or HOMA-IR was dependent on BMI, BMI z-score or percentile in four other studies with children and adolescents. The relationship between adiponectin and insulin and HOMA-IR alike was dependent on BMI in a small sample of post-pubertal Asian Indian male subjects (Vikram et al., 2004), and on BMI z-scores in a large sample of White children and adolescents (Punthakee et al., 2006). Punthakee and associates did not indicate whether or not they adjusted for puberty in their analysis. Tsou et al. (2004) found that the relationship between adiponectin and HOMA-IR was dependent on BMI in female subjects, but not in male subjects. The authors did not control for puberty, but did control for testosterone levels in the regression equation for the males. The only group to control for measures of adiposity when examining the relationship between adiponectin and HOMA-IR in a racially diverse sample found an inverse association (p=.04) in regression with race, gender or a race*gender interaction (Degawa-Yamauchi et al., 2003). The correlation was no longer significant, however, when BMI or BMI percentile were added to the analysis.

It is difficult to understand why study results vary regarding whether or not the relationship between insulin and adiponectin is independent of measures of adiposity. Studies indicating either type of result have been done with large and small samples of varied ages, comprised of male and female subjects, and most did not adjust or control for pubertal stage. More research is needed to clarify this relationship, with large, racially diverse samples.

Race

Lastly, relatively few research groups have examined the relationship between adiponectin and insulin in a racially diverse sample, although insulin sensitivity is known to be lower in Black youth (Arslanian & Suprasongsin, 1996; Gower, Nagy, & Goran, 1999; Svec et al., 1992). Moreover, studies in adult subjects have shown significant relationships between adiponectin and insulin or insulin resistance in White subjects only, and not in Black subjects (Ferris et al., 2005; Hulver et al., 2004). As in studies conducted in other racial or ethnic samples, the majority of the studies with Black and White children and adolescents have shown adiponectin to be positively related to insulin sensitivity measures and inversely related to insulin overall (Bush et al., 2005; Lee et al., 2006; Weiss et al., 2005) or in sub-groups based on overweight status (Martin et al., 2005).

Findings from studies that examined the impact of race on the relationship between adiponectin and insulin or insulin sensitivity, however, are somewhat conflicting. In a study with a small group of pre-pubertal children (n=44), Bacha et al. (2005) found that the addition of adiponectin to a regression equation with race did not alter the fact that insulin sensitivity was lower in Black children. Larger studies, however, found that adiponectin was associated with insulin sensitivity independent of race; adjustments were made for pubertal stage in each of these studies (Lee et al., 2006; Bush et al., 2005). Lee et al. (2006) also found that correlations between insulin sensitivity and adiponectin were stronger in White than Black subjects, with r-values of .61 and .51 respectively.

In summary, adiponectin and insulin appear to be inversely related overall. Few studies have been done, however, with children and adolescents in racially diverse samples. The variables of gender, measures of adiposity, and race appear to affect the relationship

between adiponectin and insulin, but findings vary in relation to overweight status as measured by BMI, and to race. Research is needed with racially diverse samples to more fully clarify the possible impact of gender, overweight status and race on the relationship between adiponectin and measures of insulin resistance.

Glucose and Adiponectin

Results concerning the relationship of adiponectin with glucose levels in adults are conflicting. Stefan et al. (2003) and Winzer et al. (2004) found that adiponectin was inversely correlated with glucose, whereas other researchers (Ryan, Berman, Nicklas et al., 2003; Xydakis et al., 2004) found no association. Few researchers have examined the relationship between adiponectin and glucose in children and adolescents (Gilardini et al., 2006; Huang et al., 2004; Singhal et al., 2005), with only one utilizing a racially diverse sample (Degawa-Yamauchi et al., 2003). Three of four of the researchers have found no significant correlations between adiponectin and glucose in children (Degawa-Yamauchi et al., 2003; Huang et al., 2004; Singhal et al., 2005). Gilardini et al. (2006) found adiponectin to be inversely correlated with glucose (r = -.21). This was the only study of the four with all subjects classified as "obese" (p. 4792), defined as a BMI greater than the 97th percentile when adjusted for age and gender. Mean glucose levels in two of the studies that found no correlation (Degawa-Yamauchi et al., 2004; Singhal et al., 2005) were similar to those in the study by Gilardini et al. (2006), and were well within normal limits; levels in the study by Gilardini et al. (2006) were 80.7 mg/dl (+/- sem of .96) for males, and 78.4 mg/dl (+/- sem of .83) for girls. The mean glucose levels in the other study that did not report a correlation between adiponectin and glucose (Huang et al., 2004) were higher (100.8 mg/dl +/- s.d. of 6.8 for boys and 97.6 +/- s.d. of 7.2 for girls), but adiponectin means were also higher for this

group in contrast to other studies. More research is needed to further clarify the relationship between adiponectin and glucose levels in a racially diverse sample. In particular, the possibility that the relationship might differ in obese versus normal weight youth should be explored, since the only significant correlation noted thus far has been found using a sample of only obese children (Gilardini et al., 2006).

Dyslipidemia

Adiponectin also appears to be related to blood levels of different types of lipids, and to their oxidation. Studies with mice have shown adiponectin to increase fatty acid oxidation (Fruebis et al., 2001; Yamauchi et al., 2001; Yamauchi et al., 2002). Results also indicate that administration of adiponectin leads to a decrease in levels of triglycerides in skeletal muscle and liver tissues, and a decrease in serum free fatty acids and triglycerides (Yamauchi et al., 2001). Weiss et al. (2003) noted an inverse correlation between adiponectin and free fatty acids in adolescents ($r = -.45$, $p < .05$), although the relationship was no longer significant when adjusted for total fat percent. In obese adolescents only, adiponectin is strongly inversely related to intramyocellular lipid content ($r = .-78$), even when controlling for total fat percent and central obesity as measured by magnetic resonance imaging (Weiss et al., 2003). Lastly, regarding the relationship between adiponectin and lipids, Retnakaran et al. (2006) conducted a factor analysis of adiponectin and factors related to the metabolic syndrome such as insulin, HDL-C, and triglycerides among others, in a sample of 236 Native Canadian adolescents. The results of this study indicated that adiponectin loaded most strongly on a factor with the lipid measures of HDL-C, Triglycerides and a ratio of apolipoprotein B to apolipoprotein A-1. Although adiponectin did have a factor loading

value of .23 for the insulin and glucose tolerance factor, it did not reach the .30 cut-point level, and also did not significantly associate with the factor reflecting adiposity measures.

Studies in adults have shown adiponectin to be positively associated with HDL-C (Abbasi et al., 2004; Pischon et al., 2004; Schulze, Rimm, Shai, Rifai, & Hu, 2004), even when controlling for BMI (Baratta et al., 2004). A recent study in adults by Vergès et al. (2006) found that adiponectin was positively associated with HDL-C ($r = .43$, $p < .05$), and also inversely associated with catabolism of apolipoprotein A-1, one of the chief apolipoproteins of HDL-C; the latter association remained significant when controlling for BMI, waist, triglycerides, HDL-C, insulin resistance, age and gender. Adult studies have also shown adiponectin to be inversely related to triglyceride levels (Pischon et al., 2004; Schulze et al., 2004). It will therefore be important to examine the relationship between adiponectin and various types of lipids in this sample of children and adolescents.

High-Density Lipoprotein Cholesterol and Adiponectin

Similar to studies in adults, adiponectin is positively associated with HDL-C in almost all of the studies in children, even when controlling for measures of adiposity such as BMI, waist circumference or percent fat mass in most studies (Asayama et al., 2003; Chu et al., 2005; Bacha et al., 2004; Bottner et al., 2004; Butte et al., 2005; Gilardini, et al, 2006; Huang et al., 2004; Nemet et al., 2003; Okada et al., 2005; Pilz et al., 2005; Singhal et al., 2005). Bacha et al. (2004) found that adiponectin explained 45% of the variance in HDL-C, independent of BMI. Correlations overall ranged from $r = .19$ (Chu et al., 2005) to $r = .52$ (Bacha et al., 2004). Vikram et al. (2004) were the only researchers who did not find a significant relationship between adiponectin and HDL-C; they found no relationship in regression with hs-CRP, BMI, %body fat, sum of skinfolds, waist circumference,

triglycerides and waist/height ratio. The authors did not report whether or not they tested for a bivariate correlation.

Effects of Gender, Race and Overweight Status on the Relationship Between Adiponectin and HDL-C

Gender

Gender may moderate the relationship between adiponectin and HDL-C. Although there was a correlation between the two variables in both male and female subjects in one study (Chu et al., 2005), relationships in other studies were primarily in female subjects only (Singhal et al., 2005; Okada et al., 2005). Singhal et al (2005) found a positive correlation between adiponectin and HDL-C in the overall subject group (p=0.01), but a borderline correlation only in females when analyzed by gender (p=.05); the p value for males was 0.07. Okada et al. (2005) noted positive correlations in female subjects only; no correlation was noted in male subjects. Female and male subjects in this study were similar in BMI and body fat measures, females had higher sum of skinfolds than males. In contrast, Asayama et al. (2003) found that there was no correlation between HDL-C and adiponectin independent of gender. Other studies adjusted for gender in their analyses (Bottner et al, 2004; Butte et al., 2005; Gilardini et al., 2006; Huang et al., 2004; Martin et al, 2005; Pilz et al., 2005), but did not analyze by gender.

Race and Overweight Status

Only one study has examined the relationship between adiponectin and HDL-C in a sample of Black and White youth (Martin et al., 2005), even though HDL-C levels are known to vary by race, with higher levels noted in Black adolescents than in White adolescents

(Cook et al., 2003; Jago et al., 2006). In addition, in adult subjects, Ferris et al. (2005) found that race may moderate the relationship between HDL-C and adiponectin. In their study, HDL-C was correlated with adiponectin only in White and Asian Indian subjects, and not in Black subjects. The one study in youth (Martin et al., 2005) controlled for race, but did not analyze to see if the relationship between adiponectin and HDL-C varied by race. Their results did show that the relationship differed by overweight status. A positive association was statistically significant in both lean (BMI less than the 85th percentile) and non-lean subject groups when adjusting for race, gender and age, but the relationship was stronger in the non-lean group (β coefficients = .47 +/- 0.01 and .94 +/- 0.13, respectively) when compared by a type of t-test (p < .0001).

In summary, there is a positive relationship between adiponectin and HDL-C in a variety of studies, but there is a need for more research in samples that include Black and White subjects since racial disparities exist regarding HDL-C. In addition, gender and overweight status may affect the relationship between the two variables.

Triglycerides and Adiponectin

Also similar to studies in adults, most studies that have examined the relationship between adiponectin and triglyceride levels in children have found the two variables to be inversely correlated (Asayama et al., 2003; Cianflone et al., 2005; Gilardini et al., 2006; Huang et al., 2004; Pilz et al., 2005; Weiss et al, 2003). Correlations ranged from r = -.07 (Chu et al., 2005) to r = -.80 (Weiss et al., 2003). In the study by Weiss et al. (2003), the relationship between adiponectin and insulin sensitivity was no longer significant when triglycerides were added to the regression equation. The inverse correlation between adiponectin and triglycerides in the study by Huang et al. (2004) was lost when the variables

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of age, gender, HDL-C were included in regression; only HDL-C and age were significantly related to adiponectin. Singhal et al. (2005) found no correlation between adiponectin and triglycerides in models with variables such as gender, insulin, HDL-C, LDL-C, leptin, and measures of vascular function. Only one study found no bivariate correlation between adiponectin and triglycerides (Vikram et al., 2004); the sample in this study was all male, and relatively lean.

Effects of Overweight Status and Gender on the Relationship Between Adiponectin and Triglycerides

Overweight Status

The relationship between adiponectin and triglycerides may vary depending on measures of adiposity. The relationship was independent of BMI or percent body fat in two studies (Pilz, et al., 2005; Weiss et al., 2005). Asayama et al. (2003) found the relationship between adiponectin and triglycerides to be independent of percent overweight and percent body fat, but dependent on visceral and subcutaneous adipose tissue in their sample of obese children and adolescents aged 6-14 years. Martin et al. (2005), the only research group to examine the relationship of triglycerides and adiponectin in a racially diverse sample, grouped subjects into lean and non-lean (>85th percentile for BMI) and used race as a covariate in analyses. They found an inverse relationship between adiponectin and triglycerides in both subject groups, but reported a stronger relationship in the non-lean subjects ($p < 0.0001$).

Gender

The relationship between adiponectin and triglycerides may also be moderated by gender. For example, Okada et al. (2005) found an inverse correlation in female subjects only. Male and female subjects in this study were similar in BMI and body fat measurements; only sum of skinfolds was higher in female subjects. Chu et al. (2005) found a small inverse correlation (r = -.07) between adiponectin and triglycerides in female subjects only. Vikram et al. (2004) explored the relationship with a sample of male subjects only, and found no correlation.

In summary, research in children indicates an inverse association between adiponectin and triglycerides overall, but gender and overweight status may influence the relationship. Only one research group has examined the relationship in a sample that includes Black and White subjects. More research is needed to explore the relationship between adiponectin and triglycerides in a racially diverse sample, and to clarify the influence of gender and overweight status.

Low-Density Lipoprotein Cholesterol and Total Cholesterol and Adiponectin

In contrast to relationships between adiponectin and HDL-C and triglycerides, researchers have found little association between adiponectin and LDL-C in adults (Hulthe et al., 2003; Schulze et al., 2004). Relatively few have examined this relationship in children (Asayama et al., 2003; Bacha et al., 2004; Huang et al., 2004; Singhal et al., 2005; Okada et al., 2005; Pilz et al., 2005). No studies have been done to examine the relationship between adiponectin and LDL-C in a racially diverse sample. Only one research group reported a significant association; Okada et al. (2005) noted an inverse correlation between adiponectin and LDL-C (r = -.19, p < .05) in female subjects only; no correlation was noted in male

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subjects. The majority of the studies seemed adequately powered, with relatively large sample sizes such as 283 in the study by Okada et al. (2005) or 294 in that of Singhal et al. (2005). Interestingly, researchers have found that adiponectin levels are positively associated with LDL particle size in adults (Hulthe et al., 2003), and small LDL particle size is a risk factor for atherosclerosis (Carmena, Duriez, & Fruchart, 2004). LDL particle size is one example of a variable that might be relevant to my model, but is not available for analysis.

Total cholesterol is another lipid variable that has little association with adiponectin. For example, no correlation was noted between adiponectin and total cholesterol in one study with adult subjects (Park et al., 2004). Relatively few research groups have examined this relationship in youth (Asayama et al., 2003; Bacha et al., 2004; Chu et al., 2005; Huang et al, 2004; Okada et al., 2005), and none have found a significant association. Huang et al. (2004) and Asayama et al. (2003), however, found inverse correlations between adiponectin and a total cholesterol/HDL-C ratio (r = -.29, p=0.001 and r = -.28, p=0.05, respectively), adjusted for age and/or gender. These correlations indicate that decreased adiponectin is associated with increased total cholesterol and/or decreased HDL-C, but the relationship is most likely driven by HDL-C levels.

In summary, adiponectin apparently has a limited relationship with LDL-C or total cholesterol, despite the evidence suggesting an association with LDL-C particle size. Gender may influence the relationship, at least for LDL-C. No one has examined the relationship between adiponectin and LDL-C, however, in a racially diverse sample. More research is needed to explore the relationship of adiponectin and LDL-C in Black and White youth, and to clarify how the relationship between adiponectin and LDL-C may possibly vary by gender.

Blood pressure and Adiponectin

Blood pressure is included in the framework as it is a well-known risk factor for CVD, and research in adults has shown it to be inversely correlated with adiponectin. Iwashima et al. (2004) found that adiponectin was significantly decreased in adult hypertensive subjects, independent of a relationship with BMI, age and cholesterol. Kazumi, Kawaguchi, Sakai, Hirano, Yoshino (2002) noted that males 18-26 years or age with high-normal blood pressure had significantly lower adiponectin levels than subjects with optimal blood pressure levels.

Relatively few researchers have examined the relationship between adiponectin and blood pressure in children and adolescents, but most studies indicate an inverse relationship with SBP and or DBP. Huang et al. (2003) found inverse relationships in their study of a 68 Taiwanese female adolescents; correlations between adiponectin and blood pressure variables in their study ranged from (r = -.29) for SBP and (r = -.30) for DBP. Bacha et al. (2004), also noted inverse relationships between adiponectin and both SBP and DBP in a study of 49 White male and female teens (r = -.39 and -.33, respectively). Other researchers, however, found adiponectin to be inversely correlated with SBP alone (Gilardini et al., 2006) or have only mentioned the correlations with SBP and not DBP in their papers (Bottner et al, 2004; Butte et al., 2005). The relationship between adiponectin and both blood pressure variables may differ somewhat by gender. Adiponectin was inversely related to both SBP and DBP in Taiwanese adolescent females (r = -.13 and -.11, respectively), but only to SBP in males (r = -.10) (Chu et al., 2005). Overweight status may also influence the relationship between adiponectin and blood pressure.

Effects of Overweight Status on the Relationship Between Adiponectin and Blood Pressure

Studies that have looked at the impact of overweight status or adiposity on the relationship between adiponectin and blood pressure in youth have shown inconsistent results. Bottner et al. (2004) examined the relationship between adiponectin and SBP and found an inverse relationship in the obese subject group (r = -.22), but not in the lean subjects. Gilardini et al. (2006) reported an inverse relationship in a sample of children and adolescents who were all obese, with BMIs > 97th percentile. In contrast, Ogawa et al. (2005) found no relationship between adiponectin and either SBP or DBP in their study of 100 Japanese male children, all of whom were obese.

Results of studies that included BMI or %fat mass as covariates in regression equations have also been somewhat contradictory. In the study by Bacha et al. (2004), adiponectin was inversely correlated with both SBP and DBP in bivariate analyses, but the correlation was no longer significant when BMI was included multiple regression. In contrast, in models that included BMI, HDL-C and insulin, adiponectin was a significant predictor of SBP, but not DBP in Taiwanese female adolescents (Huang et al, 2003). Butte et al. (2005) reported an inverse association of adiponectin with SBP that was independent of % fat mass, age and gender in their study of Hispanic children and adolescents. BMI was inversely related to adiponectin in this study, but the authors did not include it as a variable in regression equations.

Two researchers found no significant relationship between adiponectin and blood pressure, but samples for both were fairly lean (Singhal et al., 2005; Tsou et al., 2004). Singhal et al. (2005) found no correlation with SBP or DBP in their study of 294 British teens. Their sample was fairly lean, with approximately 73% of their sample having a BMI

less than the 85th percentile. Tsou et al. (2004) also found no correlation in their study of 500

Taiwanese male and female school children who took part in a national survey; these subjects

were also apparently fairly lean, with mean BMI levels well within normal limits. The group

with the highest mean BMI was the 15-18 year old girls; the mean BMI was 20.18 plus or

minus a standard deviation of 2.47.

In summary, adiponectin is inversely associated with SBP, and sometimes with DBP,

in most studies that have been done with children and adolescents. The effects of measures

of adiposity on the relationship have been inconsistent. Lastly, even though blood pressure is

higher in Black adolescents and adults than in Whites (Cook et al., 2003; Mensah et al.,

2005; Winkleby, Robinson, Sundquist, & Kraemer, 1999), no researchers have examined the

relationship of adiponectin and SBP or DBP in a racially diverse sample of children and

adolescents.

Puberty and Adiponectin

Low adiponectin is associated with insulin resistance, which increases during puberty,

especially in girls (Goran & Gower, 2001). Therefore it is important to examine the

relationship of adiponectin to pubertal level. However, relatively few researchers have

examined changes in adiponectin during puberty or its relationship with pubertal stage, and

the results have been conflicting. Moreover, no one has examined the relationship of

adiponectin and pubertal status in Black and White children and adolescents, although one

group did measure pubertal stage in a racially diverse sample (Woo et al., 2005); race was

used only as a confounding variable in this study, however, and was adjusted for in

regression models. In addition, measurement of pubertal stage has varied widely among the

studies.

Results of studies in youth are somewhat conflicting, but most have shown that adiponectin is lower in subjects who are in the latter stages or puberty, or is inversely correlated with pubertal stage (Bottner et al., 2004; Butte et al., 2005; Punthakee et al., 2006; Reinehr et al., 2004; Tsou et al., 2004; Woo et al., 2005). Some research groups found that adiponectin decreases by pubertal status, but did not examine changes over all five stages, choosing rather to group data from the five stages into two or three stages. Reinehr et al. (2005) measured pubertal stage by Tanner criteria, but grouped their subjects into two groups, a pre-pubertal group comprised of subjects in pubertal stage 1, and a pubertal group comprised of subjects in pubertal stage 2 or later. They found that adiponectin was lower in pubertal subjects than pre-pubertal, even though there were no differences overall between the two groups in a standardized BMI score, gender and percent body fat. Butte et al. (2005) measured five Tanner stages in their sample of Hispanic children and adolescents using a self-report instrument with drawings for illustration, but only compared pre-pubertal subjects to pubertal subjects. They also found that adiponectin was lower in the pubertal subjects.

Punthakee et al. (2006) also reported a negative correlation, this time examining the relationship between adiponectin and three pubertal stages. They used a self-report tool to measure three pubertal stages (pre-, intra- and post-pubertal) in a large sample of White youth aged 9, 13 and 16 years. Subjects were asked to indicate whether they had "not started, barely started, definitely started, or stopped changing…pubic hair, facial hair and voice change for boys, or pubic hair and breast development for girls," (p.5) or whether girls had begun menstruation. Subjects were classified as pre-pubertal if they had not started changing in any area, and post-pubertal if they had stopped changing or the girls had started menstruation. Any other responses were classified as intra-pubertal. The post-pubertal

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classification based on menarche is problematic because menstruation may begin in Tanner stages 3 or 4 of breast development (Faulkner and Tanner, 1986), therefore menarche would not actually signal the end of puberty. Moreover, according to information about subject characteristics, only 129 girls were classified as post-pubertal out of a total of 828. This is odd when one considers that there were 333 16-year old female subjects. Even if all 129 of the post-pubertal subjects were in the 16 year old age group, that would mean that 61% of the 16 year old girls had not started menstruation. Results of other studies have also indicated an inverse relationship between adiponectin and pubertal stage, but the relationship may be influenced or moderated by overweight status or gender.

Effect of Gender or Overweight Status on the Relationship Between Adiponectin and Pubertal Stage

Tsou et al. (2004) compared adiponectin levels in male and female subjects aged 6-18 years. They did not measure tanner stages, but rather used five chronological age groups to approximate the five pubertal stages. They found that adiponectin levels decreased at about age 10-12 years in males, whereas adiponectin levels remained stable across age groups in the female subjects. Adiponectin levels in males rebounded at approximately age 16, coinciding with an increase in testosterone. Woo et al. (2005) measured pubertal stages in their Black and White subjects by using a combination of sexual developmental milestones such as time of menarche in girls and appearance of axillary hair development in boys, and cut-points for estradiol and testosterone, to assign pubertal stage. Adiponectin levels were lower in the pubertal and post-pubertal subjects, as compared to the pre-pubertal reference group, adjusting for gender, race, BMI z-score and a logarithmically transformed HOMA-IR score. Subsequent analysis including a pubertal stage*gender interaction term indicated that

differences by pubertal stage were only evident in male subjects. Results of further analyses showed that the relationship between adiponectin and pubertal stage may vary by overweight status as well as gender.

Woo et al. (2005) examined interactions between pubertal status and gender separately in overweight (>85th percentile) and non-overweight groups. Results of this analysis showed that adiponectin levels were lower as pubertal stage increased in overweight and non-overweight boys and in overweight girls, but remained stable across pubertal stages in non-overweight girls. No other studies have examined these types of relationships in samples of varying overweight status. Two studies have, however, studied the relationship between adiponectin and pubertal stage in groups that were comprised of either only overweight children , or only lean children.

Bottner et al. (2005) looked at the relationship between pubertal status and adiponectin by gender in only the lean subjects of their sample. Similar to Woo et al. (2005), they found that adiponectin levels decreased during puberty only in boys. They further noted that pubertal stage, standardized BMI and testosterone predicted 29% of the variance in adiponectin in a regression model with age, non-standardized BMI, waist, hip, SBP, and DBP in the lean male subjects. Gilardini et al. (2006) measured pubertal stage during a physical exam by a pediatric endocrinologist. They studied male and female children and adolescents who were all obese, but unlike the study by Woo et al. (2005), found no differences by pubertal stage; they did not examine for differences by gender. More research is needed to examine the possible moderator effect of overweight status on the relationship between adiponectin and pubertal stage with samples comprised of subjects with varied overweight status.

Similar to the study by Gilardini et al. (2006), other researchers have found no difference in adiponectin by pubertal stage, or no correlation between adiponectin and pubertal stage (Singhal et al., 2005; Gilardini et al., 2006; Huang et al., 2004). Singhal et al. (2005) found no correlation between adiponectin and pubertal stage in their study of 294 British adolescents in whom the mean tanner stage was four. However, the method for measurement of pubertal stage was difficult to discern in this study. The authors stated they used a "self-assessment" (p.4616) measure and referred readers to another reference. The reference did not mention a self-assessment measure for Tanner stage, and in fact did not mention Tanner stage as a study variable. Huang et al. (2004) measured pubertal stage by self-report in Taiwanese adolescents, and also found no association between adiponectin and pubertal stage. However, most of the subjects in this study were in the middle to latter stages of puberty. Out of 230 subjects, only 6 subjects were in pubertal stage 1, and 8 in pubertal stage 2; in fact, greater than one half of the subjects were in pubertal stages 4 or 5.

In summary, adiponectin appears to decrease across pubertal stages in most studies, but results are somewhat inconsistent. The relationship between adiponectin and pubertal stage may be moderated by gender or overweight status. More research is needed to determine whether the relationship between adiponectin and puberty differs by gender or overweight status, using samples that contain both lean and overweight subjects. A wide variety of measures have been used to assess pubertal status and several have compressed data into only pre- and post-pubertal stages, and the validity of others is questionable (Singhal et al., 2005; Punthakee et al., 2006). Lastly, very little research has been done to examine these differences in a racially diverse sample even though, at least in girls, pubertal development differs by race (Wu, Mendola, & Buck, 2002). More research needs to be done

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to investigate changes in adiponectin across all five pubertal stages in racially diverse samples of children and adolescents.

Family history of CVD or T2D and Adiponectin

Both CVD and T2D have strong genetic influences and adiponectin appears to be associated with CVD and T2D by genetic mechanisms, so one might expect adiponectin to be lower in subjects with a positive family history of CVD and or T2D. An I164T mutation in the gene for adiponectin has been shown to occur more frequently in subjects with coronary artery disease and T2D than in controls of similar age and BMI (Kondo et al., 2002; Ohashi et al., 2004). Comuzzie et al. (2001) found that heredity contributed substantially to plasma concentrations of adiponectin in European adults. In another study, Lihn et al. (2003) studied adult first degree relatives of patients with diabetes, and found the adiponectin in the group of relatives was lower than in subjects in a comparable control group.

Only a few researchers have looked at the association of adiponectin levels with a family history of diabetes in children, and none have examined it in an ethnically diverse sample (Gilardini et al.,2006; Butte et al., 2005; Punthakee et al., 2006). Moreover, no researchers have investigated the relationship between adiponectin and a family history of CVD in children. Butte et al. (2005) are the only researchers that measured family history by asking about parents and grandparents, a group more likely to be older, and therefore more likely to potentially exhibit signs of or have a diagnosis of T2D. They found no difference in adiponectin by family history group, although 64% of their subjects had a positive family history of T2D, and the heritability of adiponectin was high (.93) in their sample of Hispanic children and adolescents. Punthakee et al. (2006) who studied a large sample of French Canadian youth, found no correlation between adiponectin and a history of diabetes in a

parent. Gilardini et al. (2006) found no difference in adiponectin by family history of obesity or diabetes in their sample of Italian youth who were all overweight, with BMI's $> 97^{th}$ percentile.

In summary, adult relatives of persons with diabetes have lower levels of adiponectin than those without a positive family history, and genetic mutations are more frequent in subjects with cardiac disease and diabetes. In addition, adiponectin displays substantial heritability in adults and children. In spite of these findings, studies in children thus far have shown no association between adiponectin and family history of diabetes, even in the one study (Butte et al., 2005) that assessed for a family history of diabetes in older relatives. Further research in ethnically diverse samples of children and adolescents is needed to determine whether the heritable influences of adiponectin are evident through a positive family history of diabetes and/or CVD.

Physical Fitness and Adiponectin

Physical fitness, as measured by VO_2, is included in the model because of its positive effect on CVD and T2D risk factors (Carroll & Dudfield, 2004), and possible relationship with adiponectin. Most lifestyle interventions that modify diet and physical activity and result in weight loss of even a relatively small percentage in adults or children result in increased adiponectin levels (Balagopal, George, Yarandi et al., 2005; Esposito et al., 2003; Monzillo et al., 2003; Reinehr et al., 2004). Moreover, Kriketos et al. (2004) noted an increase in adiponectin after a 10-week program of 40 minutes of aerobic exercise 4-5 times a week, even without a change in weight. A recent study by Blüher et al. (2006) tested adiponectin receptor m-RNA levels before and after an intensive three hour bike ride, and noted a 3-fold increase in AdipoR1 and a 5-fold increase in AdipoR2 in skeletal muscle.

Therefore, it would seem likely that adiponectin levels may be related to levels of physical fitness or physical activity, either directly or indirectly by promotion of weight loss.

Some researchers have looked at adiponectin and VO_2 in adults while others have assessed the relationship between adiponectin and physical activity. The findings on the effect of either on adiponectin levels in adults are somewhat contradictory. Schulze, Rimm, Shai, Rifai and Hu (2004) found no significant correlation between adiponectin and physical activity, whereas Ryan, Berman, Nicklas et al. (2003) found that $V0_2$, a measure of physical fitness, was positively associated (r = .25, p < .005) with adiponectin levels in 18-81 year old women.

Very little research has been done to describe the relationship between adiponectin and physical fitness or activity in children, and results of the few studies have either shown an inverse relationship or no relationship between adiponectin and VO_2, or physical activity. Nemet et al. (2003) examined the relationship of adiponectin to physical fitness as measured by maximum VO_2 in a small sample of adolescents. Surprisingly, these researchers found that high adiponectin was associated with lower VO_2 levels. The authors suggested this was because some of their subjects had increased adipose tissue and therefore lower adiponectin, but also had increased muscle mass that made the VO_2 higher. They explained, "our data suggest that potentially beneficial effects of exercise in obese children may be diminished in the absence of accompanying decreases in fat mass." (p.151). Butte et al. (2005) investigated the relationship between adiponectin and VO_2 peak in a large sample of Hispanic children and adolescents. The authors stated that adiponectin was inversely related to VO_2 (r value not given) in initial analyses, but the relationship was not significant when adjusting for age, gender, family membership, and percent fat mass and fat free mass as measured by DEXA.

Finally, in a large study of French Canadian children and adolescents, Punthakee et al. (2006) found no association between adiponectin and physical activity, as measured by recall of activities performed at least 15 minutes a day over 7 days. The physical activity means were very low in these subjects; with a potential maximum score of 126, means were only 9.6 +/- 7.7 in boys and 7.7 +/- 6.5 in girls.

In summary, the relationship between adiponectin and physical fitness or activity has been contradictory in adults, and few studies have examined it in children and adolescents. No researchers have investigated this relationship in samples comprised of Black and White youth. The studies that have looked at this issue in youth have either found no relationship, or an inverse relationship that was lost when controlling for demographic and adiposity measures. Further research may help to clarify the relationship between adiponectin and physical fitness in youth.

Summary of Literature Review

In conclusion, adiponectin is an adipocytokine that is protective against CVD and T2D, but decreased in obesity or overweight. Researchers have shown inverse correlations with many risk factors for CVD and T2D and positive correlations with other protective factors such as HDL in adults and youth. However, there has been little research done with samples of children and adolescents to examine the relationship between adiponectin and physical fitness or activity, or a family history of T2D or CVD. Few studies in children and adolescents have looked at how race, gender or overweight status might moderate the relationship of adiponectin with risk factors for CVD and T2D. In addition, relatively little is known about the strength of the contribution of clinically relevant measures of adiposity such

as BMI, waist circumference and sum of skinfolds to adiponectin levels, and results have been contradictory.

Moreover, relatively few research groups have examined the above relationships in racially diverse samples that include Black and White youth. Most of the studies regarding adiponectin and risk factors for CVD and T2D that have been conducted with such samples have focused on the relationship between adiponectin and insulin resistance and sensitivity, and findings are somewhat conflicting in regard to the impact of race on the relationship between adiponectin and insulin or insulin sensitivity. Only one study has explored the relationship between adiponectin and HDL-C or triglycerides in a racially diverse sample, and no studies have examined the relationship between adiponectin and blood pressure, VO_2, or family history in such samples.

This will be the first study to examine the inter-relationships between adiponectin and this many different risk factors and demographic factors in a large sample of Black and White children and adolescents. Most of the groups who examined the relationship of adiponectin to risk factors in racially diverse groups of youth used relatively small samples of 44-161 subjects. The two largest studies (Martin et al., 2005; Woo et al., 2005) were done with the same sample, and focused primarily on relationship of adiponectin to HDL-C, triglycerides and insulin or puberty, gender and overweight status. In addition, Woo et al. (2005) and Martin et al. (2005) did not mention adjustment for possible clustering effects, even though subjects in the study were selected from students who were taking part in a large school based study. Moreover, both groups adjusted for pubertal stage or studied its relationship with adiponectin, but their measure of puberty included only 3 stages, with the middle stage encompassing pubertal stages 2-4. By structuring their pubertal stage variable

in this manner, the authors effectively lost pubertal stage data during a time when many metabolic changes occur.

This study will provide a unique opportunity to expand knowledge regarding the relationship between adiponectin and risk factors for CVD and T2D in children and adolescents of racially diverse backgrounds, and how the relationships may vary by race, gender or overweight status. The findings from this study will lay the groundwork for future research concerning adiponectin and youth, as well as for the development of lifestyle interventions that may help to increase adiponectin levels and reduce the risk of chronic disease during childhood as well as in the future.

Chapter 3

METHODS

Introduction

This descriptive, cross-sectional, comparative and correlational study was a

secondary analysis of data from the Cardiovascular Health in Children and Youth III (CHIC

III) study, a study concerned with the longitudinal development of the metabolic syndrome

and cardiovascular health in children and adolescents. Baseline data were collected from

subjects between February 2000 and June 2001, and blood samples were stored at -80° C.

The existing data set includes demographic variables, and variables reflecting family history,

pubertal level, and risk factors for CVD or T2D. All of the variables for this study were

collected at the baseline time point in the CHIC III study. The General Clinical Research

Center (GCRC) at the University of North Carolina at Chapel Hill approved funding for

analysis of the stored blood samples for adiponectin. The analyses were completed in the

GCRC Bio-Analytical Core Lab, and results from the lab analyses were used in statistical

analyses to answer the research questions in this dissertation regarding the relationships

between adiponectin and risk factors for T2D and CVD.

Setting and Sample for the CHIC III Study

Introduction

Subjects were recruited from 34 elementary and high schools in three eastern North Carolina counties. Criteria for school selection included a high proportion of minority students and a rural location. Inclusion criteria for subjects were the following: a) age 8-18; b) ability to read and write English; c) no physical handicap as reported by parents, teachers, school nurse, or child; d) no serious disease such as type 1 diabetes requiring insulin, renal disease, or moderate to severe asthma as reported by parents, teachers, school nurse, or child; e) no major developmental disability as reported by parents; and f) at least one natural relative available to report family history. Data was originally collected from children of Non-Hispanic and Hispanic ethnicity, and Black, Asian/Pacific Islander, White, Native American, and 'other' race. Only 6 subjects responded as being "Non-Hispanic, Asian/Pacific Islander" when asked about ethnicity and race, and only 14 responded as being "Non-Hispanic, Other." The numbers of Hispanic, and non-Hispanic Native American subjects were also small at 49 and 33, respectively. Due to the small numbers of the subject groups mentioned above, only data from non-Hispanic Black and White children were used in this study.

Subjects in the CHIC III study were recruited by presentations given to students at school assemblies and by packets given to parents. Parental consent for the child to participate included permission to store blood for use in future studies related to obesity or cardiovascular disease. Parents were also asked to complete family information forms for data related to lifestyle habits and family history (see appendices B and C for Family Health

History Portion of Mother or Father's Questionnaire). Children received $20 and small gifts such as pencils and stickers for their participation in data collection.

Choice of subjects for current study

Subjects for this study were selected from 2211 subjects who participated in baseline data collection for CHIC III study cohorts 4 and 5. Mean values for study variables were compared to determine if there were any differences between other sample-selection based groups. Comparisons were made between groups of subjects that did and did not have stored serum samples, and between groups that had stored blood but did not have complete data on most study variables.

Of the 2211 subjects at baseline of cohorts 4 and 5, 1645 had stored blood available for analysis, and 566 did not. Mean values of other study variables were compared by these 2 groups. At an alpha level of 0.05, mean values of BMI z-score, waist circumference, sum of skin folds and VO_2 would have differed significantly. However, with a Bonferroni correction for 10 separate tests, the alpha level was reduced to 0.005. At this level of alpha, only waist and sum of skin folds remained significantly different. Subjects without stored blood had higher waist and sum of skin fold values. The differences were minimal, however, at 21% and 29% of 1 standard deviation, respectively. It is difficult to speculate as to why subjects without stored blood would have had a higher waist or sum of skin fold value. One possibility might be that extra adipose tissue might have made it more difficult to find a vein from which to draw a blood sample.

Mean results of study variables for groups of subjects who had stored blood, but did or did not have complete data on variables of interest were also compared. The 2 groups

were compared on mean values of age, waist circumference, sum of skin folds, BMI z-score, insulin, glucose, HDL-C, LDL-C, triglycerides, mean systolic blood pressure, mean diastolic blood pressure, VO_2 and by race, gender and pubertal stage. At an alpha level of 0.05, mean values of triglycerides and insulin would have differed significantly. After the alpha was adjusted based on a Bonferroni correction for 14 separate tests, there were no significant differences between the 2 groups, those with and without stored blood.

From the 1,645 subjects with stored samples, 1241 subjects were selected to have their stored serum samples analyzed for adiponectin for the current study. These subjects were selected because they had no missing data on all variables of interest, except family history, under the assumption that any missing values were completely at random or at least at random. The only variables with missing values were those that indicated whether or not a subject had a family history of either CVD or diabetes. Due to the difficulties encountered during the CHIC III study in obtaining completed family history information forms from all parents, family history data only existed for approximately 587 subjects.

In order to determine if any bias existed due to absence of data on family history, mean values of other study variables such as BMI z-score, waist, sum of skin folds, etc. were compared between groups of subjects who did and did not have complete family history data on CVD or diabetes. At an alpha level of 0.05, the only variable that was different between groups with and without data family history was LDL-C. Subjects who did not have data on family history data on CVD or diabetes had lower LDL-C levels than those who did have complete data. However, when a Bonferroni correction was utilized because of an increased number of statistical tests, the alpha level for significance became 0.0036 and the differences

in LDL-C were no longer significant. The two groups, those with and without family history

data, were therefore similar in regards to all major study variables.

Some of the 1241 subjects were deleted from the study because of problems with the

lab values obtained when analyses for adiponectin were done. Adiponectin values for 98 of

the 1241 subjects with complete data on all variables except family history were noted to be

over the standard curve of the lab assay. All but 24 samples were diluted and re-run by the

GCRC BAC lab. One of the 24 samples was not re-done because of lab technician error, and

the remaining 23 samples could not be re-run due to financial constraints. Adiponectin levels

for 2 of the 64 samples that were re-run remained above the standard curve, resulting in a

total of 26 samples with questionable results. A representative from the R&D Lab Systems

company, the company that manufactures the assay kits, was consulted regarding the validity

of the 26 values that were greater than the standard curve. Although the lab software

extrapolated the values for these subjects based on the standard curve, the R&D

representative stated the company could not guarantee the extrapolated values and advised

this investigator that the values should not be trusted. Therefore, the 26 subjects were

deleted from the data set, leaving the final sample n at 1215.

A proc univariate procedure was done, using SAS, to evaluate the normality of the

sample distribution in relation to adiponectin. The mean (sd) for adiponectin was 12.56

(6.58), the skewness value was 0.883, and the kurtosis value was 1.468. The Shapiro-Wilkes

statistic was 0.959. Taken together, these statistics indicated the distribution of adiponectin

in the sample was fairly normal. However, on inspection of the maximum extreme values,

two subjects had adiponectin values that were several points higher than the preceding

values. The values leading up to the two possible outliers were 38.25 and 39.41, and the two

highest values were 46.90 and 49.93. In order to evaluate the possible influence of the 2

extreme values, models with adiponectin as the dependent variable and all other study

variables as independent variables were therefore run with, and without, the 2 maximum

extreme values. No differences were noted in the results of the 2 models. Therefore, the full

sample of 1215 was used for subsequent analyses. This sample is described in further detail

in table 1.

Data Collection Procedures Used in the CHIC III Study

All data were collected in waves between January 2000 and February 2003 by

trained, certified research assistants (RAs) as part of the CHIC III study. RAs were trained in

the collection of blood samples, anthropometric measures and blood pressure, and they were

given written procedural manuals for use in the field. Inter-rater reliability testing for the

blood pressure and anthropometric assessments was done prior to and during data collection;

only those RAs with acceptable reliability were allowed to collect data. Subjects were called

the night before they were scheduled to have their blood drawn and reminded not to eat or

drink anything except water after 10:00 PM. Blood samples were taken from all subjects

early in the morning while the subjects were fasting. Each subject was questioned by a

research assistant before blood samples were drawn, about whether or not they had truly

fasted overnight. Breakfast was provided following collection of blood samples. Aliquots of

blood were separated into serum and plasma and labeled in the field, then placed on dry ice

until they were transferred to a freezer in the Applied Physiology Laboratory at UNC-CH for

storage at – 80 degrees centigrade until needed for lab analysis. Other physiologic data were

collected later in the day, in the following order: a) height, weight, triceps and subscapular

Table 1

Demographic Characteristics of Sample

		Number of subjects	Percent of total
Total sample		1215	100%
Age (years)			
	7-9	363	29.9%
	10-11	462	38%
	12-15	216	17.8%
	16-18	174	14.3%
Gender			
	Female	603	49.6%
	Male	612	50.4%
Race			
	Non-Hispanic Black	712	58.6%
	Non-Hispanic White	503	41.4%
Pubertal Stage			
	I	253	20.82%
	II	306	25.18%
	III	314	25.84%
	IV	282	23.21%
	V	60	4.94%

skin folds and waist circumference; b) blood pressure (after sitting quietly for at least 2 minutes); and c) aerobic power, using multiple stations. The anthropometric, blood pressure and aerobic power, or cardiovascular fitness data were recorded on the "Physiological Data" form, shown in appendix D. Subjects completed a gender specific Pubertal Development Scale (see appendices E and F) with the assistance of an RA of the same gender as the subject.

Data were entered by two different research assistants into a SAS database, then compared for accuracy; inaccuracies were corrected after examining original subject records. Data related to all of the original CHIC III variables has been cleaned and verified. This existing data set is available for analysis in the current study. The University of North Carolina at Chapel Hill Institutional Review Board has given approval for this study, assigning it an exempt status.

Variables From the CHIC III Study and Their Measurement

CHIC III Study variables that were used in this study are summarized in Table 2 (Summary of CHIC III Variables and Levels of Measurement), with procedural details about the measurement of the variables given below. The variables were used in analyses in a continuous and/or categorical manner, depending on the research question to be answered.

Total Cholesterol, LDL-C, HDL-C and Triglycerides

In the original CHIC III study, HDL-C, triglyceride, total cholesterol, and LDL-C levels were measured in fasting plasma samples by the UNC Hospitals Core Laboratory, which is certified by the College of American Pathologists. Cholesterol and triglyceride

Table 2

Summary of CHIC III Variables and Their Measurement

Domain	Name of Variable	Scale[1]	Description of Variable	When Recorded
Demographic factors				
	Age	C	Age of subject in years	baseline
	Race	N	Non-Hispanic Black, Non-Hispanic White	baseline
	Gender	N	Subject's sex	baseline
	Family History	N	Family history of CVD or diabetes in a parent, grandparent, aunt or uncle	baseline
	Puberty	O	Pubertal stages I-V	baseline
Anthropometric Measures				
	Height	C	Height in meters	baseline
	Weight	C	Weight in kilograms	baseline
	BMI	C	Wt in kg./ht in meters^2	baseline
	Waist	C	Waist circumference in cm.	baseline
	Sum of Skin folds	C	Sum of triceps and sub-scapular skin folds	baseline
Risk Factors for CVD & T2D				
	Fitness level	C	VO$_2$ max	baseline
	Insulin	C	Insulin (μU/L)	baseline
	Glucose	C	Glucose	baseline
	HDL-C	C	HDL cholesterol (mg/dl)	baseline
	LDL-C	C	LDL cholesterol (mg/dl)	baseline
	Triglycerides	C	Triglyceride (mg/dl)	baseline
	Blood pressure	C	Systolic and diastolic blood pressure	baseline

[1]Scales: N=nominal labels, O=ordinal, C=continuous

levels were determined using the automated Boehringer-Mannheim Corporation (BMC) Cholesterol/HP and triglyceride/GV coupled-enzymatic procedures, respectively, on a BMC Hitachi 911 analyzer. HDL-C was determined using the BMC HDL-C (direct) enzymatic colorimetric test, also on a BMC/Hitachi 911 analyzer. Triglyceride tests were glycerol-blanked. If triglyceride was $\leq$ 400 mg/dl and the subject reported fasting status, LDL-C was calculated using the Friedewald formula (Friedewald, Levy, & Fredrickson, 1972). If triglyceride was greater than 400 mg/dl, no LDL-C result was calculated, because a level that high was considered to be unlikely in this population. Procedures for cholesterol, HDL-C and triglyceride were evaluated and certified quarterly through the CDC/NHLBI Lipid Standardization Program.

Insulin

Insulin was measured in a fasting plasma sample of venous blood. Aliquots of plasma were separated, labeled and placed on dry ice in the field, then stored at -80°C. The frozen plasma was shipped on dry ice to Linco Labs in St. Louis, MO for analysis of insulin levels using a radioimmunoassay method. Fasting insulin was used as a proxy measure for insulin resistance, as opposed to the HOMA-IR index (defined as 'fasting insulin X fasting glucose/22.5'). Fasting insulin is strongly correlated with the HOMA-IR index in large samples of adults and children, with correlations ranging from r = .98 to r = .99 (Allard et al., 2003; Haffner, Miettinen, & Stern, 1997). Fasting insulin has also been found to be strongly and inversely correlated with insulin sensitivity, a reciprocal measure to insulin resistance, as measured by frequently sampled intravenous glucose tolerance testing (Conwell, Trost, Brown, & Batch, 2004); the spearman correlation in this study was -.91 (p<.01). Gungor, Saad, Janosky and Arslanian (2004) studied Black and White children, and found similarly

strong correlations between fasting insulin and insulin sensitivity as measured by euglycemic clamp; correlation values were -.87 (p<.01) and -.91 (p<.01), respectively for Black and White subjects.

Glucose

Glucose was measured in fasting plasma samples by the UNC Hospitals Core Laboratory. The accuracy of all tests was routinely monitored using the quality control procedures established by the College of American Pathologists and CLIA guidelines. The lab used the Johnson & Johnson (J&J) vitros dry slide method for glucose (glucose oxidase/peroxidase) on a J&J 950 automated chemistry system. All values above 126 mg/dl, the cut-point for a provisional diagnosis of diabetes at that time (ADA, 2000), were reported to parents and it was suggested that parents discuss the elevated values with the child's physician.

Blood Pressure

Systolic and diastolic blood pressure was measured by trained research assistants with a random zero mercury sphygmomanometer to control for investigator bias. Subjects sat quietly for at least 2 minutes prior to measurement. Systolic and diastolic blood pressure was measured three times, with at least 1 minute between measurements. The three measurements for each type of blood pressure were recorded on the "Physiologic Data Form," shown in appendix D. The means of the three measurements for systolic and diastolic blood pressure were calculated by computer, for use in data analysis.

Measures of Overweight/obesity

Body mass index (BMI, Height, and Weight)

Height was measured to the nearest 0.1 cm on a stadiometer (Perspective Enterprises, Kalamazoo, MI); subjects were clothed, but shoeless. Weight was measured to the nearest 0.1 kg with an electronic Pro Plus metric scale (Healthometer Medical, Bridgeview, IL. The BMI was calculated as weight in kg/height in meters^2.

Triceps and subscapular skin folds

Triceps and subscapular skin folds were each measured three times to the nearest mm using a Lange skin fold caliper. Calipers were calibrated prior to data collection at each school. The triceps skin fold measurement was taken at the midpoint between the acromion and the olecranon processes with the elbow bent at 90 degrees. The subscapular measurement was taken 1 cm below the inferior angle of the scapula. These sites were recommended by the National Center for Health Statistics (National Center for Health Statistics, 1987). The three skin fold measurements taken from the tricep and subscapular sites were recorded on the "Physiological Data Form" (appendix D), and the three values were averaged by computer. The triceps and sub-scapular skin fold measurement means were summed for use in statistical analyses.

Waist circumference

Waist circumference was measured in centimeters using guidelines from the National Heart, Lung and Blood Institute (National Heart Lung and Blood Institute, 1988). The waist was measured in the horizontal plane at the level of the iliac crest at the mid-axillary line for both males and females, with subjects wearing a t-shirt and shorts.

Cardiovascular Fitness

Cardiovascular fitness was measured in terms of the subjects' predicted VO_2 max using a sub-maximal cycle ergometer test, the Physical Work Capacity (PWC_{195}). This test has a correlation of 0.81 to 0.95 and a mean error of ~8% (4 ml/kg/min) when compared to direct measures of VO_2max (McMurray, Guion, Ainsworth, & Harrell, 1998). The PWC_{195} consists of three, 3-minute stages on the cycle ergometer (Mocellin, Lindemann, Rutenfranz, & Sbresny, 1971). Subjects pedaled at 60 rpms on a Monark 818 cycle ergometer (Country Technology, Gay Mills, WI), while the ergometer provided resistance. Resistance was dependent on body weight and heart rate response to the exercise.

Pubertal Level

Pubertal stage was determined in the CHIC III study using the Pubertal Development Scale (PDS) (Petersen, Crockett, Richards & Boxer, 1988), a self-administered questionnaire with two 5-item subscales, one for each gender (see Appendices E and F). Subscales consisted of specific developmental characteristics such as growth spurt, pubic hair, and skin change for both boys and girls; facial hair growth and voice change for boys; and breast development and menarche for girls. They were coded on a 4-level ordinal response scale, and results from selected items were used in a prediction equation to produce a score that corresponded to five Tanner pubertal stage levels. Internal consistency of the PDS was acceptable in the CHIC III study, with a median alpha of .77 (range .63 to .88). High correlations have been found between the PDS and physician ratings of sexual maturity based on the Tanner scale (r = .61 to .67), and between the PDS and adolescent self-reports based on Tanner pictures (r =.72 to .80) (Brooks-Gunn & Warren, 1988).

Demographic Variables

*A*ge and gender were determined directly from subjects. Subjects were asked to describe their race by checking the appropriate box on a subject information form used by the original study (see Appendix G). The categories on the form (Asian/Pacific Islander, Black, Native American, White and Other) were based on US Census categories. Subjects were also asked to indicate whether or not they considered themselves to be of Hispanic or Non-Hispanic ethnicity.

Variables Created for the Current Study from CHIC III Variables

Several of the variables described above were used in the creation of new variables for the current study. These new variables were primarily used for the classification of subjects by risk level in relation to risk factors for CVD and diabetes such as the different lipids, insulin, glucose, cardiovascular fitness, and family history. Information on cut-points for the different variables that reflect risk for CVD and T2D may be found in Appendix H (Description of Risk Categories for Risk Factor Variables); these cut-points were used when categorizing subjects by risk levels. The subject groups formed according to risk level category were used in analyses to determine whether mean adiponectin levels differ by the level of risk related to selected risk factors. For example, an analysis was run to determine if mean adiponectin levels were different in subjects with high HDL-C, as opposed to those with low HDL-C. Results of all analyses will be given in chapter 4. The process used to create the new variables is described in the following sections.

Risk Categories for Lipids

For analyses that required subjects to be classified based on risk levels related to HDL-C, LDL-C and triglycerides, the guidelines of the National Cholesterol Education Program (NCEP) Expert Panel on Blood Cholesterol Levels in Children and Adolescents (American Academy of Pediatrics, 1992) were used to create variables to indicate subjects with normal and low HDL-C, and normal and high triglycerides. Subjects whose HDL-C levels were greater than 35 mg/dl were considered to have normal HDL-C levels, and those with levels less than or equal to 35 mg/dl were classified as having low HDL-C levels. Subjects whose LDL-C levels were less than 110 mg/dl were considered to have normal, or acceptable, levels of LDL cholesterol, and those with an LDL-C of 110-129 were classified as borderline. Subjects with LDL-C levels greater than or equal to 130 mg/dl were assigned to the high risk group. Subjects with triglyceride levels less than 150 mg/dl were considered to have levels in the normal range, and those with levels greater than or equal to 150 mg/dl were classified as having high triglyceride levels.

Insulin Risk Categories

Currently, there are no universally agreed upon cut-points for fasting insulin for use in defining hyperinsulinemia in children and adolescents. Some researchers have used various insulin percentiles determined by analyzing data collected from the subjects in their samples, while others have used actual insulin level cut-points. Several research groups have used the 75th percentile of their data (Goodman, Daniels, Morrison, Huang, & Dolan, 2004; Lambert et al., 2004), while others have used the 95th percentile (Freedman, Dietz, Srinivasan, & Berenson, 1999). Janssen et al. (2005) used the top quintile, or the 80th percentile for insulin values in their sample. All three of these research groups used samples

that were representative of the population including subjects with a range of BMI, not just obese or non-obese subjects.

Other groups have suggested actual cut-points for hyperinsulinemia. The American Heart Association recommends a level of 20µU/L, but do not give a rationale for that particular cut-point (Williams et al., 2002). Gidding et al. (2004) reported using a cut-point of 20 mU/mL. This value is the same as the value mentioned above except that milli-units per milliliter were used for the concentration, instead of micro-units per liter. Gidding et al. referenced an article by the American Diabetes Association, but the article they referenced did not specifically mention any cut-points for hyperinsulinemia. Viner et al. (2005) used different values for pre-pubertal and mid-pubertal children, 15 µU/l and 30 µU/l respectively, and referenced Goran and Gower (2001). Again, the referenced article did not mention specific cut-points. Moreover, the sample utilized by Goran and Gower had only 60 subjects, not a very large sample when considering a norm for insulin.

In summary, there is no specific cut-point for classification of subjects to normal or high insulin groups. The level of fasting insulin that represented the 75th percentile in this study (18µU/L) was used as a cut-point for hyperinsulinemia in the current study. Subjects with a fasting insulin level less than 18µU/L were considered to have normal insulin levels, and those with levels greater than or equal to 18µU/L were classified as having hyperinsulinemia.

Glucose Risk Categories

The most recent guidelines of the American Diabetes Association (ADA, 2006) were used to create a variable to indicate subjects with normal, borderline and high fasting glucose levels. Subjects with a fasting blood glucose level of less than 100 mg/dl were considered to

be normal, and those with a fasting glucose of greater than or equal to 100 mg/dl and less than 126 mg/dl were categorized as pre-diabetic. Any subject with a fasting glucose greater than 125 mg/dl was considered to have diabetes.

Blood Pressure Risk Categories

When analyses required that subjects be classified into groups based on blood pressure levels, the guidelines of the 2004 Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents (National High Blood Pressure Education Program Working Group, 2004) were used to create variables that classified subjects as having normal, pre-hypertensive, or hypertensive blood pressure levels. Subjects were classified into the 3 groups based on their systolic and/or diastolic blood pressure percentiles. The National High Blood Pressure Education Working Group provided equations, and gender-adjusted regression coefficients based on blood pressure measurements from an NHANES data set, for use in the creation of blood pressure percentiles. The equations, using the coefficients, subject age, and height percentile (adjusted for age and gender), were used to identify subjects' expected systolic and diastolic blood pressures. Height percentiles were created with the use of a SAS program, available at the Centers for Disease Control website (CDC, 2006). This program created height percentiles based on data the same NHANES data set used by the National Working Group to create the regression equation coefficients. The expected blood pressure values derived from the National Working Group equations were then converted to z-scores for male and female systolic and diastolic blood pressures, by subtracting the expected blood pressure from the observed value and dividing the result by standard deviations provided by the National Working Group. Finally, systolic and diastolic blood pressure percentiles were

created by multiplying a subject's "$\Phi(z\_bp)$", the area to the left of the z-score under a standard distribution curve, by 100%.

Subjects with systolic and diastolic blood pressures less than the 90th percentile were classified as normal. Subjects were classified as pre-hypertensive if their systolic or diastolic blood pressure measurements were equal to or greater than the 90th percentile, but less than the 95th percentile. Hypertension was defined as a systolic or diastolic blood pressure greater than or equal to the 95th percentile.

Categories Related to Measures of Adiposity

BMI Risk Categories and BMI Z-scores

When analyses were conducted that required categorization of subjects into different groups according to overweight status, the Centers for Disease Control (Centers for Disease Control, 2003) guidelines were used to create a variable to indicate subjects with 3 levels of BMI risk. In an initial step, a SAS program obtained from the same Centers for Disease Control website mentioned above (Centers for Disease Control, 2006) was used to calculate the BMI percentile. These calculations were based on national BMI means from NHANES data for youth aged 2-20 years. After the BMI percentiles were obtained, the variable indicating BMI risk level was created. Subjects with a BMI that was less than the 85th percentile for age and gender were considered to have a normal BMI. Subjects were considered at risk of overweight if their BMI was greater than or equal to the 85th percentile for age and gender, but less than the 95th percentile. Subjects were classified as overweight if their BMI was greater than or equal to the 95th percentile for age and gender.

When analyses called for a continuous variable to represent BMI, a BMI z-score adjusted for age and gender was used. This measure took into account the differences in

growth and development during childhood and adolescence. BMI z-scores were calculated

with the same SAS program from the Centers for Disease Control (Centers for Disease

Control, 2006) that was used to calculate the BMI percentiles. The z-scores were therefore

based on national means and associated z-score values for males and females aged 2-20

years.

Waist Circumference

A variable was also needed to indicate a subject's risk level according to their waist

circumference. In order to create this variable, waist circumference percentiles specific to

age, gender and race from a large multi-ethnic population based sample (Fernandez et al.,

2004), were used to classify subjects into normal and high waist circumference groups. The

percentiles developed by Fernandez et al. (2004) were based on waist measures taken at the

"uppermost lateral border of the right ilium" (p.440), the same location as was used by the

CHIC III study, and therefore deemed appropriate for use in creating the waist circumference

risk variable for this study. A waist circumference measure greater than or equal to the 90th

percentile was considered high, and measures less than the 90th percentile were classified as

within normal limits.

Cardiovascular Fitness and Fitness Levels

To account for differences in body mass due to adiposity, the analyses for this study

used VO_2 results expressed as ml/kg/min, and also as ml/kg of lean body mass/min to control

for the increased correlation between VO_2 and body weight. Percent lean body mass was

calculated from total body mass and percent body fat, calculated from triceps and

subscapular skin fold thicknesses using the method of Slaughter et al. (1988) and Lohman

(1992). Lean body mass in kilograms was calculated by multiplying the percent lean body

mass times the weight in kilograms. The expression of VO_2 in ml/min was then calculated by multiplying the ml/kg/min by the subject's weight in kilograms. Lastly, a variable representing VO_2 was created by dividing VO_2 in ml/min by kilograms of lean body mass.

A variable was also created to indicate fitness levels based on VO_2 tertiles. A SAS program was run to identify the 33rd and 66th percentile for VO_2. These percentiles were used to define 3 tertiles for the VO_2 variable, then the tertiles were used to classify subjects based on cardiovascular fitness. The upper tertile of VO_2 values were considered to indicate a high level of fitness, and the lower tertile indicated a low fitness level; the lower and upper tertiles were used for comparison in statistical analyses.

Family History Variables

Mothers and/or fathers whose children took part in the CHIC III study were asked to complete the Personal Family Health History Scale (found in Appendices B and C). Parents were requested to give medical family history information related to cardiovascular diseases and diabetes. Only results from biological family members were used to assess family health risks. From this data, 2 variables were created for the current study. One variable indicated whether or not a subject had a family history of CVD, and the other whether or not a subject had a family history of diabetes. A positive family history of CVD was defined as a report by parents of angioplasty, heart attack, angina, cardiac bypass surgery, high BP, stroke in any of the subject's parents or grandparents, or aunts or uncles. A positive family history of diabetes was defined as a report of diabetes in a subject's parents, grandparents, aunts or uncles.

Age-Group Categories

Subjects were also assigned to one of 2 age-group categories. Subjects younger than 13 years old were assigned to one group, and subjects 13 years of age and older were assigned to the other group. The age-group variable was then used in analyses designed to compare mean adiponectin levels in the young and older subjects.

Laboratory Procedures for Analysis of Adiponectin in Serum Samples from the CHIC III Study

The serum samples collected from the CHIC III subjects were thawed and analyzed for adiponectin by using an enzyme-linked immunosorbent assay (ELISA). All lab analyses were completed in the General Clinical Research Center (GCRC) Bio-Analytical Core Laboratory (BAC Lab) in the School of Dentistry at UNC Chapel Hill. The GCRC agreed to fund the adiponectin analyses as a 'lab only' protocol. Methods of laboratory analysis are described below.

Analysis of adiponectin by this investigator

Prior to the analyses completed in the GCRC BAC Lab, this investigator performed an ELISA analysis of adiponectin on serum samples from 18 subjects randomly selected from the fourth cohort of CHIC III at a time-point other than baseline. The analysis was completed in the Bio-behavioral Lab in the SON at UNC-Chapel Hill in June of 2006. The staff in the Bio-behavioral Lab provided the investigator with training, and with assistance while performing the procedures needed for analysis. Training included the use of electronic pipettes and the proper techniques for plating and analysis of samples using ELISA kits from the R&D Systems labs. The ELISA kits are identical to those the GCRC BAC Lab used for

analysis of adiponectin for this study, and analyzed in triplicate. Subject serum samples that had been stored at -80 C were thawed for the analyses. The steps of the ELISA protocol are detailed in the assay protocol brochure, available on the R&R Lab Systems website, at: http://www.rndsystems.com/pdf/drp300.pdf.

The pilot experience in the BBL was very helpful in giving this candidate an opportunity to practice the required techniques and to become familiar with the ELISA assays that were used in this study. Results of adiponectin standards testing fit the standard curve with little deviation. High, medium and low controls were each plated in triplicate on two rows, for a total of six wells for each type of control. Values obtained were within the ranges for medium and low controls in the ELISA protocol booklet. The values for the high control fit within the high control range with removal of one outlier value. The results obtained for adiponectin in subject samples were within the range of mean levels as reported in the literature. The opportunity gave me an understanding of what kinds of errors may potentially be involved with the lab analysis of adiponectin, such as errors in pipette technique or deviation from the assay protocol. These types of errors should have been minimized in the study as all analyses were completed by experienced laboratory personnel in the GCRC BAC Lab.

Analysis of Total Adiponectin by Enzyme-Linked Immunosorbent Assay (ELISA)

Serum adiponectin was measured in the stored serum of the 1215 study subjects, using the same ELISA that was used in this investigator's pilot analyses. The ELISA analyses for the full study were done by lab personnel experienced in analysis of cytokines, in the GCRC BAC Lab located in the School of Dentistry at UNC-Chapel Hill. Before analysis, the serum samples were stored at -80 C in freezers in the Applied Physiology lab in

the Fetzer building here at UNC-Chapel Hill. All samples were collected at baseline time-points during CHIC III data collection. In preparation for the lab analyses, the location (box number and row number) of the selected samples were identified from CHIC databases by the CHIC Project Coordinator. The samples were then placed in boxes for transport to the GCRC BAC Lab. An Excel file was created that contained blood id numbers for the stored serum samples, for use by the GCRC BAC lab.

The lab in turn, created lab numbers that were linked to the blood id numbers. After the analyses were completed, the results were placed into a verified Excel data base by the GCRC BAC lab staff, and given to this candidate. Of the 1215 samples, 126 from the first seven plates were analyzed in duplicate. Results of five samples were saturated, or at a level too high for the plate reader to interpret. The duplicate readings for these five samples were also saturated, so the coefficient of variation between these two readings was zero. Otherwise, the coefficient of variation values ranged from 0.1 to 16.2, with a mean of 3.94. The median value was 3.0. The lab protocol booklet indicated that the intra-assay coefficient of variation for the adiponectin ELISA ranged from 5.8 to 6.9.

Data Management

The adiponectin results from the ELISA assay were merged with the existing data set from the CHIC III study. The merged data set was used for statistical analyses. Data were stored on a password protected computer at the UNC School of Nursing, on a drive that is backed up every 24 hours. An archive copy of the data and statistical analyses was also be kept under lock at an off campus location.

Research Aims and Questions and Methods of Analysis

The research aims for this study were examined and the questions answered using data from a large, racially diverse sample of children and adolescents from elementary and high schools in eastern North Carolina. Statistical methods for analysis of specific research questions are described following each question in section 3.6.2. The SAS program for statistical analysis was used for all analyses. Overall, Generalized Estimating Equations (GEE) were used to run different regression models to examine the relationship between adiponectin and risk factors for CVD and T2D. GEE is an extension of the general linear model that is designed to account for the increased correlation of clustered data (Stokes, Davis & Koch, 2000). The GEE method of analysis was used to account for the fact that the original CHIC III data were collected from clusters (schools) of children.

Adjustment for Clustering

The data for this study were collected from students in clusters of 34 elementary and high schools in 3 rural counties in eastern North Carolina. There were 964 subjects from elementary schools and 391 subjects from high schools. The number of children per school cluster ranged from 2 – 115, while the mean number of subjects per cluster of was 35.74. One school had 2 subjects, or 0.16% of the total number of subjects. After that, there were 16 schools with groups of 11 – 30 subjects. The subjects from these schools made up 25.68% of the total number of subjects. Ten schools had groups of 31 – 50 subjects each, and contributed 29.55% of the total number. Seven schools had groups of greater than 50 subjects each; these schools contributed 44.61% of the total number of subjects.

Since data were collected from subjects within school units, or clusters, the possibility existed that the subjects' data may be correlated due to close proximity and similarities in

lifestyle habits and genetic background. Norton, Bieler, Ennett and Zarkin (1996) describe

clustered subjects as being "more similar to each other than to individuals in other clusters

because they are exposed to a common set of circumstances and tend to interact with each

other" (p.919). For example, habitual physical activity or usual dietary intake may be similar

in subjects from the same school. These similarities might lead to increased correlation, or

clustering, of physiological or anthropometric measurements such as weight or BMI. In turn,

differences in levels of overweight could affect adiponectin levels. If the increased

correlation between clustered subjects for an outcome variable such as adiponectin is

ignored, the standard error will appear smaller than it really is, thereby leading to "increased

Type 1 errors" (Norton et al., 1996, p.919). In order to avoid such error, statistical analyses

for this study took into account the possibility of this increased correlation. More

specifically, the intra-class correlation coefficient (ICC) was considered.

The ICC has been defined as "the degree of similarity among responses within a

cluster" (Donner & Klar, 2000, p. 1), or as "a statistical measure of…intra-cluster

dependence." (Campbell, Thomson, Ramsay, MacLennan, & Grimshaw, 2004 p. 114). The

formula for the ICC, or ρ, is as follows: $\rho = \sigma^2_b / (\sigma^2_b + \sigma^2_w)$. The symbol σ^2_b stands for the

between cluster component of variance, and the symbol σ^2_w stands for the within cluster

component of variance. ICC values can range from 0 to 1. An ICC value of 0 indicates there

is no correlation among subjects in the cluster on the specified variable, i.e., all subjects'

values are independent of one another. An increased ICC reflects a decrease in within cluster

variance, in relation to the between cluster variance. Decreased variance in a cluster means

there is increased similarity among subjects in the cluster. In the case of an ICC value of 1,

the values of subjects in the cluster would be perfectly correlated. In this instance, the

effective sample size for analysis would technically be decreased. For example, even if there were 50 subjects in a cluster, an ICC of 1.0 that indicated perfect correlation, would mean they essentially represented the same value. In essence, measuring one subject would give the same value as would be obtained when measuring another subject. In regards to overall sample size, the closer an ICC is to 0, the better, because this would mean the subject values were independent of one another.

In addition to affecting the effective sample size, clustering of outcome variables results in an inflation of the actual variance in analyses; the extent of this inflation is denoted as the "design effect" (Norton et al., 1996, p.920), or "Variance Inflation Factor" (VIF) (Donner & Klar, 2000). The formula for the VIF is as follows: $VIF = 1 + (m-1) \rho$, where 'm' is the average cluster size, and 'ρ' is the ICC value. Depending on the average cluster size, an ICC as low as 0.01 in the previous equation would result in a large inflation of variance. There is no information in the current literature concerning an ICC for adiponectin, therefore an ICC for adiponectin in this sample was calculated using GEE, or 'proc genmod' in SAS. The ICC calculated for adiponectin alone was 0.07, and 0.10 when adjusted for race and gender, either of which indicated enough correlation to be of concern unless accounted for by statistical analysis method.

To illustrate this possibility, the ICC values of 0.07 and 0.10 were inserted for ρ in the VIF equation above, with the average cluster size of 36. The resulting design effects were 3.45 and 4.36, respectively. These design effects were high enough to alter results if a statistical method that accounted for clustering, such as GEE, was not used. Indeed, when a model was run using the GEE method then repeated using multiple regression, there were differences in the results that also reinforced the need to use GEE. Adiponectin was the

dependent variable in the model, and independent variables were race, gender, HDL-C, insulin, systolic blood pressure and waist circumference. Except for systolic pressure and HDL-C, standard errors were smaller with the multiple regression analysis than with GEE. In addition, insulin was significantly related to adiponectin in the multiple regression analysis, but was not significant in the GEE analysis. These results, and the results above concerning the design effect, reinforced the need for using GEE to adjust for the effects of clustering. All analyses for this study were done with GEE.

Although GEE is often used in analysis of longitudinal linear models, it may also be used in analysis of clustered data to account for high correlations based on close proximity of subjects (J. A. Hanley, Negassa, Edwardes, & Forrester, 2003; Preisser, Young, Zaccaro, & Wolfson, 2003; Zorn, 2001). The GEE method is useful with continuous, binary or categorical data (Norton, Bieler, Ennett & Zarkin, 1996). When using GEE, the "structure of the working correlation matrix" must be specified before analyses are run (Zorn, 2001). A compound symmetric, or exchangeable, matrix was specified for this study. This type of matrix assumes that all values of the outcome variable "co-vary equally across all observations within a cluster" (Zorn, 2001, p.473). According to Stokes et al. (2000), this type of matrix is "appropriate when cluster sampling is involved" (p.476). GEE will still offer appropriate results if the correlation matrix of the study sample does not match the chosen working correlation matrix exactly, although the best possible match is desirable (Stokes et al., 2000). As Zorn (2001) explains, "the parameter estimates obtained through application of these models are robust to misspecification of those correlations, an important trait, since our understanding of those relationships is often imperfect at best." (p.474). The GENMOD procedure in SAS was used to fit the GEE models.

Research Aims and Questions

Aim 1.

Examine the differences in mean adiponectin levels by demographic factors and pubertal stage.

Question 1a

Do adiponectin levels differ by the demographic factors of gender, race (non-Hispanic Black and White), age (> 13 years, and $\geq$ 13 years), and family history of CVD or diabetes?

The sample as a whole, and by demographic factors such as age, gender, race, and family history, was described by descriptive statistics such as means, standard deviations and ranges for adiponectin. After these analyses, regression models were run with GEE, by using proc genmod in SAS, to compare mean adiponectin levels between gender, race, and age groups, and family history of CVD or diabetes groups. When significant differences were found, the variables were treated as confounding variables in subsequent analyses. The only exception had to do with the family history variables; these variables were not used in subsequent analyses because a large number of subjects were missing family history data, and information from subjects with missing family history was ignored when models were run with GEE, thereby limiting the total sample information available for analysis. Therefore, the only analyses that utilized the family history variables were those that compared adiponectin means by the 2 types of family history groups.

Question 1b

Do adiponectin means differ by pubertal stages I-V, controlling for gender or race, and does this relationship differ by race, gender or overweight status?

Adiponectin means, standard deviations and ranges were obtained in relation to the different pubertal stages. Regression models were run to compare means among pubertal stages I-V, adjusting for any possible confounding variables that were significant in question 1a. Age was not be included as a confounding variable because of its high correlation with pubertal stage ($r = .75$, $p < .0001$). Contrasts were done to test for a trend across pubertal stages, because pubertal stage is an ordinal variable.

Regression models were also run with interaction terms to test for possible interactions between puberty and variables such as race, gender and overweight that might moderate the relationship between puberty and adiponectin. Overweight status was included here because results of previous studies suggest it might function as a moderator of the relationship between puberty and adiponectin (Woo et al., 2005); the model also tested for the possibility of a main effect of overweight status on adiponectin levels. The race variable referred to non-Hispanic Black or White. The overweight status variable had 3 levels: normal weight (<85th percentile for age and gender), at risk for overweight ($\geq$85th to <95th percentile) and overweight ($\geq$ 95th percentile). In order to achieve the most parsimonious model, the full model was reduced by deleting variables that were not significantly related to adiponectin, starting with interaction terms. The resulting reduced model was compared to the full using the QIC criterion. The model with the lowest QIC number was considered to be the best fitting model. No interaction terms were significant before or after model reduction, therefore interaction plots were not needed for this question.

Aim 2.

Determine which of the three different measures of adiposity is the best predictor of adiponectin.

Question 2a

Of BMI z-score, waist circumference and sum of skin folds, which measure of adiposity is the best predictor of adiponectin levels, when controlling for demographic factors or puberty?

As a first step, bivariate correlations between adiponectin and 3 measures of adiposity, (BMI z-score, sum of skin folds, and waist circumference) were determined. Next, each adiposity variable that was significantly correlated with adiponectin was regressed separately on adiponectin, adjusting for the potential confounding variables of race and gender because they were significantly related to adiponectin in question 1. The models were run separately because of increased correlation among the three measures of adiposity, as shown in chapter 4. The models were compared by using the Quasi likelihood under the Independence model Criterion (QIC) (Pan, 2001). This statistic was obtained because GEE will not generate the Akaike Information Criteria (AIC) or the Bayesian Information Criteria (BIC) for use in model comparison or selection. The QIC number has been found to be a useful and valid method to use when comparing models run in GEE (Hardin & Hilbe, 2004; Pan, 2001). The model with the lowest QIC number was considered the best fitting model. Models with QIC numbers differing by greater than approximately 2 were considered to be truly different from one another (personal communication, Wei Pan, March 2007).

Aim 3.

To examine the relationship between adiponectin and risk factors for CVD and T2D, and to determine if the relationship is moderated by race, gender or overweight status.

Question 3a

Do mean adiponectin levels differ by risk categories for the following risk factors for CVD and T2D, when controlling for demographic factors: HDL-C, glucose, insulin, LDL-C, triglycerides, systolic or diastolic BP, and level of fitness?

Regression models were run in GEE in order to compare mean adiponectin levels among groups of children who were in different categories of risk in relation to the risk factors mentioned above. Models that included potential confounding variables such as gender or race were also run to account for the possible effects of those variables on the relationships among adiponectin and the risk factor category variables.

Question 3b

What is the relationship of adiponectin to the following risk factors for CVD and T2D (HDL-C, LDL-C, triglyceride, total cholesterol, glucose, insulin, BP and fitness level) when operationalized as continuous variables, when controlling for demographic factors and measures of adiposity?

Bivariate correlations were assessed between adiponectin and the following variables: HDL-C, LDL-C, triglyceride, total cholesterol, glucose, insulin, systolic and diastolic BP and VO_2 level. In addition, subjects that might have an influential effect on analyses were identified by use of a SAS diagnostic program designed by Hammill and Preisser (2003). Leverage is defined as "a measure of how extreme an observation or cluster is with respect to

the predictors" (Hammill & Preisser, 2003, p.1199). According to Garson (2007), any

subject with a leverage value greater than 0.5 is considered to "have undue leverage". Three

subjects were noted to have a leverage values greater than 0.05 (see figure 5). Regression

models were run in samples with and without the subjects with high leverage values. No

differences were noted in the results for the 2 models, so subsequent analyses included all

1215 subjects.

Next, regression models were run with adiponectin as the dependent variable, and all

of the risk factors that were significantly correlated with adiponectin in a bivariate manner as

independent variables. This was done to further clarify the relationship between adiponectin

and the risk factors. The full model with all significant variables was reduced by a backward

stepwise process with the goal of achieving the most parsimonious model. The full model

was compared to the reduced model with QIC numbers. In relation to the QIC, the model

with the lowest QIC number was considered the best model. In addition, contrast statements

for the deleted variables were used to test the hypothesis that the β-coefficients for the

deleted variables were essentially equal to zero. A p value for the Chi-square test was greater

than 0.05, that indicated that the β-coefficients for the deleted variables were not different

from zero, and therefore the reduced model was more parsimonious than the full model.

The next step was to put all risk factor variables that remained in the reduced model

into a regression model that also included the adiposity measure from the model in question

2a that resulted in the lowest QIC score. This was to be done in order to determine if the

measure of adiposity attenuated the relationship between adiponectin and the risk factors.

However, the QIC scores for the models with the adiposity measures BMI z-score and waist

circumference in question 2a were very close. Therefore, the regression model with all

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significant risk factors plus an adiposity measure was actually run twice, once with the BMI z-score variable and again with the waist circumference variable. The waist circumference and BMI z-score variables were highly correlated with one another ($r = .68$), so two separate models were required to avoid multi-collinearity concerns. Both models adjusted for potential confounding variables such as gender and race. Both models were reduced to the most parsimonious model, and compared by QIC numbers and Chi-square statistics as described above. A p value greater than 0.05 for the Chi-square statistic indicated that the reduced model was the best model, and the model with the lowest QIC number was considered the best.

Question 3c

Do the relationships of adiponectin to the risk factors in question3b differ by race (non-Hispanic Black or White), gender or overweight status?

A regression model was run with the variables that were related to adiponectin in question 3b models, in addition to race, gender, BMI risk level and interaction terms between the risk factor variables and race, gender or overweight status. Interaction terms were included to determine if race, gender or overweight status moderated the relationships between adiponectin and the variables. The risk factor variables used in the model were HDL-C, systolic blood pressure and insulin.

Again, in order to achieve the most parsimonious model, the full model was reduced by deleting variables that were not significantly related to adiponectin, starting with interaction terms. The reduced model was compared to the full model by using the QIC procedure, and a Chi-square statistic test with a contrast statement that included the deleted variables, as described above. The model with the lowest QIC number was considered

Figure 5. Observation Level Leverage Values
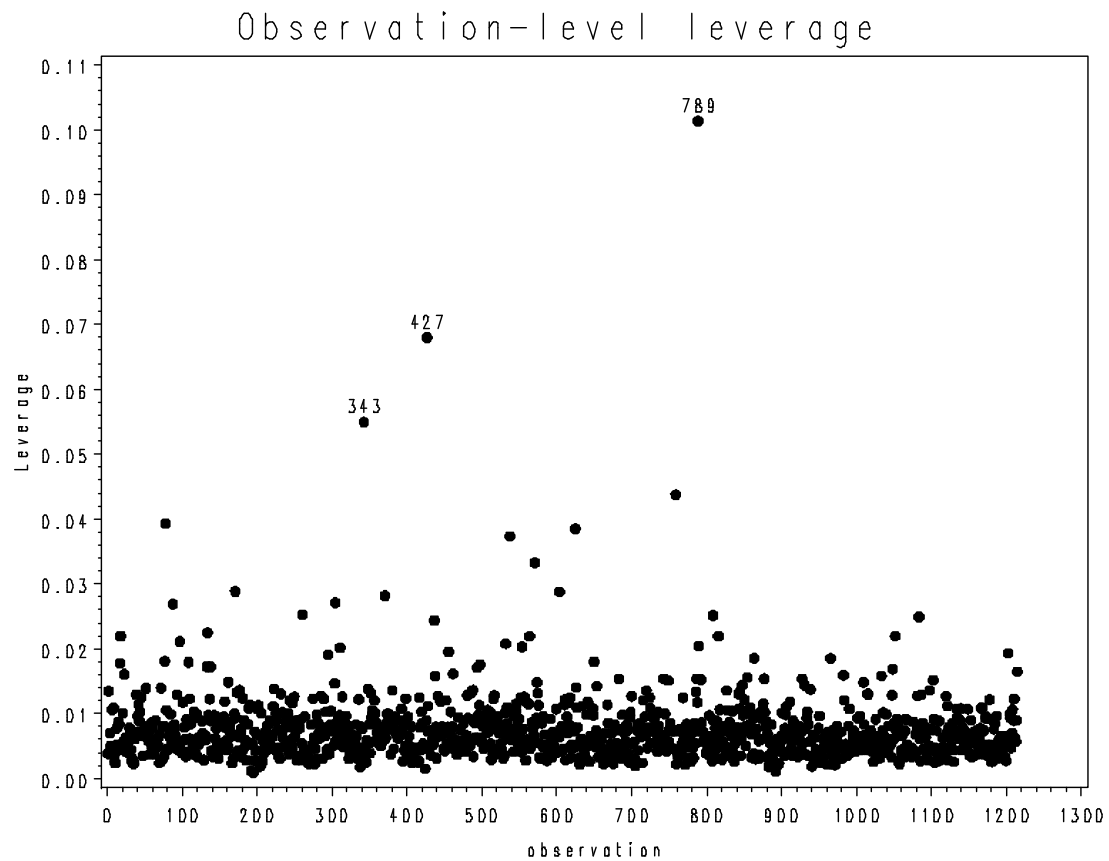
Note: In order to protect subject confidentiality observation numbers replace subject id numbers in above plot.

the best model, and if the p value for the Chi-square statistic test was greater than 0.05, the reduced model was considered the better model.

A significant interaction effect between hdl and gender was illustrated by plotting an interaction graph for the model: adiponectin $= \beta_0 + \beta_1 HDL + \beta_2 gender + \beta_3\ HDL*gender$ (see figure 6). The interaction shown in the graph falls somewhere between the subtle and and substantial interaction graphs shown in figure 7, therefore the models were run in separate groups stratified by gender.

Summary of Methods

Data collected from 1215 subjects who took part in the CHIC III study were used for analysis of research questions for this descriptive, comparative and correlational study. Data included variables that were originally collected from subjects during the CHIC III study, additional variables that were created from CHIC III variables, and results from laboratory analysis of stored serum samples for adiponectin. The GEE method was used for all analyses to account for clustering of subjects within schools. Results of these analyses are presented in the following chapter.

Figure 6

Interaction Plot for HDL*Gender Interaction
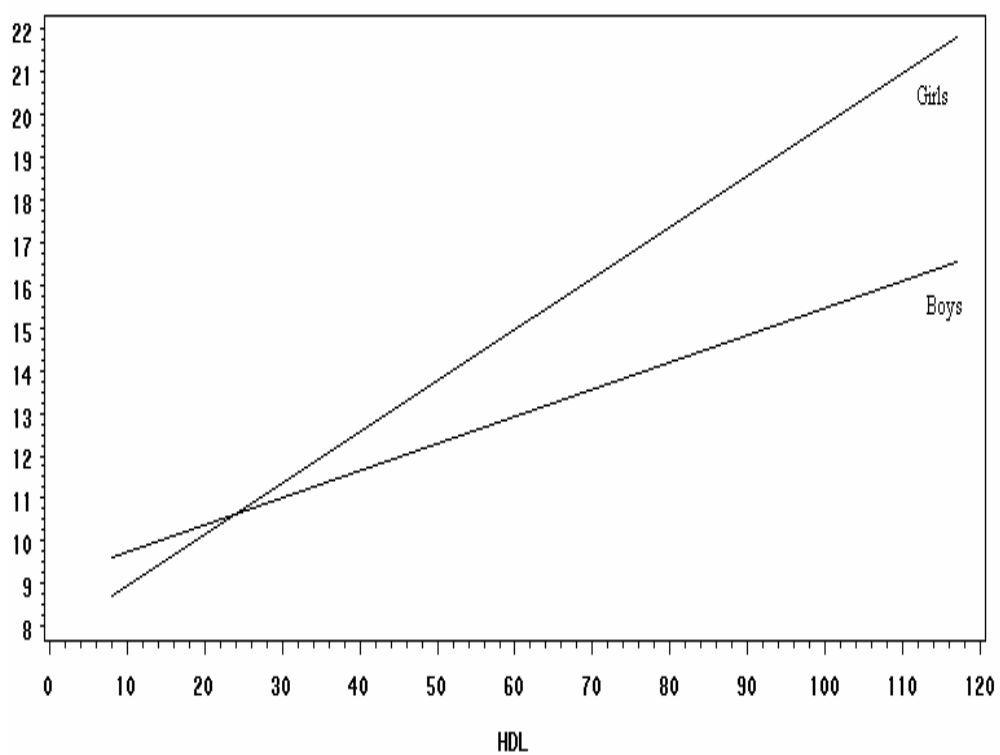
Figure 7

Possible Interaction Graphs
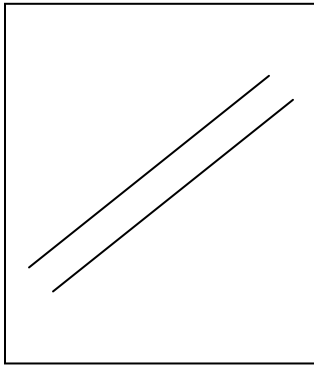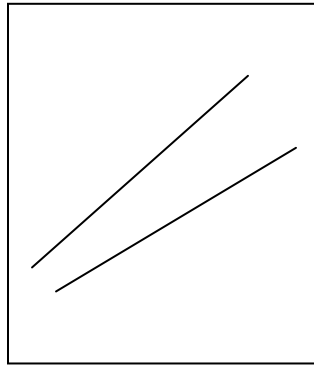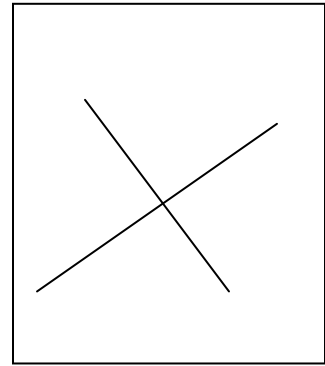
a   b   c.

Chapter 4

Results

Introduction

The purpose of this research study was to explore the relationships between adiponectin and multiple risk factors for CVD and T2D in a large, racially diverse sample of children and adolescents, and to determine if the relationships are moderated by gender, race and overweight status. Results from all analyses will be given in this chapter, beginning with basic descriptive statistics and then progressing to results from each research question in turn.

General Descriptive Results for Adiponectin and other Study Variables

Descriptive statistics such as the mean, standard deviation and range for adiponectin in the sample as a whole, by demographic factors and by pubertal status are given in Table 3, and by separate risk factor categories in Table 4. The mean for adiponectin in the overall sample was 12.56 µg/ml, and ranged from 10.14 µg/ml to 14.03 µg/ml in the demographic, pubertal stage and risk factor categories. Similar descriptive statistics for the remaining study variables are given in Table 5. Bivariate correlations between adiponectin and the other study variables are given in Table 6. The correlations with adiponectin that were significant at the $p < .05$ level ranged from $r = -.10$ to $r = -.34$.

Table 3

Descriptive Statistics for Adiponectin for Demographic Variables and Pubertal Stages

Variable	group	Mean (µg/ml)	SD	Minimum	Maximum
Full Sample		12.56	6.58	1.29	49.93
Age-group	< 13 years	13.05	6.72	1.38	49.93
	> 13 years	11.39	6.10	1.29	32.80
Race**	Black	11.63	6.36	1.29	39.41
	White	13.87	6.68	1.57	49.93
Gender	Male	12.11	6.61	1.29	46.90
	Female	13.02	6.53	1.84	49.93
Pubertal	1	13.96	6.60	1.38	46.90
Stage	2	12.69	6.56	1.43	38.25
	3	12.32	6.62	1.29	49.93
	4	11.67	6.32	1.84	39.41
	5	11.35	6.82	2.85	32.80
Fam. Hx.	yes	12.59	6.52	1.38	49.93
of CVD	no	13.19	6.41	3.33	30.58
Fam. Hx.*	yes	11.97	6.41	1.38	49.93
of Diabetes	no	13.57	6.54	1.63	38.25

Difference by group: *p < .05, ** p < .001

Table 4

Descriptive Statistics for Adiponectin in Risk Factor Categories

Risk Factor	Category	Mean(μg/ml)	SD	Max	Min
BMI risk**, [§]	normal	14.03	6.55	1.29	46.90
	at risk	12.01	6.32	1.43	49.93
	overweight	9.41	5.62	1.38	39.41
Waist risk**	Normal	13.33	6.59	1.29	49.93
	High	9.36	5.53	1.38	31.03
Blood Pressure	normal	12.72	6.56	1.29	49.93
	pre-hypertnsive	11.67	6.217	1.84	39.41
	hypertensive	11.89	7.53	2.12	36.79
HDL-C**	normal	12.88	6.66	1.29	49.93
	Low	10.29	5.55	2.37	24.80
LDL-C	normal	12.61	6.52	1.29	46.90
	borderline	12.42	6.12	3.33	30.73
	High	12.17	8.26	1.38	49.93
Triglycerides	normal	12.57	6.59	1.29	49.93
	High	12.06	6.43	3.87	23.51
Insulin**	normal	13.43	6.68	1.29	49.93
	High	10.14	5.64	1.38	24.98
Glucose	normal	12.64	6.51	1.29	46.90
	pre-diabetic	11.72	7.41	1.44	49.93
	diabetic	11.489	6.27	2.38	19.36
VO$_2$ (ml/kg/min)**	lowest tertile	11.81	6.84	1.84	49.93
	highest tertile	13.29	6.39	1.63	46.90
VO$_2$ (ml/kg lbm/min)	lowest tertile	12.68	6.49	1.57	46.90
	highest tertile	12.44	6.95	1.29	49.93

Different by group, **adjusting for race and gender**: *p < .05, ** p < .001

All levels of 3-level risk variable different: [§] (p < .01)

Table 5

Means and Standard Deviations for Study Variables Other Than Adiponectin

Variable	Total Sample	Black	White	Male	Female
Age	11.39 (2.85)	11.37 (2.80)	11.40 (2.92)	11.30 (2.81)	11.47 (2.89)
HDL-C (mg/dl) *	49.00 (13.71)	50.86 (14.43)	46.38 (12.18)	49.57 (13.84)	48.43 (13.58)
LDL-C (mg/dl)	91.53 (26.07)	93.17 (26.90)	89.21 (24.67)	91.15 (27.29)	91.92 (24.77)
Triglycerides (mg/dl) *, §	63.30 (35.65)	57.86 (32.38)	71.00 (38.59)	59.78 (32.59)	66.87 (38.22)
Insulin (μU/L) *, §	14.95 (9.51)	16.60 (10.81)	12.61 (6.63)	13.24 (8.70)	16.68 (9.98)
Glucose (mg/dl) §	89.33 (9.06)	88.97 (9.49)	89.83 (8.40)	90.31 (8.75)	88.33 (9.26)
Systolic BP (mm/Hg) *	106.18 (11.98)	106.87 (11.58)	105.20 (12.46)	106.76 (12.54)	105.59 (11.35)
Diastolic BP (mm/Hg)	64.20 (10.08)	64.45 (10.05)	63.85 (10.12)	63.75 (10.49)	64.67 (9.63)
VO$_2$ Max (ml/kg/min) §	36.88 (10.81)	36.09 (10.67)	37.10 (10.92)	40.89 (10.75)	32.82 (9.23)
VO$_2$ Max (ml/kg lbm/min) §	47.61 (11.26)	47.07 (10.90)	48.38 (11.71)	49.40 (11.20)	45.80 (11.03)
BMI z-score*	0.77 (1.10)	0.92 (0.08)	0.55 (1.09)	0.73 (1.08)	0.80 (1.12)
Waist (cm)	71.24 (13.80)	72.20 (14.35)	69.89 (12.87)	70.72 (14.01)	71.77 (13.57)
Sum of Skin folds (mm)*, §	26.57 (15.03)	28.90 (16.49)	23.27 (11.95)	22.55 (13.12)	30.64 (15.75)
BMI percentile (%)*	70.50 (27.24)	74.23 (25.84)	65.20 (28.31)	69.80 (27.22)	71.20 (27.27)

* different by race (p<.05), § different by gender (p<.05)

Table 6

Bivariate Correlations with Adiponectin

Variable	r-value*
BMI z-score	-0.29**
Waist circumference (cm)	-0.34**
Sum of Skin folds (mm)	-0.27**
HDL-C (mg/dl)	0.25**
LDL-C (mg/dl)	0.01
Triglycerides (mg/dl)	-0.14**
Insulin (µU/L)	-0.26**
Glucose (mg/dl)	-0.02
Mean Systolic Blood Pressure (mm/Hg)	-0.22**
Mean Diastolic Blood Pressure (mm/Hg)	-0.10*
VO$_2$ Max (ml/kg/min)	0.10*
VO$_2$ Max (ml/kg lean body mass/min)	0.02
Age (in years)	-0.14**
Pubertal Status (Tanner stage)	-0.12**

* $p < .05$, **$p < .0001$

Research Questions

Aim 1

Question 1a.

Question 1a was, "Do adiponectin levels differ by the demographic factors of gender, race (non-Hispanic Black and White), age (elementary age vs. high school age), and family history of CVD or diabetes?" In order to answer this question, regression models were run with GEE to compare mean adiponectin levels by demographic factors such as age-group, gender, race and family history of CVD or diabetes. No significant differences in mean adiponectin levels were found between subjects who were less than 13 years of age, and those who were 13 years old and older (p=.11). There were also no differences in male and female subjects (p=.053). When race was added to the model with gender, however, both race and gender groups were found to differ significantly on mean adiponectin levels (p<.05); Black subjects had lower mean adiponectin levels than White subjects, and male subjects had lower adiponectin levels than female subjects. An interaction term between race and gender was not significant. In a model that included only race, mean adiponectin levels were also lower in Black subjects than in White subjects (p<.0001) (see Table 7 for parameter estimates for race and gender models).

In the initial part of post-hoc analyses to determine if adiponectin differed by the four race/gender groups adiponectin, means differed by gender in Black subjects, but not in Whites. With a Bonferroni correction for the 6 contrast tests, however, the only significant differences were between Black males and White subjects of either gender, and between Black females and White females (see Table 8 for parameter estimates for race/gender models). There was no longer a difference in adiponectin means between Black males and females (see Table 9 for race/gender means). When the model was re-run to include BMI z-

Table 7

Parameter Estimates for Race and Gender Models

Variable	B-coefficient	SD	p value
	Gender		
Gender (male)	- 0.9421	0.45	0.0538
	Race		
Race (Black)	- 2.6993	0.42	0.0001
	Gender and Race		
Gender (male)	- 1.0171	0.44	0.0341
Race (Black)	- 2.7337	0.43	0.0001

Table 8

Parameter Estimates for Models with Race/Gender Variable, With and Without BMI z-score

Variable	B-coefficient	SD	p value
Model with only Race_gender Variable			
Race_gender (Black males)	- 3.2954	0.55	0.0012
Race_gender (Black females)	- 2.1278	0.54	
Race_gender (White males)	- 0.8414	0.58	
Race_gender (White females)	0.000	0.00	
Model with Race_gender and BMI z-score			
Race_gender (Black males)	- 3.1380	0.57	0.0009
Race_gender (Black females)	- 1.5147	0.52	
Race_gender (White males)	- 0.3728	0.56	
Race_gender (White females)	0.0000	0.00	
BMI z-score	- 1.7377	0.17	< 0.0001

scores, adiponectin levels in Black boys differed from Black girls and from White subjects of either gender, even when correcting for a Bonferroni adjustment (see Table 8 for parameter estimates for race/gender models). Adiponectin means were lower in Black boys than any other race/gender group (see Table 9 for race/gender means).

Table 9

Descriptive Statistics for Adiponectin by Race/Gender Group

Race/Gender	Mean(μg/ml)	SD	Min.	Max.
Black Males	11.02	6.24	1.30	36.08
Black Females	12.19	6.43	1.84	39.41
White Males	13.48	6.81	1.57	46.90
White Females	14.32	6.50	1.90	49.93

In the subset of subjects who had data available regarding family history of CVD or diabetes (n=588 and n=587, respectively), there were no significant differences noted between the family history of CVD groups (p=.58), but subjects who had a family history of diabetes had lower mean adiponectin levels than subjects who reported no family history of diabetes (p<.05).   The latter finding remained true even when adjusting for race and gender (p<.05); all three variables were significant in that model.

Because of the significant differences in adiponectin by family history of diabetes group, regression models with and without the family history of diabetes variable were run using the subset of 587 subjects who had available data on family history of diabetes to determine the influence that family history of diabetes might contribute to subsequent models. Adiponectin was the dependent variable for each model, and independent variables

other than family history of diabetes were BMI z-score, age, HDL-C, LDL-C, triglycerides, insulin, glucose, mean systolic and diastolic blood pressure, and VO_2. Adiponectin did not differ by the family history of diabetes variable in these 2 models, and there were no differences in significant findings otherwise. It was therefore concluded that information about whether or not a subject had a family history of diabetes contributed no additional influence to the model, and subsequent models were run without the family history of diabetes variable.

Question 1b

Question 1b was,"Does adiponectin differ by pubertal stages I-V, controlling for gender or race, and does this relationship differ by race, gender or overweight status?" Mean adiponectin levels were compared by pubertal stage for this research question. The adiponectin means for pubertal stages 1-5 may be found in Table 3 (Descriptive Statistics for Adiponectin). Pubertal stage was not a significant independent variable in a regression model with adiponectin as the dependent variable (p=.42). In addition, no differences were noted in mean adiponectin levels among pubertal stages when contrasts for trend were run. When race and gender were added to the model, both variables were significant (p<.0001 and p<.05, respectively), but pubertal stage remained non-significant (p=.24). Black subjects and male subjects had lower adiponectin than White subjects or female subjects, respectively. Contrasts for trend by pubertal stage in the model with race and gender did show that adiponectin means were higher in stage 1 than in stages 3 and 4 at an alpha level of 0.05, but the findings were not significant when a Bonferroni correction was made for the 10 contrast tests (alpha level 0.005).

When interaction terms between pubertal stage and race, gender or the 3 BMI risk levels were added to the model, pubertal stage remained non-significant (p=.57). The main effects of race and BMI risk level were significant (p<.01 and p<.05, respectively), but the main effect of gender (p=.06) and all interaction terms were non-significant. In order to obtain a more parsimonious model, backward statistical selection was used to delete variables one at a time, starting with the higher order interaction terms. The variable with the highest p value was removed and the model was reviewed, then the process was repeated until p values for most variables were significantly related to adiponectin; variables considered theoretically important to the model were not removed. The final model included only the main effects of race, gender and BMI risk; pubertal status was not significant. Again, race and gender were significant (p<.001 and p<.05, respectively), with adiponectin means being lower in Black subjects and male subjects than in White or female subjects. Subjects with lower BMI risk had higher adiponectin levels (p<.0001) (see Table 10 for parameter estimates for full model with 5 pubertal stages and interaction terms, and Table 11 for reduced model).

Lastly, because several studies in the literature used a 2-stage variable for puberty (pre-pubertal, and pubertal and beyond), a variable that indicated 2 stages of puberty in the current study was created, and used in the same models as described above instead of the 5 stage variable. There were no differences in mean adiponectin between the 2 pubertal stages when in a model without covariates (p=0.052). However, when the 2 pubertal stage variable was used in a model with race and gender, there were significant differences by race (p<.0001), gender (p<.05), and pubertal stage (p<.05); subjects who were pre-pubertal had higher adiponectin levels than subjects who were pubertal or post-pubertal. In a model with interaction terms between the 2-stage variable and race, gender or BMI risk level, pubertal

Table 10

Parameter Estimates for Full Model with 5 Pubertal Stages and Interaction Terms

Variable	B-coefficient	SD	p value
Pubertal stage 1	1.3405	2.21	0.5701
Pubertal stage 2	2.5661	2.53	
Pubertal stage 3	1.7376	2.29	
Pubertal stage 4	2.4779	2.37	
Race (B)	0.4503	1.73	0.0063
Gender (M)	-2.4582	0.89	0.0609
BMI risk (NL)	6.1031	2.15	0.0169
BMI risk (AR)	1.9469	1.67	
Pubertal Stage 1 *Race (B)	-3.1245	1.85	0.6108
Pubertal Stage 2 *Race (B)	-2.7417	1.94	
Pubertal Stage 3 *Race (B)	-2.5561	1.99	
Pubertal Stage 4 *Race (B)	-2.6798	2.22	
Pubertal Stage 1 *Gender (M)	1.6500	1.09	0.3129
Pubertal Stage 2 *Gender (M)	1.5207	1.21	
Pubertal Stage 3 *Gender (M)	2.1066	1.16	
Pubertal Stage 4 *Gender (M)	-0.3188	0.98	
Pubertal Stage 1 *BMI risk (NL)	0.2473	2.22	0.2157
Pubertal Stage 2 *BMI risk (NL)	-1.9643	2.28	
Pubertal Stage 3 *BMI risk (NL)	-2.0066	2.13	
Pubertal Stage 4 *BMI risk (NL)	-2.1409	2.45	
Pubertal Stage 1 *BMI risk (AR)	1.8976	1.90	
Pubertal Stage 2 *BMI risk (AR)	-0.5734	2.06	
Pubertal Stage 3 *BMI risk (AR)	1.3499	1.94	
Pubertal Stage 4 *BMI risk (AR)	0.3988	1.98	

*NL = BMI < 85th percentile, AR = BMI 85th to < 95th percentile (adj. for age and gender)

Table 11

Parameter Estimates for Reduced Models with 5 Pubertal Stages and Interaction Terms

Variable	B-coefficient	SD	p value
Pubertal Stage 1	0.8276	1.39	0.5913
Pubertal Stage 2	0.3869	1.38	
Pubertal Stage 3	0.1000	1.2641	
Pubertal Stage 4	-0.4271	1.13	
Race (B)	-2.1889	0.43	0.0002
Gender (M)	-1.3102	0.46	0.0153
BMI risk (NL)	4.5617	0.37	< 0.001
BMI risk (AR)	2.5196	0.48	

*NL = BMI < 85th percentile, AR = BMI 85th to < 95th percentile (adj. for age and gender)

Table 12

Parameter Estimates for Model with 2 Pubertal Stage Groups, Including Race, Gender, BMI

risk level and interaction term

Variable	B-coefficient	SD	p value
Pubertal Group 1 (Tanner 1)	-1.0879	0.49	0.6465
Pubertal Group 2 (Tanner 2 – 5)	0.0000	0.00	
Race (B)	-2.1949	0.43	0.0002
Gender (M)	-1.1543	0.42	0.0135
BMI risk (NL)	4.1575	0.42	< 0.0001
BMI risk (AR)	2.2398	0.61	
Pubertal Group 1*BMI risk (NL)	2.2533	0.67	0.0291
Pubertal Group 1*BMI risk (AR)	1.5687	1.16	

*NL = BMI < 85th percentile, AR = BMI 85th to < 95th percentile (adj. for age and gender)

stage was no longer significant (p=.65), but race and gender remained significant (p<.0001 and p<.05, respectively). BMI risk level was also significant (p<.0001), as was the interaction term between BMI risk level and the 2-stage puberty variable (p<.05) (see Table 12 for parameter estimates for model with 2 pubertal stages, race, gender and BMI risk level). Subjects with lower BMI risk levels had higher adiponectin levels. The interaction was interpreted as indicating that pre-pubertal subjects with lower BMI risk levels had higher adiponectin levels (see Table 13 below for adiponectin means by BMI risk level and 2 pubertal stages).

Table 13

Adiponectin means (μg/ml) and standard deviations by 2 puberty groups and BMI risk

	Puberty Group 1 (Tanner 1)	Puberty Group 2 (Tanner 2-5)
BMI risk = NL	15.61 (6.62)	13.54 (6.46)
BMI risk = AR	12.82 (5.51)	11.83 (6.49)
BMI risk = OW	8.61 (3.93)	9.55 (5.87)

*NL = BMI < 85th percentile, AR = BMI 85th to < 95th percentile, OW = BMI > 95th percentile (percentiles adjusted for age and gender)

Aim 2

Question 2a

Question 2a was "Of BMI z-score, waist circumference and sum of skin folds, which measure of adiposity is the best predictor of adiponectin levels, when controlling for demographic factors or puberty?" Pearson correlations between adiponectin and each of the 3 adiposity variables (BMI z-score, waist circumference and sum of skin folds) were all inverse and significant (p<.0001, see results in table 6). Correlations ranged from r = -0.27

for skin folds to r = -0.34 for waist circumference. Each of the 3 adiposity variables were then used in regression models with adiponectin as the dependent variable; QIC scores were obtained for each model. The models were run separately because of the increased correlation between the 3 adiposity measures; the Pearson R ranged from 0.68 for the correlation between waist and BMI z-score, and 0.79 for the correlation between waist and sum of skin folds (p value <.0001 for all correlations). Each model was significant (p<.0001), and each adiposity variable was inversely related to adiponectin, as was noted in bivariate correlation results. The QIC score was technically lowest for the waist circumference variable, but the difference between the QIC numbers for the model with BMI z-score and the one with waist was only 0.34, indicating that the 2 models were similar. Models with a difference of approximately 2 or more between QIC numbers are considered to be different form one another (personal communication, Wei Pan, March 2007). The QIC number for the model with sum of skin folds was 1.20 points higher than the model with BMI z-score and 1.54 points higher than the model with waist, so it could be considered somewhat different from the former 2 models (personal communication, Wei Pan, March 2007). The parameter estimates for all models with BMI z-score, waist circumference and sum of skin folds are presented in Tables 14, 15, and 16, respectively.

When race and gender were added to the models described above, the adiposity measures remained significant and inversely related to adiponectin. The race and gender variables were also significant. As noted in previous models, Black subjects and male subjects had lower adiponectin levels. Again, the model with waist circumference had the lowest QIC number and the model with sum of skin folds had the highest number, but all 3 QIC numbers were fairly close.

Table 14

Parameter Estimates and QIC Values for Aim 2 Models with BMI z-score

	β-coefficient (SE)	p value	QIC value
Model 1			
BMI z-score	-1.8003 (0.15)	<.0001	1219.59
Model 2			
BMI z-score	-1.7130 (0.17)	<.0001	1224.43
Race (B)	-2.1863 (0.45)	0.0005	
Gender (M)	-1.0877 (0.43)	0.0172	
Model 3			
BMI z-score	-1.7209 (0.17)	<.0001	1228.28
Race (B)	-2.1286 (0.45)	0.0004	
Gender (M)	-1.0757 (0.43)	0.0190	
Age	-0.3629 (0.10)	0.0010	

Table 15

Parameter Estimates and QIC Values for Aim 2 Models with Waist Circumference

	β-coefficient (SE)	p value	QIC value
	Model 1		
Waist Circumference	-0.1624 (0.01)	<.0001	1219.25
	Model 2		
Waist Circumference	-0.1596 (0.01)	<.0001	1223.51
Race (B)	-2.4339 (0.43)	0.0001	
Gender (M)	-1.0887 (0.40)	0.0113	
	Model 3		
Waist Circumference	-0.1596 (.01)	<0001	1228.12
Race (B)	-2.4339 (.43)	0.0001	
Gender (M)	-1.0888 (.41)	0.0115	
Age	0.0018 (.11)	0.9870	

Table 16

Parameter Estimates and QIC Values for Aim 2 Models with Sum of Skin folds

	β-coefficient (SE)	p value	QIC value
Model 1			
Sum of Skin folds	-0.1252 (0.01)	<.0001	1220.79
Model 2			
Sum of Skin folds	-0.1370 (0.01)	<.0001	1225.60
Race (B)	-2.4091 (.41)	0.0002	
Gender (M)	-1.9797 (.49)	0.0009	
Model 3			
Sum of Skin folds	-0.1342 (0.01)	<.0001	1231.34
Race (B)	-2.3700 (.40)	0.0002	
Gender (M)	-1.9479 (.50)	0.0011	
Age	-0.2465 (.11)	0.0284	

Lastly, the same models were run with the addition of age. Results were similar in that waist circumference continued to have the lowest QIC number. Model results among the 3 models were also similar in relation to the adiposity variable, race and gender information, but age was only significant in the models with BMI z-score and sum of skin folds. Age was inversely related to adiponectin in those 2 models (p<.01 in the model with BMI z-score and p<.05 in the model with sum of skin folds). In the model with race, gender, waist circumference and age, age was not significantly related to adiponectin; only the former variables were significantly related. In summary, the models with waist circumference were consistently the best models. The models with the sum of skin folds measured tended to have the highest QIC numbers, indicating they were not the best models.

Aim 3

Question 3a

Question 3a was, "Do mean adiponectin levels differ by risk categories for the following risk factors for CVD and T2D, when controlling for demographic factors: HDL-C, glucose, insulin, LDL-C, triglycerides, systolic or diastolic BP, and level of fitness?" Separate regression models were run using GEE to determine if adiponectin means differed by categorical risk factor level. Adiponectin was the dependent variable and variables that indicated subject risk factor category level were the independent variables. Definitions for risk factor category cut-points are given in Appendix H. Mean adiponectin levels did not differ significantly by categorical risk level for the following risk factors: triglycerides, LDL-C, glucose or VO_2 as measured in 'ml/kg lean body mass/min' units. Adiponectin means did differ significantly by risk level for HDL-C (p<.001), insulin (p<.0001), blood pressure (p<.05), waist circumference (p<.0001), BMI (p<.0001), and VO_2 when expressed

as 'ml/kg/min.' According to the model estimates, adiponectin was higher in subjects who were in the lower risk categories (see mean adiponectin levels by risk category in table 4). When contrast tests were done to examine for differences in adiponectin means by the 3 levels of risk for blood pressure, a significant difference was noted between subjects with normal blood pressure and those with pre-hypertension ($p<.05$), but when a Bonferroni correction was made for the 3 contrast tests there was no longer a difference at the decreased alpha level of 0.017. Contrasts for the 3 levels of BMI, however, did show significant differences in adiponectin means between all 3 levels even with the decreased alpha level. As the subjects' risk category levels decreased, adiponectin consistently increased.

When race and gender were added to the models described above, the results were fairly similar; in addition to adiponectin means differing significantly by race and gender in all models ($p<.001$ and $p<.05$, respectively), they also differed by the risk level of HDL-C, insulin, waist circumference, and BMI ($p<.0001$ for all risk factors). Contrasts for trend with BMI risk levels remained significant in the models with race and gender ($p<.001$). As in the previous models, parameter estimates indicated that adiponectin was higher in the subjects who were in the lower risk level categories. There was no difference in mean adiponectin by LDL-C, glucose, triglycerides or VO_2. One difference from the previous models was that adiponectin means no longer differed by risk level for blood pressure when race and gender were added to the model.

Question 3b

Models with Continuous Risk Factor Variables

Question 3b was, "What is the relationship of adiponectin to the following risk factors for CVD and T2D (HDL-C, LDL-C, triglyceride, total cholesterol, glucose, insulin, BP and

fitness level) when operationalized as continuous variables, when controlling for demographic factors and measures of adiposity?" Bivariate correlations among adiponectin and risk factor variables are presented in table 6. All risk factor variables that were significantly correlated with adiponectin were included as independent variables in a model with adiponectin as the dependent variables; these variables were race, gender, age, HDL-C, triglycerides, insulin, and systolic and diastolic blood pressure. Race, gender, HDL-C, insulin and systolic blood pressure were significantly related to adiponectin in this model, but age, triglycerides, and diastolic blood pressure were not. The model was reduced to a simpler model by backwards deletion. First, the variable with the largest p value was deleted and results were reviewed. This process was repeated, one variable at a time, until a more parsimonious model was obtained. Independent variables for this model included race, gender, HDL-C, insulin and systolic blood pressure. The full and reduced models were compared by the use of QIC numbers, as were the models in Aim 2. The full and reduced models in this question were also compared by the use of a model with contrasts for deleted variables that tested the hypothesis that the β-coefficients of the deleted variables were essentially equal to 0, and the resulting Chi-square statistic. If the Chi-square probability was greater than 0.05, this was interpreted as an indication that the coefficients were no different from 0, and the reduced model was better than the full model (see table 17 for β coefficients, standard errors, and p values for the full and reduced models, and the QIC numbers and Chi-square probability statistics used to compare the models).

Adiposity Measures added to Models

When the variables race, gender, HDL-C, insulin and systolic blood pressure were included in models with waist circumference, insulin and systolic blood pressure were no

Table 17

Parameters for First Full and Reduced Models in Question 3b with Risk Factors only

Variable	β Coefficient	SE	p value	QIC#	Pr > Chi^2
			Full Model		
Race (B)	-2.8645	0.4940	0.0002	1232.74	
Gender (M)	-1.4779	0.4105	0.0017		
Age	-0.1123	0.1103	0.3034		
HDL-C	0.0983	0.0102	<.0001		
Trig.	-0.0121	0.0062	0.0645		
Insulin	-0.0935	0.0264	0.0002		
SBP	-0.0464	0.0207	0.0359		
DBP	-0.0075	0.0180	0.6788		
			Reduced Model		
Race (B)	-2.6757	0.4805	0.0002	1226.46	0.1191
Gender (M)	-1.4063	0.4103	0.0021		
HDL-C	0.1056	0.0114	<.0001		
Insulin	-0.1049	0.0259	<.0001		
SBP	-0.0550	0.0156	0.0030		

longer significant. When the model was reduced to a more parsimonious version, the final model included race, gender, HDL-C and waist circumference. According to the model, adiponectin was lower in Black or male subjects, and in subjects with higher waist circumference measures. Adiponectin was higher in subjects who had higher HDL-C levels. The final model was compared to the full model in the same manner as for previous models (see table 18 for β coefficients, standard errors, and p values for the full and reduced models with waist circumference, and the QIC numbers and Chi-square probability statistics used to compare the models).

In a model with BMI z-score as opposed to waist circumference, only systolic blood pressure was no longer significant; insulin remained significantly related to adiponectin. Systolic blood pressure was removed from the model, and the resulting model was compared to the full model by using the QIC numbers and Chi-square probability statistics as described above (see table 19 for β coefficients, standard errors, and p values for the full and reduced models with BMI z-score, and the QIC numbers and Chi-square probability statistics used to compare the models). This model was interpreted in a similar manner as the model with waist circumference; adiponectin was lower in Black or male subjects, and in subjects with higher insulin levels or BMI z-scores. Adiponectin was higher in subjects with higher HDL-C levels.

Question 3c

Models to Test Interaction Terms

Question 3c was, "Do the relationships of adiponectin to the risk factors in question3b differ by race (non-Hispanic Black or White), gender or overweight status?" In order to answer this question, the variables that remained significantly related to adiponectin in the

Table 18

Parameters for Full and Reduced Models in Question 3b With Risk Factor Variables and

Waist Circumference

Variable	β Coefficient	SE	p value	QIC#	Pr > Chi^2
		Full Model			
Race (B)	-2.6951	0.4665	0.0001	1227.02	
Gender (M)	-1.2769	0.3987	0.0032		
HDL-C	0.0863	0.0111	0.0001		
Insulin	-0.0443	0.0276	0.0792		
SBP	-0.0260	0.0157	0.1140		
Waist	-0.0987	0.0181	0.0004		
		Reduced Model			
Race (B)	-2.8867	0.4688	<.0001	1224.12	0.1056
Gender (M)	-1.1915	0.4031	0.0061		
HDL-C	0.0900	0.0110	0.0001		
Waist	-0.1246	0.0122	<.0001		

Table 19

Parameters for Full and Reduced Models in Question 3b With Risk Factor Variables and

BMI z-score

Variable	β Coefficient	SE	p value	QIC#	Pr > Chi^2
			Full Model		
Race (B)	-2.4927	0.4856	0.0003	1226.92	
Gender (M)	-1.3386	0.4051	0.0028		
HDL-C	0.0916	0.0106	0.0001		
Insulin	-0.0663	0.0244	0.0047		
SBP	-0.0344	0.0161	0.0511		
BMI-z	-0.9892	0.1897	0.0001		
			Reduced Model		
Race (B)	-2.5301	0.4903	0.0004	1226.16	0.0511
Gender (M)	-1.4199	0.4195	0.0026		
HDL-C	0.0929	0.0110	<.0001		
Insulin	-0.0737	0.0255	0.0034		
BMI-z	-0.0798	0.1901	<.0001		

reduced model with only risk factors in question 3b, and interaction terms between those variables and race, gender and BMI risk category, were all used as independent variables in a model with adiponectin as the dependent variable. BMI risk level was included as a categorical main effect variable in this model, as opposed to the use of the continuous BMI z-score variable in the previous question, in order to determine if the relationships among adiponectin and the risk factor variables were moderated by BMI risk. Race and gender were also used in the model to examine the main effects of race and gender. All three categorical variables were used with risk factor variables to create interaction terms for use in testing for moderation effects. After the full model was run, a more parsimonious model was selected by using backward statistical selection. The full model and final model were compared by using QIC numbers and a Chi-square statistic, as described previously. Significant variables in the final model were race, BMI risk category, HDL-C, systolic blood pressure, insulin, and an interaction between HDL-C and gender. According to the model, adiponectin was lower in Black children, children at a higher level of BMI risk category, and in subjects with higher systolic blood pressure or higher insulin. Adiponectin was higher in subjects with higher HDL-C levels. An interaction between HDL-C and gender was also significant, meaning that gender functioned as a moderator of the relationship between HDL-C and adiponectin. The result of the moderating effect was interpreted as the relationship between adiponectin and HDL-C being weaker in males. Gender was not significant in the final model, but it was left in the model because of the significant interaction term. Race and BMI risk level did not function as moderators for the relationship between adiponectin and HDL-C, insulin, or systolic blood pressure. See tables 20 and 21 for β coefficients, standard errors, p values, QIC numbers and Chi-square probability statistics for the full and reduced models.

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Table 20

Parameters for Full Model With Interaction Terms

Variable	β Coefficient	SE	p value	QIC#	Pr > Chi^2
				1236.62	
Race (B)	1.4933	3.8598	0.7024		
Gender (M)	0.1930	4.0252	0.9618		
BMI risk (NL)*	7.5407	4.0482	0.2089		
BMI risk (AR)*	2.9466	5.3022			
HDL-C	0.1631	0.0343	<.0001		
SBP	0.0090	0.0419	0.2255		
Insulin	-0.1691	0.0531	0.0552		
Hdl*race (B)	-0.0205	0.0293	0.4970		
Hdl*gender (M)	-0.0520	0.0243	0.0383		
Hdl*BMI risk (NL)	-0.0461	0.0317	0.3174		
Hdl*BMI risk (AR)	-0.0006	0.0460			
Insulin*race (B)	0.1075	0.0481	0.0818		
Insulin*gender (M)	0.0294	0.0351	0.4021		
Insulin*BMI risk (NL)	0.0119	0.0455	0.5624		
Insulin*BMI risk (AR)	0.0723	0.0597			
SBP*race (B)	-0.0424	0.0296	0.1699		
SBP*gender (M)	0.0056	0.0342	0.8698		
SBP*BMI risk (NL)	-0.0276	0.0336	0.7230		
SBP*BMI risk (AR)	-0.0249	0.0493			

*NL = BMI < 85th percentile, AR = BMI 85th to < 95th percentile (adj. for age and gender)

Table 21

Parameters for Reduced Model With Interaction Terms

Variable	β Coefficient	SE	p value	QIC#	Pr > Chi^2
				1227.72	0.6014
Race (B)	-2.5200	0.4666	0.0002		
Gender (M)	1.5326	1.0329	0.1458		
BMI risk (NL)	2.7341	0.5325	0.0017		
BMI risk (AR)	1.4794	0.5397			
HDL-C	0.1197	0.0149	<0.0001		
SBP	-0.0379	0.0168	0.0377		
Insulin	-0.0591	0.0270	0.0187		
Hdl*gender (M)	-0.0584	0.0206	0.0087		

*NL = BMI < 85th percentile, AR = BMI 85th to < 95th percentile (adj. for age and gender)

Models in Subject Groups Stratified by Gender

The final, or reduced, model described above was run in subject groups stratified by gender because of the interaction between HDL-C and gender (see figure 6). The gender variable and the interaction term between HDL-C and gender were removed when the models were run in the separate groups. Race, BMI risk category and HDL-C were the only significant variables when the model was run using male subjects (n=612); systolic blood pressure and insulin were not significant. The parameter estimates indicated that adiponectin was lower in Black males, and higher in males with higher HDL-C levels. Subjects who were in a lower BMI risk level category also had higher adiponectin levels.

In the model run with female subjects (n=603), all variables except systolic blood pressure remained significant. According to parameter estimates, Black females and the

females with higher insulin levels had lower adiponectin levels. Females in a lower BMI

risk level category or those who had higher HDL-C levels had higher adiponectin levels. The

β coefficients, standard errors, and p values for the models in males and females are

presented in table 22.

In summary, adiponectin means were lower in Black subjects and in male subjects

overall, and specifically lower in Black males than in other race/gender groups. Adiponectin

means were also lower in subjects with a family history of diabetes, but there were no

differences in adiponectin means by Tanner stage or fitness level. BMI z-score, waist

circumference and sum of skinfolds were each inversely related to adiponectin, but BMI z-

score and waist circumference provided the best models for prediction of adiponectin when

compared by the Quasi-Likelihood in Independence Model Criterion (QIC). Adiponectin

was positively associated with HDL-C and inversely associated with insulin and systolic

blood pressure in multivariate regression with other risk factors, but the relationships with

insulin and systolic blood pressure were dependent on one or more measures of adiposity.

There was an interaction between HDL-C and gender, in that the relationship between

adiponectin and HDL-C was stronger in female than in males. Insulin was only related to

adiponectin in female subjects.

Table 22

Parameter Estimates for Interaction Models Stratified by Gender

Variable	β Coefficient	SE	p value
Males			
Race (B)	-2.7916	0.6297	0.0007
BMI risk (NL)	3.5844	0.7959	0.0036
BMI risk (AR)	1.2218	0.6566	
HDL-C	0.0648	0.0187	0.0133
SBP	-0.0204	0.0221	0.3847
Insulin	-0.0256	0.0316	0.3944
Females			
Race (B)	-2.0707	0.5633	0.0041
BMI risk (NL)	1.6439	0.9174	0.2011
BMI risk (AR)	1.5055	0.8631	
HDL-C	0.1196	0.0167	<0.0001
SBP	-0.0525	0.0260	0.0646
Insulin	-0.0971	0.0409	0.0146

*NL = BMI < 85th percentile, AR = BMI 85th to < 95th percentile (adj. for age and gender)

Chapter 5

Discussion

Introduction

An inflammatory process is now considered to be the pathophysiological basis for CVD and T2D (Berg & Scherer, 2005; Pickup, 2004; Ross, 1999). Obesity, a common risk factor for both diseases, may play a role because of the numerous inflammatory factors secreted by adipocytes (Trayhurn & Beattie, 2001). Prolonged exposure to these factors in conditions of increased adiposity is thought to contribute to a chronic "pro-inflammatory milieu" (Lyon et al., 2003) that may lead to endothelial dysfunction and insulin resistance, and eventually to the disease states of T2D and CVD. However, not all substances secreted by adipocytes are harmful. Adiponectin, a protein secreted primarily by adipocytes, acts in an anti-inflammatory manner, and it appears that higher levels of adiponectin afford protection against atherosclerosis and insulin resistance (Furukawa et al., 2004; Ouchi et al., 2001; Ouchi et al., 2003; Ukkola & Santaniemi, 2002). However, adiponectin levels are lower in obese persons, a possible link to the increased CVD and T2D incidence in the obese. Adiponectin is positively correlated with protective factors such as HDL-C, and inversely related to many of the negative risk factors related to CVD and T2D such as increased adiposity, triglycerides, blood pressure and insulin levels in adults and youth (Asayama et al., 2003; Bacha et al., 2004; Bottner et al., 2004; Chu et al., 2005; Cnop et al., 2003; Gilardini et

al., 2006; Huang et al., 2003; Pischon et al., 2004). However, relatively few researchers have examined the relationships among adiponectin and multiple risk factors for CVD and T2D in a racially mixed sample.

This study is the largest of the studies that have examined adiponectin and its relationships with risk factors for CVD and T2D in a sample of Black and White youth. The majority of the studies in the literature have been focused on the relationship of adiponectin to measures of adiposity, insulin or insulin sensitivity, and lipids, and except for investigation regarding insulin, most researchers have only included race as a confounding variable. The literature also suggests that relationships between adiponectin and certain risk factors may differ by gender or overweight status. This study examined the relationships among adiponectin and multiple metabolic risk factors such as HDL-C, insulin and glucose, as well as other risk factors for CVD and diabetes, such as blood pressure, family history, and physical fitness levels. This racially diverse sample was also used to determine if relationships were moderated by race, gender or a subject's overweight status. Findings from this study are discussed in the following sections.

Race and Gender

Race was significantly related to adiponectin in this study of 1215 Black and White children and adolescents; adiponectin means were lower in Black subjects than in White subjects. These results are similar to those of other studies in the literature that compared Black and White youth (Bacha et al., 2005; Bush et al., 2005; Degawa-Yamauchi et al., 2003; Lee et al., 2006; Woo et al., 2005). The lower levels in Black subjects in this study may stem partly from the fact that there were also differences in some adiposity measures by race. Black children and adolescents had higher BMI z-scores and sum of skinfolds

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measures than White children and adolescents. However, there were no differences in waist circumference means by race, even though some studies have shown that central adiposity may influence adiponectin levels (Bacha, Saad, Gungor and Arslanian, 2004; Park et al., 2004). Further research is needed to more fully investigate the relationship between adiponectin and race, in regards to different adiposity measures

The significant relationship between adiponectin and race extended throughout all analyses. Adiponectin means differed by race even in multivariate regression equations with variables such as age, gender, HDL-C, LDL-C, triglycerides, insulin, and systolic and diastolic blood pressure. Most of the other study variables differed by race as well. Black youth had higher BMI z-scores, systolic blood pressures, HDL-C and insulin levels, and lower triglyceride levels than White youth. However, even though Black children had higher BMI z-scores, race was significantly related to adiponectin independently of BMI z-score. Race is clearly an important variable to consider in analyses regarding adiponectin, and also appears to play a role in the relationship between adiponectin and gender.

There were no differences in adiponectin means by gender in initial analysis of the relationship between adiponectin and gender alone, although the p value of 0.0511 was close to significance with an alpha level of 0.05. These results are not out of line with results in the literature, where about half of researchers found higher adiponectin levels in female subjects (Butte et al., 2005; Chu et al., 2005; Gilardini et al., 2006; Huang et al., 2004; Kim et al., 2006; Nemet et al., 2003; Punthakee et al., 2006 Singhal et al., 2005; Woo et al., 2005), and others have found no differences by gender (Asayama et al., 2003; Bacha et al., 2004; Bottner et al., 2004; Bush et al., 2005; Cianflone et al., 2005; Lee et al., 2006; Okada et al., 2005; Pilz et al., 2005; Stefan et al., 2002). When race was included in the equation for

analysis in this study, however, gender was found to be significantly related to adiponectin.

Further post-hoc analyses by the four different race/gender groups showed that race was an important consideration in regards to the relationship between adiponectin and gender. In an initial analysis, it appeared that adiponectin means might differ by gender in Black subjects, that is adiponectin means for Black males were lower than for Black females; there were no gender differences in White subjects. With a Bonferroni correction for the 6 contrast tests, however, the only significant differences were that adiponectin means in Black males were lower than those for White subject of either gender, and adiponectin means for Black females were lower than White females. There was no longer a difference in adiponectin means between Black males and females.

Only one other research group has examined adiponectin means by different race/gender groups, but they also included BMI in their model. Their results were somewhat different from these findings, as they found a difference between Black males and females, when adjusting for BMI (Degawa-Yamauchi et al., 2003). Black males in their study had lower adiponectin levels than Black females or White subjects of either gender. Their study was small, with a sample of 86 subjects. The subjects' ages ranged from 12-21 years, somewhat older than for this study. BMI percentiles were fairly similar, although Black females and males were slightly heavier in the current study. When the model examining differences in adiponectin means by race/gender levels in this study was repeated including BMI z-scores, the results matched those found by Degawa-Yamauchi et al. (2003); adiponectin means for Black males were lower than those for Black females, and also lower than means for White subjects of either gender, even when correcting for a Bonferroni adjustment.

This study adds to existing knowledge concerning differences in adiponectin means by race or gender, with information from a large sample with subjects ranging in age from 7 to 18. Information about race and gender is important in light of the fact that racial disparities are known to exist in the prevalence of CVD and T2D, and in the prevalence of related risk factors in adults and children (Brancati et al., 1996; Cook et al., 2003; Freedman et al., 2006; Haffner et al., 1999; Jago et al., 2006; Mensah et al., 2005). Although adiponectin means are lower in black youth overall analyses, the lower levels are primarily in black males, when adjustments are made for BMI z-score. Measures of race, gender and also adiposity are, therefore, also important to consider when studying the relationships among adiponectin and other risk factors for CVD and diabetes in youth.

Adiponectin and Measures of Adiposity

Adiponectin levels are lower in children who are overweight or obese in many studies, but most authors who have studied this relationship have only compared adiponectin means by two risk groups (Asayama et al., 2003; Bacha et al., 2004; Stefan et al., 2002). One group has usually been defined as having a normal weight and the other as being obese or overweight. The current study adds to existing knowledge by showing that adiponectin means decrease significantly as the 3 levels of risk defined by the Centers for Disease Control guidelines (CDC, 2003) increase. Adiponectin was also shown to differ by waist circumference risk groups, with mean levels lower in subjects in the higher waist risk groups. These results help to more clearly define the picture of risk associated with lower adiponectin levels.

The relationships among adiponectin and the 3 adiposity measures, BMI z-score, waist circumference and sum of skinfolds, were also examined in this study with an aim of

determining which was the best predictor of adiponectin when adjusting for demographic variables. Adiponectin was inversely related to BMI z-score, waist circumference and sum of skinfolds. Each adiposity measure remained significantly related to adiponectin when adjusted for race, gender and age. The inverse relationships between adiponectin and waist circumference or BMI z-score were also significant in later models that included other risk factors for CVD and diabetes that were significantly related to adiponectin, such as HDL-C, insulin, and systolic blood pressure. When the separate models were compared by using the QIC number (Pan, 2001), the models with waist circumference were technically the best models, with the smallest QIC number. QIC numbers for the models with BMI z-score were very close to the waist circumference numbers, only differing by 0.16 to 0.92, depending on which of the age, race or gender variables were also in the model. Models with QIC numbers that differ by less than approximately 2 are considered to be similar models (personal communication, Wei Pan, March 2007). The models with sum of skinfolds were not as good as those that included waist circumference or BMI z-score. The QIC numbers for the models with sum of skin folds were greater than the ones for waist circumference by a range of 1.54 to 3.22, and greater than the BMI z-score values by a range of 1.20 to 3.06.

Results in the literature have been conflicting in studies that compared the different adiposity measures (Bottner, et al., 2004; Huang et al., 2004; Singhal et al., 2005; Woo et al., 2005; Vikram et al., 2004). Vikram and colleagues found that BMI was related with adiponectin, but not waist circumference or sum of skinfolds in their sample of 62 male adolescents. Singhal and colleagues found the opposite, that waist and not BMI or sum of skinfolds was significantly related to adiponectin. Their sample was fairly large, but somewhat lean overall. Results have also been contradictory in studies that compared only

the two measures of BMI and waist circumference to one another (Bottner et al., 2004; Huang et al., 2004; Woo et al., 2005). Most of the models, except in the study by Woo et al. (2005), included different adiposity measures in the same model. This is concerning in light of the fact that the adiposity measures were highly correlated in the current study. Separate models were therefore used for each adiposity measure in this study, to avoid problems with multi-collinearity. Woo and colleagues were the only group to compare models with BMI z-score or waist circumference using criteria such as the Bayesian Information Criterion (BIC). The model with BMI z-score had the highest BIC value, which indicated that it was the best model. The current study indicates waist circumference and BMI z-scores to be the best predictors of adiponectin, and they may convey different influences on the significance of the other variables, as well be discussed in the following sections.

Relationships Among Adiponectin and Bio-Behavioral Risk Factors for CVD and Diabetes

In addition to waist circumference, BMI z-scores and BMI risk level, the variables HDL-C, insulin and systolic blood pressure were the most consistently related to adiponectin, by bivariate correlation and in most multivariate models. Triglycerides, LDL-C, glucose, diastolic blood pressure and physical fitness variables were less likely to be associated with adiponectin, especially in multivariate models. The relationship of each variable with adiponectin, and any interaction effects are discussed below. Effects of the adiposity measures on risk factor variables are also discussed.

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HDL-Cholesterol

Similar to the variable of race, HDL-C was related to adiponectin throughout all analyses. The relationship was in the positive direction in each analysis, that is, higher adiponectin levels were related to higher levels of HDL-C. This relationship was found in bivariate correlations, and in multivariate regression models with demographic variables, other risk factor variables, and with either of the adiposity variables of waist circumference, BMI z-score or BMI risk level. Adiponectin means also differed by the risk level related to HDL-C, in that subjects with HDL-C levels greater than 35 mg/dl had higher adiponectin means that subjects with lower HDL-C levels; this relationship was also independent of race and gender.

The positive relationship between adiponectin and HDL-C is similar to most other studies that have been done with children and adolescents (Asayama et al., 2003; Chu et al., 2005; Bacha et al., 2004; Bottner et al., 2004; Butte et al., 2005; Gilardini, et al, 2006; Huang et al., 2004; Martin et al., 2005; Nemet et al., 2003; Okada et al., 2005; Pilz et al., 2005; Singhal et al., 2005). Vikram et al. (2004) were the only researchers who found no relationship between adiponectin and HDL-C, in their group of 62 post-pubertal males. The sample was fairly lean as well, as opposed to the sample for this study.

The only study that examined the relationship between HDL-C and adiponectin in a sample of Black and White youth also noted a positive correlation between adiponectin and HDL-C, but race was only used as a covariate in that study (Martin et al., 2005). No other researchers have investigated the possibility that an interaction might exist between race and HDL-C in relation to adiponectin in children or adolescents, that is, that race might moderate the relationship. One study in adults found that adiponectin was only related to HDL-C in

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White subjects, and not in Black or Indian subjects (Ferris et al., 2005). No interactions were noted in the current study, even though the study was well-powered. There was a fairly even distribution according to race and gender, and a wide range of age and BMI levels. HDL-C and adiponectin means both differed by race, but the interaction between race and HDL-C was not significant.

This study also included analyses to determine if an interaction between gender and HDL-C existed in relation to adiponectin. A significant interaction was found in a model with race, gender, insulin, systolic blood pressure, BMI risk level, and other interaction terms between risk factors and race, gender or BMI risk level. The interaction indicated that the relationship between HDL-C and adiponectin was stronger in females than in males. Very few studies have examined for differences in this relationship by gender.

Results from one study showed that bivariate correlations between adiponectin and HDL-C were only significant in female subjects (Okada et al., 2005), whereas other studies have found the correlation to be significant in both genders (Chu et al., 2005). Another group found that although HDL-C was related to adiponectin in the overall sample of 294 British youth, it was not related to adiponectin in males, and there was a borderline level (p=0.05) relationship noted in females (Singhal et al., 2005). The model in the study by Singhal and associates did not include measures of adiposity, and the subjects in were relatively lean in comparison to the subjects in this study.

Adiponectin levels were higher in females in this study when race was included in the models, but there was no difference in HDL-C means by gender. A significant interaction between HDL and gender in relation to adiponectin is important because it indicates the possibility that adiponectin may behave differently in females as opposed to males. This will

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be important to remember in future research that includes subjects of different genders, and possibly in research regarding the development of therapeutic interventions that involve adiponectin.

This study also examined the effects of adiposity measures on the relationship between HDL-C and adiponectin, and results indicated that HDL-C was related to adiponectin independently of adiposity measures. Models included either waist circumference, BMI z-score, or BMI risk level, along with race, gender and other risk factor variables. No interaction was found, however, between HDL-C and BMI risk level, in relation to adiponectin. These results are in contrast to a study by Martin et al. (2005), where results indicated that the relationship between adiponectin and HDL-C was stronger in subjects who were obese than in lean subjects; obese was defined as a BMI greater than the 85th percentile for age and gender. It is unclear why the results of the current study differ; both studies included a large number of subjects and included a fairly even distribution of subjects from both genders and both Black and White races. One difference might be that subjects in the study by Martin and associates were somewhat leaner than subjects in the current study, although the differences were minor. Thirty-seven percent of subjects had BMI values greater than the 85th percentile, as opposed to 41.5% in the current study. There was also no adjustment for clustering in the study by Martin and colleagues, even though data were collected from subjects recruited from school settings.

Triglycerides

Triglycerides were inversely correlated with adiponectin in bivariate correlation tests in this study. However, the variable was no longer related to adiponectin in a multivariate model with other risk factor variables. It was therefore not used for in subsequent analyses

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with adiposity variables. Several research groups also found that triglycerides were inversely correlated with adiponectin (Asayama et al., 2003; Gilardini et al., 2006; Okada et al., 2005). Also similar to this study, the relationship between adiponectin and triglycerides was lost in models with variables such as age, gender, HDL-C (Huang et al., 2004). The relationship was, however, independent of BMI in one study (Pilz, et al., 2005), but the BMI was not adjusted for age and gender. In addition, over half of the 240 subjects were very obese, with BMI values greater than the 97th percentile. In another study, correlations were stronger in subjects with BMI values greater than the 85th percentile, adjusting for pubertal stage, gender and race (Martin et al., 2005). Subjects in that study were similar overall in race and gender to the current study, but were somewhat leaner, although only to a minor degree. No correlation between adiponectin and triglycerides was found at all in one study (Vikram et al., 2004). The sample in the study by Vikram and associates was all male, and relatively lean in comparison to the current study. The current study indicates that the effects of other demographic and risk factor variables in relation to adiponectin seem to outweigh the relationship of triglycerides to adiponectin.

LDL-Cholesterol

There was no significant bivariate correlation between LDL-C and adiponectin in the current study. Likewise, there were no differences in adiponectin means by LDL-C risk levels. Therefore, this variable was not used for further multivariate analyses. Most of the other studies that examined the relationship between adiponectin and LDL-C also found no relationship between the two variables in bivariate or multivariate analyses (Asayama et al., 2003; Bacha et al., 2004; Huang et al., 2004; Singhal et al., 2005; Pilz et al., 2005) in bivariate or multivariate analyses. The only exception was one study where a significant

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correlation was found in female subjects only (Okada et al., 2005). All subjects in the study by Okada and associates were Asian, and were younger compared to the sample used in the current study. Results of this study, and most other studies in the literature that examined LDL-C, indicate that it is not closely related to adiponectin.

Insulin

Insulin was strongly and inversely related to adiponectin in bivariate correlation, and in most multivariate models in this study. These results are similar to most of the studies in the literature that found an inverse relationship with insulin or insulin resistance, or a positive relationship with insulin sensitivity (Bacha et al., 2004; Bottner et al., 2004; Bush et al., 2005; Chu et al., 2005; Degawa-Yamauchi et al., 2003; Lee et al., 2006; Tsou et al., 2004). The inverse relationship between insulin and adiponectin was expected as adiponectin appears to have protective properties in regard to T2D and insulin resistance (Combs, Berg, Obici, Scherer and Rossett, 2001; Yamauchi et al., 2001; Yamauchi et al., 2002). Adiponectin has also been shown to predict insulin resistance and T2D in human adults and children (Cruz et al., 2004; Snehalatha et al., 2003; Spranger et al., 2003;Yamamoto, Hirose, Saito, Nishikai, & Saruta, 2004). In addition to examining the relationship between insulin and adiponectin, this study also included analyses to determine if the relationship between insulin and adiponectin was moderated by race, gender or overweight status, or if the relationship was attenuated by the addition of waist circumference or BMI z-score to regression models.

Relatively few researchers have examined the relationship between adiponectin and insulin in a racially diverse sample of youth, although insulin sensitivity is known to be lower in Black youth (Arslanian & Suprasongsin, 1996; Gower, Nagy, & Goran, 1999; Svec et al.,

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1992). Findings from studies that examined the impact of race on the relationship between adiponectin and insulin or insulin sensitivity, are somewhat conflicting (Bacha et al., 2005; Bush et al, 2005; Lee et al, 2006). In the current study, both insulin and race were significantly related to adiponectin in regression models with gender, HDL-C, systolic blood pressure, BMI risk level, and a term for the interaction between HDL-C and gender. The significant correlation independent of race is different from one smaller study in the literature. Race and fat mass, but not adiponectin were significantly related to insulin sensitivity in a study with a small group of 44 pre-pubertal children (Bacha et al., 2005). The current study did not adjust for fat mass, but did include other adiposity measures in the models. In larger studies with a wider age variation similar to this study that included pubertal ages, adiponectin was inversely associated with insulin in bivariate correlations, and positively related to insulin sensitivity independent of race in studies by Lee et al. (2006) and Bush et al. (2005).

Lee and associates also reported stronger bivariate correlations between insulin sensitivity and adiponectin in White subjects than in Black subjects, with r-values of 0.61 and 0.51, respectively. No interaction between insulin and race in relation to adiponectin were evident in this study with a large sample of Black and White youth, although adiponectin and insulin mean levels differed by race. Results of studies in adults suggest that race may moderate the relationship between adiponectin and insulin (Hulver et al., 2004), but no other studies have been done in children and adolescents to examine this possibility.

This study also sought to determine if any interactions between gender and insulin existed in relation to adiponectin. As noted above, insulin was inversely related to adiponectin in a reduced model with race, BMI risk level, systolic blood pressure, HDL-C

and a term for the interaction between HDL-C and gender. The model was run again, in groups stratified by gender, because the HDL-C and gender interaction was significant. The terms for gender and the HDL-C*gender interaction were removed from the repeated models. When the models were run in males and females separately, insulin remained inversely and significantly related to adiponectin in female subjects, but was not significantly related to adiponectin in male subjects. This was somewhat surprising, as the interaction term for a possible interaction between insulin and gender in relation to adiponectin was not significant in previous models.

The results are similar, though, to a few other studies. Chu et al. (2005) found adiponectin to be inversely correlated with insulin in both boys and girls in bivariate analyses in their large sample of 12 to 16 year old subjects, but only in girls in regression models with other covariates, including BMI. Singhal et al. (2005) noted an inverse correlation between adiponectin and fasting insulin in their overall sample or 13 to 16 year olds, but only in females when the analyses were run stratified by gender group. The age range for a study by Tsou et al. (2004) was more similar to that of the current study, and the authors noted gender differences in the relationship between adiponectin and insulin in certain age groups. Adiponectin was inversely correlated with insulin in females overall and at ages 11to14 years, but only at ages 15 to18 in males. This finding may have been due to differences in timing of puberty onset for males and females, but they did not measure puberty directly; subjects were instead grouped into 5 chronological age groups.

Results from the current study indicate the possibility that adiponectin may behave differently in relation to insulin in females than in males. This will be important to consider in future research regarding adiponectin and insulin in different gender groups, but variable

effects of different adiposity measures on the relationship between insulin and adiponectin will also be important to consider.

As stated above, insulin was inversely related to adiponectin in this study. This relationship remained significant in when adjusting for either BMI z-score or BMI risk level. It was no longer significant, however, when adjusting for waist circumference. The finding that adiponectin and insulin were not related independent of waist circumference is in contrast to findings from other studies. The relationship between adiponectin and insulin or HOMA-IR was found to be independent of waist measurement in 2 studies (Singhal et al. 2005; Vikram et al., 2004). Subjects in the sample by Singhal and colleagues were fairly lean overall, however, and Vikram and associates studied only male post-pubertal subjects. The two samples may not have had the range in waist circumference found in this study's large sample of males and females. The ranges for waist circumference were not given in either study, but based on a calculation of the mean +/- 3 standard deviations, waist circumference ranged from 43.4 to 99.8 cm. in the subjects in the study by Singhal and associates. Based on similar calculations, the range for the male subjects in the study by Vikram and colleagues was 40.1 to 115.3 cm. In the current study, waist circumference range from 46.2 cm. to 144 cm. in male subjects, and 46.3 to 144.5 in females, a wider range than in the other studies and possibly enough to affect regression results.

Results of studies that examined whether the relationship of adiponectin and insulin, HOMA-IR or insulin sensitivity was independent of BMI or related measures such as BMI z-score or percentile were more variable. Similar to this study's findings, the majority of studies showed that adiponectin and insulin variables were significantly related, independent of BMI or BMI z-score (Bacha et al., 2004; Bottner et al., 2004; Butte et al., 2005; Chu et al.,

2005; Cruz et al., 2004; Pilz et al., 2005). In contrast, the relationship between adiponectin

and insulin or HOMA-IR was dependent on BMI, BMI z-score or percentile in other studies.

For example, the relationship between adiponectin and insulin and HOMA-IR alike was

dependent on BMI in a small sample of post-pubertal Asian Indian male subjects (Vikram et

al., 2004), and on BMI z-scores in a large sample of White children and adolescents

(Punthakee et al., 2006). The subjects in the study by Punthakee and colleagues were,

however, lean in comparison to subjects in the current study. Only 23% of boys and 22% of

girls in the study by Punthakee and associates had BMI values greater than the 85th percentile

for age and gender. This is in contrast to the 39% of males, and 44% of females with BMI

values greater than the 85th percentile in the current study. Degawa-Yamauchi (2003) was

the only group to control for measures of adiposity when examining the relationship between

adiponectin and insulin or HOMA-IR in a racially diverse sample. In an initial model, they

found that a HOMA-IR index was significant in a model with race, gender and a race/gender

interaction, but it not significant when adjusting for BMI or BMI percentile, indicating that

the effect of BMI was stronger in relation to adiponectin than was HOMA-IR.

Lastly, the only study to examine the relationship between adiponectin and insulin by

BMI risk found that the relationship was only significant in subjects whose BMI values were

greater than the 85th percentile (Martin et al, 2005). This is in contrast to the current study,

where there was no significant interaction between insulin and overweight status.   The

current study, however, classified subjects into 3 BMI risk groups according to the current

CDC guidelines. The study by Martin and colleagues used only 2 risk groups, and their

sample was only somewhat leaner than the current sample; 37% of subjects had BMI values

greater than the 85th percentile versus 42% in the current study.

In summary, insulin was inversely associated with adiponectin in most analyses in this large sample of Black and White youth. No interaction was found between race and insulin in relation to adiponectin, and insulin was associated with adiponectin independently of race. However, gender was shown to be an important moderator when considering the relationship between insulin and adiponectin, in that insulin was only associated with adiponectin in female subjects. In addition, measures of adiposity had varying effects on the relationship between insulin and adiponectin. Insulin was associated with adiponectin independently of BMI z-score and BMI risk level, both reflective of overall adiposity. The relationship was, however, not independent of waist circumference, a measure of central adiposity. Taken together, the results of this study reinforce findings from the majority of smaller studies on the effects of race on the relationship between insulin and adiponectin.

Glucose

There was no significant bivariate correlation between glucose and adiponectin in this study. Therefore, the glucose variable was not used for further multivariate analyses. In addition, there were no differences in adiponectin means by glucose risk category. These results are the same as those found in other studies with children and adolescents (Degawa-Yamauchi et al., 2003; Huang et al., 2004; Singhal et al., 2005). Gilardini et al. (2006) was the only group to find a significant correlation between adiponectin and glucose; adiponectin was inversely related to glucose. All subjects in that sample had BMI values greater than the 97th percentile, so they were heavier than the sample for the current study. It is not clear why adiponectin would not be related to glucose, in light of the correlation with insulin, but it may be due to the normal range of glucose values found in 91% of this study's sample. Glucose was apparently still well-controlled even though 26% of the subjects in this study had high insulin levels. This fits with evidence that normal glucose levels are common in the early

stages of insulin resistance, well before a diagnosis of diabetes is made (Beck-Nielson &

Groop, 1994).

Systolic and Diastolic Blood Pressure

Relatively few researchers have examined the relationship between blood pressure

and adiponectin in youth, and no one has looked at it in a sample consisting of both Black

and White children. It was be expected that adiponectin would be related to blood pressure,

as research with animal and human cells as shown that a globular form of adiponectin has

been shown to increase production of nitric oxide in bovine aortic endothelial cells, and to

up-regulate endothelial nitric oxide synthase in human aortic endothelial cells (Hattori,

Suzuki, Hattori, & Kasai, 2003). Nitric oxide is known to be a potent vasodilator. Systolic

and diastolic blood pressures were both correlated with adiponectin in bivariate analyses in

this study, but the diastolic measurement was no longer significantly related to adiponectin in

a multivariate model with other risk factor variables. Similar to this study, systolic and

diastolic blood pressure were both correlated with adiponectin in a study where the sample

included 49 White male and female subjects who were obese and non-obese (Bacha et al,

2004). The correlation was lost when adjustments were made for BMI, perhaps due to the

small sample size or the lack of racial diversity. Systolic blood pressure, but not diastolic,

was correlated with adiponectin in another study where the sample included only obese

subjects (Gilardini et al., 2006).

No researchers have examined the effect of race on the relationship between

adiponectin and blood pressure in children. Results of this study are the first to demonstrate

the lack of significant interaction between race and systolic blood pressure in relation to

adiponectin. Interaction effects between race and diastolic blood pressure were not explored

because diastolic blood pressure was not significantly related to adiponectin in regression with other variables. Although blood pressure differs by race, even in youth (Cook et al., 2003: Mensah et al., 2005), race does not appear to moderate the relationship between blood pressure to adiponectin.

The effects of gender on the relationship between blood pressure and adiponectin are not well understood. Few researchers have studied the relationships between adiponectin and systolic or diastolic blood pressure in youth, and only one examined correlations by gender (Chu et al., 2005). Systolic blood pressure was related to adiponectin in both males and females, but diastolic pressure was only related to adiponectin in females. No studies have tested for interactions between gender and blood pressure in relation to adiponectin. In the current study, systolic blood pressure was included in a model to test for interactions because if was significantly related to adiponectin in multivariate analyses. Diastolic blood pressure was not included because it was not related to adiponectin beyond bivariate correlation. No interaction was found between gender and systolic blood pressure in relation to adiponectin.

Systolic blood pressure was one of the variables significantly related to adiponectin in a final reduced model with race, BMI risk level, insulin, HDL-C and a term for the interaction between HDL-C and gender. The model was run in groups stratified by gender, because the HDL-C and gender interaction was significant. The terms for gender and the HDL-C by gender interaction were removed from the model when run in the stratified groups. In addition to the differences in the relationship between HDL-C and adiponectin by gender, systolic blood pressure was no longer significantly related to adiponectin in either gender group, and insulin was not significant was not in male subjects, as described earlier.

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It is unclear why systolic blood pressure was no longer significant in the models run in the different gender groups, without the gender and HDL-C by gender term.

No one has investigated the effects of waist or BMI risk level on the relationship between blood pressure and adiponectin. This study examined the effects of different adiposity measures on the relationship between systolic blood pressure and adiponectin. Systolic pressure was related to adiponectin in multivariate analyses, but was no longer significant when either waist circumference or BMI z-score was added to the models. This indicated that the contribution of the two adiposity measures to the variance in adiponectin outweighed the contribution of systolic blood pressure   Systolic blood pressure was, however, still related to adiponectin in a model with the BMI risk group variable.

The relationships among adiponectin, blood pressure and BMI have been examined in only two studies in the literature. One study found results similar to the current study, and the other study showed conflicting results. Huang et al. (2003) found that systolic blood pressure, but not diastolic, was inversely related to adiponectin in female adolescents in regression models that included BMI, HDL-C and insulin. In contrast, results from a study by Bacha et al. (2004) showed that systolic and diastolic blood pressure were related to adiponectin in bivariate correlation analyses in their sample of White youth, but were no longer significant when BMI was added to the model. The current study also used the BMI z-score variable, to account for the effect of age and gender on BMI values in the developing child or adolescent. This study's findings reinforced the results of Bacha and colleagues, but also provided information from a large sample of youth who varied by race. This study also provides the first results indicating that the relationship between adiponectin and blood

pressure is no longer significant when waist circumference or BMI risk levels are taken into account.

In summary, this study is the first to provide results concerning the relationship between blood pressure and adiponectin in a sample of Black and White youth. Both systolic and diastolic blood pressures were inversely correlated with adiponectin, but only systolic pressure remained significantly related in a model with other risk factors. Gender and race did not function as moderators of the relationship between blood pressure and adiponectin. There was also no interaction with the categorical variable of BMI risk level, but the relationship between systolic blood pressure and adiponectin was dependent on the continuous measures of waist circumference or BMI z-score.

Physical Fitness

The relationship between adiponectin and VO_2 max was examined in this study with VO_2 measured in terms of 'ml/kg/min,' and also as 'ml/kg of lean body mass/minute'. The second measure of VO_2 took the subjects' body fat weight into account, and was used to avoid any correlation that might have been significant solely because of the known relationship between adiponectin and adipose tissue. Adiponectin was inversely correlated with the first measure of VO_2, and the mean was higher in the highest VO_2 tertile. However, when the measure with lean body mass was used, the correlation was no longer significant and the mean did not differ by tertile. Two other studies have examined the relationship between adiponectin and physical fitness level, and results were similar although different measures were used for VO_2.

Nemet et al. (2002) used the 'ml/kg/min' measure, and noted an inverse correlation with adiponectin. Butte et al. (2005) used "absolute" VO_2 initially, and found a negative correlation, but the correlation was no longer significant when adjusted for "family

membership, age, gender, FFM, and percent FM" (p.4173). The units for the absolute VO_2 in the initial analyses were not given, but are presumed to have been 'ml/min.' The results with the adjustment that followed can not be directly compared to those in the current study, but both studies suggest the relationship between adiponectin and VO_2 is influenced by the amount of body fat. It is not clear why adiponectin would not be related to VO_2 since they both appear to confer protective effects in relation to CVD and diabetes. The protective effects of fitness may be achieved via different pathways or mechanisms.

Pubertal Stage and Demographic Variables Other than Race and Gender

Pubertal Stage

Adiponectin means did not differ by the five pubertal stages initially used for analysis in this study. Results in the literature are somewhat conflicting. Some researchers have found that adiponectin levels are lower in the latter stages of puberty, or that pubertal stage is inversely related to adiponectin (Butte et al., 2005; Punthakee et al., 2006; Reinehr et al., 2004; Woo et al., 2005). However, there was considerable variation in the measurement of pubertal stage in the studies in the literature. Several of the research groups mentioned above only used two or three pubertal stage levels in their analyses, even though they may have measured all five stages. Results of studies by other groups that utilized all five pubertal stages in analyses, were similar to the results of this study, i.e., there were no differences in adiponectin means by pubertal stage (Bottner, et al., 2004; Gilardini et al., 2006; Huang et al., 2004; Singhal et al, 2005). The samples in these studies were composed of either all obese subjects (Gilardini et al, 2006), all lean subjects (Bottner, et al., 2004), or subjects who were mostly in the later stages of puberty (Huang et al, 2004; Singhal et al., 2005). There

was a wide range of values for age, pubertal status and BMI for subjects in the current study sample, but still no differences were noted by the five pubertal stages.

The difference in results from the previous studies may likely have been due to the number of pubertal stages used in analyses. Due to the variation in the literature, I also compared adiponectin means by two groups in post-hoc analyses; subjects in one group were pre-pubertal, or Tanner stage 1. Subjects in the other group were in Tanner stages 2 to 5. Similar to results of the former studies mentioned above, adiponectin means were higher in pre-pubertal subjects, but this difference was only noted when gender was also in the model. Apparently, division of puberty into two stages provides a more distinct change in adiponectin than is seen over five stages, perhaps due to differences in body fat levels.

The Effect of Race, Gender and Overweight Status on the Relationship Between Adiponectin and Pubertal Stage

No other researchers have examined the relationships between adiponectin and pubertal stage to see if they differed by race, even though the timing of pubertal development appears to differ by race, at least in females (Wu, Mendola & Buck, 2002). Moreover, Woo et al. (2005) were the only researchers to examine this relationship in a sample consisting of Black and White youth; they adjusted for race in their study. As noted above, results from the current study of Black and White youth showed there were no differences in adiponectin means by pubertal stage, except when analyzed with 2 pubertal stages. In addition, no interactions were noted between pubertal stage and race whether analyzed using two groups (Tanner stage 1 vs. Tanner stages 2 to 5), or all five Tanner stage groups.

Woo et al. (2005) examined the relationship between adiponectin, gender and puberty, by adding an interaction term between puberty and gender, and found that

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adiponectin decreased in males but not in females. They further clarified this relationship by showing that adiponectin remained stable in lean female subjects, and not in the subjects with BMI values greater than the 85th percentile. No interactions were found between pubertal stage and gender in relation to adiponectin in the current study, even when only 2 pubertal stages were used for analysis. Woo and associates, however, used 3 pubertal stages; that may explain some of the difference in results with the current study. Otherwise, it is unclear why the results differ from the current study. The two samples are fairly similar in distribution by gender, and the distributions by race or BMI are only somewhat different. The only other difference is the fact that Woo and colleagues did not use statistical methods would have adjusted for the potential effects of clustering, even though their subjects were drawn from school settings.

Age

Most researchers have found that adiponectin is inversely correlated with age. Results in this study were similar to a point, in that adiponectin was inversely correlated with age in bivariate correlation analysis, but not in multivariate regression. Butte et al. (2005) noted that adiponectin decreased by age, but decreased most sharply from ages 4-10 years. Age ranged from 7 -18 years in this study. Age may have contributed more to multivariate analyses if the range had extended downward to preschool years.

Family History of CVD and Diabetes

Research indicates that the frequency of coronary artery disease and diabetes is increased in subjects with polymorphisms in the gene for adiponectin (Kondo et al., 2002; Ohashi et al., 2004) and adiponectin displays substantial heritability in adults and children (Butte et al., 2005; Comuzzie et al ., 2001). It would be expected, therefore, that adiponectin

would be related to a positive family history of CVD or diabetes. Relationships between adiponectin and either type of family history have been noted in adult subjects. Patel, Srinivasan, Xu, Chen and Berenson (2006) found lower adiponectin levels in subjects with a family history of coronary heart disease or hypertension or diabetes in young adults, and adiponectin levels were lower in adults with a first degree relative with diabetes (Lihn et al., 2003).

No researchers have examined this type of relationship in a racially diverse sample. Three studies have examined the relationship between adiponectin and a family history of diabetes in either Caucasian or Hispanic children only, but no significant relationships have been found (Butte et al., 2005, Gilardini et al., 2006, Punthakee et al., 2006T). This is in contrast to the significant correlation between adiponectin and family history of diabetes found in the current study. The incidence of a positive family history of diabetes may have had some effect on the correlation; Punthakee and associates reported that 6% of subjects had a positive family history of diabetes, much lower than the 56% of subjects in the current study. The incidence of diabetes in subject families was not reported in the other studies.

No researchers have investigated the relationship between adiponectin and a positive family history of CVD in children. This study provided the first results, indicating there was no relationship between adiponectin and a family history of CVD. Almost all (90%) of the subjects with family history data, reported a positive family history of CVD, which may have affected the potential correlation.

The Importance of Statistical Adjustment for Clustering

One final point of discussion is the importance of statistical adjustment for clustering when subjects are recruited from school or family settings. When subjects are recruited from

such settings, there is a chance that physiological or anthropometric data collected from subjects within schools, or clusters, may be more highly correlated in one school or family than in another. This may be due to being "exposed to a common set of circumstances." (p.919, Norton, Bieler, Ennett and Zarkin, 1996). For example, subjects in a certain school may have similar dietary or physical activity habits, or they may be related to one another, leading to increased correlation for genetic reasons. If this increased correlation is ignored, the standard error will appear smaller than it really is, thereby leading to "increased Type 1 errors" (Norton et al., 1996, p.919). Statistical techniques such as mixed models or GEE are needed to adjust for the potential increased correlation within clusters.

Only three of the fourteen studies in the literature whose subjects were recruited from schools or families reported the use of statistical analyses that accounted for clustering (Butte et al., 2005; Chu et al., 2005; Punthakee et al., 2006). Chu et al. (2005) reported the use of mixed models in SAS to adjust for clustering by school, and Butte et al. (2005) used GEE for clustering within families. The description given by Punthakee et al. (2006) concerning the statistical methods used for analyses was puzzling however. The authors apparently used a type of mixed models to adjust for clustering, but it was difficult to determine. At one point the authors stated they used "hierarchical maximum likelihood linear regression" and "clustering between subjects in the same school was treated as a random effect." Yet, in a further comment they said, "Because of the complex survey design, sampling weights and clustering effects were estimated and incorporated into all computations except correlations and regression models."

The lack of attention to the effects of clustering in analyses with adiponectin is concerning. The ICC for adiponectin in this study was 0.07, well above the level of 0.01 that

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is considered high enough to inflate variance in statistical analyses. Moreover, the ICC for adiponectin when adjusting for race and gender was 0.10. When the design effect was calculated, using the ICC values of 0.07 and 0.10 and the average cluster size of 36, the design effects were 3.45 and 4.36, respectively. These design effects are high enough to alter results if a statistical method that accounts for clustering, such as GEE, is not used. Further analysis reinforced the need to account for clustering. When a model was run using the GEE method, then repeated using multiple regression, results showed that standard errors overall were smaller with the multiple regression analysis than with GEE. In addition, insulin was significantly related to adiponectin in the multiple regression analysis, but was not significant in the GEE analysis.

The results obtained from comparison of the two statistical methods reinforce the need for using GEE to adjust for the effects of clustering. High ICC values can lead to an inflation of variance and over-estimation of statistical significance, or type I error, if statistical methods are used that don't adjust for clustering. Results from the studies in the literature that did not adjust for clustering may therefore be questionable. Future studies regarding adiponectin, that use data collected from subjects in clustered samples, should use statistical methods that adjust for the effects of clustering.

Limitations in the Current Study

Limitations in the current study include the fact that secondary data was used for analysis, and the relatively localized geographic area from which subjects were recruited. First, almost all of the data used for this study was collected in a previous research study. The only data original to this study was the data obtained from analysis of adiponectin. The main limitations in using secondary data are that the researcher has no control over the

design and procedures used in the original research or over what variables are available for study. The design for the CHIC III study was well researched and planned, and procedures were carried out with a great deal of care and attention to detail. The study design and procedures are therefore more of a strength for the current study than a limitation.

The other limitation related to analysis of secondary data concerns the variables available for study. There are a few variables that may have fit well with the framework for this study, and contributed information regarding the relationships among adiponectin and risk factors for CVD and diabetes. For example, as mentioned in the literature review chapter, adiponectin levels are positively associated with LDL particle size in adults (Hulthe et al., 2003), and small LDL particle size is a risk factor for atherosclerosis (Carmena, Duriez, & Fruchart, 2004). LDL-C was not related to adiponectin in this study, but a measure of LDL particle size may have contributed more specific information. It was, however, not readily available for analysis in this study.

A measure of fat distribution (visceral vs. subcutaneous) by DEXA or CT scan would also have been a relevant addition, as adiponectin levels are thought to be higher in individuals with higher visceral, or central adiposity (Hulthe et al., 2003; Park et al., 2004). Waist circumference is also reflective of central adiposity, but a more specific measure of central adipose tissue may have been helpful in explaining relationships between adiponectin and risk factor variables in this large sample of youth. Such measures are expensive, though, and the chances of having secondary data on those types of measure in a large number sample of youth are small.

Measures of variables that might influence the inflammatory or metabolic processes in the body may also have fit well in the framework for this study. Examples might be stress

or depression measures, as both are correlated with CVD and diabetes. Dental disease, or more specifically periodontitis, is known to be related to the inflammatory process and CVD (Beck, Offenbacher, Williams, Gibbs & Garcia, 1998). Dental disease is very common in children and adolescents (Krol, 2003). A variable indicating the degree of periodontitis may have been helpful in clarifying the relationship between adiponectin and risk factors for CVD and diabetes. Measures of other adipocytokines may have also fit well in the framework used for this study, due to the possibility of effects on or from adiponectin. Examples might be C-Reactive protein, or TNF-alpha and IL-6, two cytokines secreted by adipose tissue (and other types of tissue) that contribute to suppression of adiponectin levels (Bruun, et al., 2003; Fasshauer, et al., 2003). These variables could possibly have been measured in the stored serum samples, but only at great expense.

The variables mentioned above might have added helpful information to this study, but many other variables were available for analyses concerning the relationships between adiponectin and risk factors for CVD and diabetes. Variables that have not been studied in relation to adiponectin in samples of Black and White youth, including systolic and diastolic blood pressure, family history of CVD or diabetes, and physical fitness as measured by VO_2 were readily available for use in this study. The availability of frozen serum for analysis of adiponectin levels, and multiple risk factor variables for analysis of relationships between adiponectin and risk factors for CVD and diabetes, as well as possible interaction effects in this large sample of Black and White youth was a significant strength.

Another possible limitation for this study was the localized geographic area from which subjects were recruited. The youth in this study were recruited from three rural counties in eastern North Carolina, so study findings may not be generalizeable to urban or

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suburban areas, or to other parts of the country. Even so, the study adds to existing knowledge regarding the relationships among adiponectin and risk factors for CVD and diabetes in a large, racially diverse sample.

Conclusions

This study of a very large sample of youth who were diverse in race, gender, age, and adiposity allowed a comprehensive examination of the relationships among risk factors for CVD and diabetes and adiponectin, an anti-inflammatory protein secreted by adipocytes. This is the first study to include this many risk factor variables as well as possible interaction terms for analysis in a sample of Black and White children. Race, gender and HDL-C were the variables that were most consistently related to adiponectin in the different analyses used in this study. Race was related to adiponectin throughout all analyses no matter what other variables were included in study models. This even held true for inclusion of measures of general and central adiposity; race was related to adiponectin independently of BMI z-score and waist circumference. Gender was related to adiponectin in a manner independent of adiposity for almost all models. The relationship between adiponectin and race or gender was further clarified by the finding that the main differences in adiponectin means by race and gender were the lower adiponectin levels in Black males as opposed to Black females or White subjects of either gender. This study also provides the new information that race does not seem to function as moderator of the relationship between insulin, HDL-C or systolic blood pressure and adiponectin in youth.

Various adiposity measures, HDL-C and Insulin were also related to adiponectin in this study. Waist circumference, BMI z-score and BMI risk level and insulin were all inversely associated with adiponectin, adjusting for race and gender. In contrast, HDL-C was

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consistently related to adiponectin in a positive manner in all study models. Subjects with higher adiponectin levels had higher HDL-C levels, even when adjustments were made for race, gender and adiposity measures. Relationships were, however, stronger in female subjects than in males. These differences by gender may contribute to differences in CVD incidence by gender, but this cross-sectional study did not allow for causal inference. The relationship between insulin and adiponectin differed depending on which measure of adiposity was included in the model, and was not significant when a measure of central adiposity was included. Gender was also an important variable to consider regarding the relationship between insulin and adiponectin; a relationship was only noted in female subjects. This study supports results from smaller studies on the effects of race on the relationship between insulin and adiponectin, and adds to the current knowledge concerning the effects of gender and adiposity measures, by providing information from a large, racially diverse sample of youth with a wide range of adiposity.

No studies in racially diverse youth have examined the relationship between adiponectin and blood pressure. The current study indicates an inverse relationship between adiponectin and systolic or diastolic blood pressure, but only with systolic pressure when demographic factors or other risk factors such as HLD-C are considered. The relationship between systolic blood pressure and adiponectin was superseded by the effects of central or overall adiposity. The study also provides information about the relationship between adiponectin and variables that have had limited study thus far in samples of any race, such as family history of CVD or diabetes, or physical fitness levels.

This study was the first to examine whether or not adiponectin means differed by whether or not a child or adolescent had a family history of CVD, and added to sparse

findings regarding a family history of diabetes. Subjects with a family history of diabetes had lower adiponectin levels than subjects without diabetes in their families. There were no differences based on a family history of CVD, although studies in young adults suggest that subjects with a family history or coronary artery disease or hypertension have lower adiponectin levels (Patel, Srinivasan, Xu, Chen and Berenson, 2006). These findings give further clarification concerning the risk associated with lower adiponectin levels. The study also adds to the limited literature regarding the relationship between adiponectin and physical fitness as measured by VO_2 levels. The importance of adjusting the VO_2 measure for body fat was demonstrated. Differences in mean adiponectin levels by fitness group that were noted when using the 'ml/kg/min' formula were no longer significant when adjustments were made for body fat, by using the lean body mass term in the formula. Use of this formula for VO_2 gives the researcher a clearer picture of the relationship between adiponectin and fitness level.

Lastly, the relationship between pubertal stage and adiponectin was also examined, and it was noted that the choice of measurement of pubertal stage may affect its relationship with adiponectin. In this study, differences were only noted when two pubertal stage groups were measured, as opposed to all five Tanner stage groups. This will be an important consideration in future research regarding adiponectin that is conducted in children or adolescents.

In the future, nurse researchers and other investigators that examine the relationship between adiponectin and risk factors for CVD and diabetes are encouraged to include variables that reflect the process of common psychological and physiological health concerns such as stress, depression or dental disease. Measures such as parental education or family

income may also be helpful as covariates, to help sort out issues related to racial disparity versus socio-economic status. Research done in broader geographic areas could also contribute valuable information regarding the relationships studied here. Future intervention research regarding improvement of cardiovascular or metabolic health in youth should also consider the possibility that successful interventions may lead to increases in adiponectin. Lastly, all future research done with samples of children and adolescents that are recruited from school settings should adjust for the effects of clustering.

Clinical practitioners should be aware that an inflammatory process underlies the pathophysiology that leads to CVD and T2D, and that higher levels of the anti-inflammatory protein adiponectin are associated with a more favorable risk factor profile in regards to CVD and T2D even in children and adolescents. In particular, Black males in this study had the lowest levels of adiponectin, and may be therefore be more at risk than Black females, or White youth. In addition, it will be important to be aware that dental disease, a common problem in youth (Krol, 2003), may also contribute to the inflammatory processes associated with CVD and T2D. Dental disease, or more specifically periodontitis, is known to be related to the inflammatory process and CVD (Beck, Offenbacher, Williams, Gibbs & Garcia, 1998). Clinical practice should routinely include screening for increased adiposity and dental disease in young patients. In this study, waist circumference and BMI z-scores were both related to adiponectin levels, and waist circumference influenced the relationship between adiponectin and insulin. Both measures are easily obtained in a clinical setting, and may add to the practitioner's knowledge of a patient's risk, in regards to having low adiponectin levels and increased risk for CVD or T2D. Lastly, clinical intervention to decrease or prevent overweight or obesity and associated risk profiles must begin in youth.

In conclusion, adiponectin is an important variable to consider in relation to the inflammatory process that is thought to lead to CVD and T2D; according to the literature, adiponectin appears to confer a protective effect against either illness. This study of the relationships between adiponectin and risk factors for CVD and T2D in a large sample of Black and White youth demonstrates that adiponectin is related to various measures of adiposity, HDL-C and insulin when adjusting for race and gender, and that race and gender are also important variables to consider in research related to adiponectin. The pathophysiology related to development of CVD and diabetes begins in youth, and the incidence of T2D in youth is increasing. Knowledge of the relationships among adiponectin and risk factors for CVD and diabetes in children and adolescents reinforces the need for the prevention CVD and T2D to begin at early ages.

Appendices

Appendix A: Funding Sources

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6. Sigma Theta Tau Small Grants Award (2006)

Appendix B: Family Health History Portion of Mother's Questionnaire

STUDY OF CARDIOVASCULAR HEALTH IN CHILDREN AND YOUTH
MOTHER'S QUESTIONNAIRE

1. What is the social security number of the child in this study?

    ☐☐☐ - ☐☐ - ☐☐☐☐

2. Are you the natural mother of the child in this study?

    _____a. Yes
    _____b. No

3. Is the natural father of this child alive?

    _____a. Yes
    _____b. No
    _____c. I don't know

4. Was this child born approximately when he or she was due?

    _____a. Yes, within one or two weeks of due date
    _____b. Yes, within two or three weeks of due date
    _____c. No, he or she was three or more weeks early
    _____d. No, he or she was three or more weeks late
    _____e. I do not know if this child was born early or late

5.   Was this child a single birth or multiple birth (for example, is this child a twin?)
      _____a. Single birth
      _____b. Multiple birth (twin)
      _____c. Multiple birth (other)

6.   What was this child's birth weight? _____lbs _____oz

7.   How often does this child live with you?
      (Check the one response that best answers the question.)

      _____a. all the time
      _____b. all the time EXCEPT every other weekend
      _____c. every other weekend
      _____d. school year
      _____e. summer
      _____f.   other (please specify) _____
      _____g. child does not live with me

8.   a. How many people live in your house?   _____

      b. How many of the people living in your house
             are younger than 18 years old (*including the child in this study*)?   _____

9.   What is your total family income?
      (Check the one response that best answers the question.)

      _____a.   less than $5000
      _____b. $5,000-$9,999
      _____c. $10,000-$19,999
      _____d. $20,000-$29,999
      _____e. $30,000-$39,999
      _____f. $40,000-$49,999

_____g. $50,000-$74,999
_____h. $75,000-$100,000
_____i.   above $100,000

*The next questions are about **you** and your health habits*

10.   What is the highest grade you finished in school?
      (Check the one response that best answers the question.)

      _____a.   Sixth grade or less
      _____b.   Junior high (7th g-9th grade)
      _____c.   Some high school (10th or 11th grade)
      _____d.   High school graduate
      _____e.   Some college or specialized training
      _____f.   College or university graduate
      _____g.   Graduate professional training (graduate degree)

11.   How old are you? _____years old.

12.   How tall are you? _____feet and _____inches.

13.   How much do you weigh? _____lbs.

14.   Do you consider yourself Hispanic (ancestors from Mexico, Puerto Rico, Central or South America, or any other Spanish culture, regardless of race)?

      _____a. Yes
      _____b. No

15. Which of these best describes your race?
    (Check the one best response that answers the question).

    _____a. **Asian or Pacific Islander** - (ancestors from the Far East, Southeast Asia, the Indian Subcontinent or the Pacific Islands).
    _____b. **Black** (African American - ancestors from the black racial groups of Africa).
    _____c. **Native American** (American Indian - ancestors from the original peoples of North America who keep tribal affiliation or community recognition).
    _____d. **White** (Caucasian - ancestors from Europe or the Middle East).
    _____e. **Other** (specify_____)

16. How active are you at work (or, if you don't work, How active are you during the day at home)?
    (Check the one response that best answers the question.)

    _____a. **Inactive** - spend most of my time sitting.
    _____b. **Slightly active** - spend some time sitting, walking and stair climbing
    _____c. **Active** - on my feet most of the day.
    _____d. **Very active** - on my feet most of the day and do manual labor.

17. In the last 6 months, about how often did you participate in one or more physical activities that lasted 20-30 minutes? *(Check the one response that best answers the question.)*

    _____a. Not at all
    _____b. Less than once a month
    _____c. About once a month
    _____d. 2-3 times a month
    _____e. 1-2 times a week
    _____f. 3 or more times a week

18. Do you smoke?

_____a. Yes
_____b. No, never smoked
_____c. No, quit during the last month
_____d. No, quit 2-6 months ago
_____e. No, quit 7-12 months ago
_____f.   No, quit more than 1 year ago

19. Is your cholesterol over 200?

_____a. Yes
_____b. No
_____c. I don't know

20. Have you ever had any of these? *(Please circle your answer.)*

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**
Diabetes	**YES**	**NO**	**I Don't Know**
Heart Attack	**YES**	**NO**	**I Don't Know**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**
High Blood Pressure	**YES**	**NO**	**I Don't Know**
Stroke	**YES**	**NO**	**I Don't Know**

21.	Circle how many times DAILY you eat....

FRUIT-- fresh or canned or FRUIT JUICES	2+	1	0
VEGETABLES-- fresh, canned or frozen	2+	1	0

22. Circle how many times WEEKLY you eat . . .

SNACK CHIPS like potato chips, pretzels pork skins or cheetos	0 1 2	3 4	5+
ICE CREAM	0 1	2	3+

23. For each of the foods listed:
    a) Note the standard serving size in PART 1.
    b) In the spaces of PART 2 write in the usual **number** of times each day, week, month, **OR** year you eat the food.
    c) Try not to leave any foods blank. If you don't eat the food, just put a check in the Rarely/Never space.

<div align="center">PART 1   PART 2</div>

FOOD	STANDARD SERVING SIZE (M)		HOW OFTEN EATEN?				
			DAY	WEEK	MONTH	YEAR	RANRE EVLE YR
EXAMPLE: Hamburgers, cheeseburgers, meatloaf	3-4 oz ➜			2			
Hamburgers, Cheeseburgers, Meatloaf	3-4 oz.						
Beef steaks, Roasts	3-4 oz.						
Pork, including chops, roast	2 chops or 4oz.						
Hotdogs	2 dogs						
Ham, lunch meats	2 slices						
Bacon/Sausage	2 slices or patties						
Fried fish or fish sandwich	4 oz. or 1sandwich						
Fried chicken	4 oz. or 1 sandwich						
Chicken, not fried	2 small or 1 large						
Whole Milk not including on cereal	1-8 oz. glass						
Cheese, excluding cottage	2 slices or 2 oz.						

Doughnuts, cookies, cake, pastries	1 piece or 3 cookies					
Eggs	2 eggs					
White bread, rolls, bagels, etc. including on sandwiches	2 slices, 3 crackers					
Dark bread, such as whole wheat, Rye, Pumpernickel	1 slice					
Margarine	2 pats					
Butter	2 pats					
Salad Dressing or Mayonnaise	2 Tbsp					
French fries, fried potatoes, other fried vegetables	3/4 cup					
Breakfast cereal	3 oz.					

*The next set of questions are about **your** family's health.*

The first two are about <u>your mother</u> *(**the child's grandmother**)*.

24.   Has your mother *(**the child's grandmother**)* ever had any of these conditions?
(Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**
Diabetes	**YES**	**NO**	**I Don't Know**
Heart Attack	**YES**	**NO**	**I Don't Know**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**
High Blood Pressure	**YES**	**NO**	**I Don't Know**
Stroke	**YES**	**NO**	**I Don't Know**

25.   If she had or has any of the conditions above, was she <u>under **60** years old</u> when they first appeared?
(Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**	**She never had this**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**	**She never had this**
Diabetes	**YES**	**NO**	**I Don't Know**	**She never had this**
Heart Attack	**YES**	**NO**	**I Don't Know**	**She never had this**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**	**She never had this**
High Blood Pressure	**YES**	**NO**	**I Don't Know**	**She never had this**
Stroke	**YES**	**NO**	**I Don't Know**	**She never had this**

The next 2 questions are about your father (*your child's grandfather*).

26. Has your father (*the child's grandfather*) ever had any of these conditions?
(Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**
Diabetes	**YES**	**NO**	**I Don't Know**
Heart Attack	**YES**	**NO**	**I Don't Know**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**
High Blood Pressure	**YES**	**NO**	**I Don't Know**
Stroke	**YES**	**NO**	**I Don't Know**

27. If he had or has any of the conditions above, was he <u>under **55** years old</u> when they first appeared?
(Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**	**He never had this**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**	**He never had this**
Diabetes	**YES**	**NO**	**I Don't Know**	**He never had this**
Heart Attack	**YES**	**NO**	**I Don't Know**	**He never had this**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**	**He never had this**
High Blood Pressure	**YES**	**NO**	**I Don't Know**	**He never had this**
Stroke	**YES**	**NO**	**I Don't Know**	**He never had this**

The last question is about <u>your sisters or brothers</u> *(the child's aunts or uncles)*.

28.   Do you have a blood-related brother or sister who has ever had any of the following conditions? *(Please circle your answer)*

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**
Diabetes	**YES**	**NO**	**I Don't Know**
Heart Attack	**YES**	**NO**	**I Don't Know**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**
High Blood Pressure	**YES**	**NO**	**I Don't Know**
Stroke	**YES**	**NO**	**I Don't Know**

29.   If any of your sisters or brothers *(the child's aunts or uncles)* had or have any of the conditions above, were they <u>under **60** years old</u> when the conditions first appeared?
       (Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**	**Never had this**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**	**Never had this**
Diabetes	**YES**	**NO**	**I Don't Know**	**Never had this**
Heart Attack	**YES**	**NO**	**I Don't Know**	**Never had this**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**	**Never had this**
High Blood Pressure	**YES**	**NO**	**I Don't Know**	**Never had this**
Stroke	**YES**	**NO**	**I Don't Know**	**Never had this**

Appendix C: Family Health History Portion of Father's Questionnaire

STUDY OF CARDIOVASCULAR HEALTH IN CHILDREN AND YOUTH
FATHER'S QUESTIONNAIRE

1.   What is the social security number of the child in this study?

☐☐☐ - ☐☐ - ☐☐☐☐

2.   Are you the natural father of the child in this study?

_____a. Yes
_____b. No

3.   Is the natural mother of this child alive?

_____a. Yes
_____b. No
_____c. I don't know

4.   How often does this child live with you?
(Check the one response that best answers the question.)

_____a. all the time
_____b. all the time EXCEPT every other weekend
_____c. every other weekend
_____d. school year
_____e. summer
_____f. other (please specify) _____
_____g. child does not live with me

5. a. How many people live in your house? _____

    b. How many of the people living in your house
          are younger than 18 years old (*including the child in this study*)? _____

6. What is your total family income?
    (Check the one response that best answers the question.)

    _____a. less than $5000
    _____b. $5,000-$9,999
    _____c. $10,000-$19,999
    _____d. $20,000-$29,999
    _____e. $30,000-$39,999
    _____f. $40,000-$49,999
    _____g. $50,000-$74,999
    _____h. $75,000-$100,000
    _____i. above $100,000

*The next questions are about **you** and your health habits*

7. What is the highest grade you finished?
    (Check the one response that best answers the question.)

    _____a. Sixth grade or less
    _____b. Junior high (7th -9th grade)
    _____c. Some high school (10th or 11th grade)
    _____d. High school graduate
    _____e. Some college or specialized training
    _____f. College or university graduate
    _____g. Graduate professional training (graduate degree)

8. How old are you? _____years old.

9. How tall are you? _____feet and _____inches.

10. How much do you weigh? _____lbs.

11. Do you consider yourself Hispanic (ancestors from Mexico, Puerto Rico, Central or South America, or any other Spanish culture, regardless of race)?

   _____a. Yes
   _____b. No

12. Which of these best describes your race?
   (Check the one best response that answers the question).

   _____a. **Asian or Pacific Islander** - (ancestors from the Far East, Southeast Asia, the Indian Subcontinent or the Pacific Islands).
   _____b. **Black** (African American - ancestors from the black racial groups of Africa).
   _____c. **Native American** (American Indian - ancestors from the original peoples of North America who keep tribal affiliation or community recognition).
   _____d. **White** (Caucasian - ancestors from Europe or the Middle East).
   _____e. **Other** (specify_____)

13. How active are you at work (or, if you don't work, How active are you at home during the day)?
   (Check the one response that best answers the question.)

   _____a. **Inactive** - spend most of my time sitting.
   _____b. **Slightly active** - spend some time sitting, walking and stair climbing
   _____c. **Active** - on my feet most of the day.
   _____d. **Very active** - on my feet most of the day and do manual labor.

14. In the last 6 months, about how often did you participate in one or more physical activities that lasted 20-30 minutes? *(Check the one response that best answers the question.)*

   _____a. Not at all
   _____b. Less than once a month
   _____c. About once a month
   _____d. 2-3 times a month
   _____e. 1-2 times a week
   _____f. 3 or more times a week

15. Do you smoke?

   _____a. Yes
   _____b. No, never smoked
   _____c. No, quit during the last month
   _____d. No, quit 2-6 months ago
   _____e. No, quit 7-12 months ago
   _____f. No, quit more than 1 year ago

16. Is your cholesterol over 200?

   _____a. Yes
   _____b. No
   _____c. I don't know

17.   Have you ever had any of these?   *(Please circle your answer.)*

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**
Diabetes	**YES**	**NO**	**I Don't Know**
Heart Attack	**YES**	**NO**	**I Don't Know**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**
High Blood Pressure	**YES**	**NO**	**I Don't Know**
Stroke	**YES**	**NO**	**I Don't Know**

18.   Circle how many times DAILY you eat....

FRUIT-- fresh or canned or FRUIT JUICES	**2+**	**1**	**0**
VEGETABLES-- fresh, canned or frozen	**2+**	**1**	**0**

19. Circle how many times WEEKLY you eat . . .

SNACK CHIPS like potato chips, pretzels pork skins or cheetos	0 1 2	3 4	5+
ICE CREAM	0 1	2	3+

20. For each of the foods listed:
   a) Note the standard serving size in PART 1.
   b) In the spaces of PART 2 write in the usual **number** of times each day, week, month, **OR** year you eat the food.
   c) Try not to leave any foods blank. If you don't eat the food, just put a check in the Rarely/Never space.

	PART 1		PART 2				
FOOD	**STANDARD SERVING SIZE (M)**		**HOW OFTEN EATEN?**				
			DAY	**WEEK**	**MONTH**	**YEAR**	**RARELY OR NEVER EVER LE YR**
EXAMPLE: Hamburgers, cheeseburgers, meatloaf	3-4 oz ➔			2			
Hamburgers, Cheeseburgers, Meatloaf	3-4 oz.						
Beef steaks, Roasts	3-4 oz.						
Pork, including chops, roast	2 chops or 4oz.						

Hotdogs	2 dogs						
Ham, lunch meats	2 slices						
Bacon/Sausage	2 slices or patties						
Fried fish or fish sandwich	4 oz. or 1sandwich						
Fried chicken	4 oz. or 1 sandwich						
Chicken, not fried	2 small or 1 large						
Whole Milk not including on cereal	1-8 oz. glass						
Cheese, excluding cottage	2 slices or 2 oz.						
Doughnuts, cookies, cake, pastries	1 piece or 3 cookies						
Eggs	2 eggs						
White bread, rolls, bagels, etc. including on sandwiches	2 slices, 3 crackers						
Dark bread, such as whole wheat, Rye, Pumpernickel	1 slice						
Margarine	2 pats						
Butter	2 pats						
Salad Dressing or Mayonnaise	2 Tbsp						
French fries, fried potatoes, other fried vegetables	3/4 cup						
Breakfast cereal	3 oz.						

*The next set of questions are about **your** family's health.*

The first two are about <u>your mother</u> *(**the child's grandmother**)*.

21.   Has your mother *(**the child's grandmother**)* ever had any of these conditions?
        (Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**
Diabetes	**YES**	**NO**	**I Don't Know**
Heart Attack	**YES**	**NO**	**I Don't Know**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**
High Blood Pressure	**YES**	**NO**	**I Don't Know**
Stroke	**YES**	**NO**	**I Don't Know**

22.   If she had or has any of the conditions above, was she <u>under **60** years old</u> when they first appeared?
        (Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**	**She never had this**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**	**She never had this**
Diabetes	**YES**	**NO**	**I Don't Know**	**She never had this**
Heart Attack	**YES**	**NO**	**I Don't Know**	**She never had this**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**	**She never had this**
High Blood Pressure	**YES**	**NO**	**I Don't Know**	**She never had this**
Stroke	**YES**	**NO**	**I Don't Know**	**She never had this**

The next 2 questions are about your father *(your child's grandfather)*.

23.   Has your father *(the child's grandfather)* ever had any of these conditions?
      (Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**
Diabetes	**YES**	**NO**	**I Don't Know**
Heart Attack	**YES**	**NO**	**I Don't Know**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**
High Blood Pressure	**YES**	**NO**	**I Don't Know**
Stroke	**YES**	**NO**	**I Don't Know**

24.   If he had or has any of the conditions above, was he under **55** years old when they first appeared?
      (Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**	**He never had this**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**	**He never had this**
Diabetes	**YES**	**NO**	**I Don't Know**	**He never had this**
Heart Attack	**YES**	**NO**	**I Don't Know**	**He never had this**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**	**He never had this**
High Blood Pressure	**YES**	**NO**	**I Don't Know**	**He never had this**
Stroke	**YES**	**NO**	**I Don't Know**	**He never had this**

The last question is about <u>your sisters or brothers</u> *(the child's aunts or uncles)*.

25.   Do you have a blood-related brother or sister who has ever had any of the following conditions? *(Please circle your answer)*

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**
Diabetes	**YES**	**NO**	**I Don't Know**
Heart Attack	**YES**	**NO**	**I Don't Know**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**
High Blood Pressure	**YES**	**NO**	**I Don't Know**
Stroke	**YES**	**NO**	**I Don't Know**

26.   If <u>any</u> of your sisters or brothers *(the child's aunts or uncles)* had or have any of the conditions above, were they <u>under **60** years old</u> when the conditions first appeared?
       (Please circle your answer)

Angina or Chest Pain	**YES**	**NO**	**I Don't Know**	**Never had this**
Angioplasty (balloon procedure)	**YES**	**NO**	**I Don't Know**	**Never had this**
Diabetes	**YES**	**NO**	**I Don't Know**	**Never had this**
Heart Attack	**YES**	**NO**	**I Don't Know**	**Never had this**
Heart Bypass Surgery	**YES**	**NO**	**I Don't Know**	**Never had this**
High Blood Pressure	**YES**	**NO**	**I Don't Know**	**Never had this**
Stroke	**YES**	**NO**	**I Don't Know**	**Never had this**

Appendix D: Physiological Data Form

PHYSIOLOGICAL DATA Cohort 5 Time 1

1) Name: _____   ID_____

                                                School ID_____

2) Date: __ __/__ __/__ __

3) Skinfolds and circumferences:

    Right mid-arm circumference   (cm) _____

    Waist (nearest mm)   (cm) _____

    Hips   (cm) _____

To nearest 0.5 mm.	Tricep	1)___ ___.___
		2)___ ___.___
		3)___ ___.___
	Scapula	1)___ ___.___
		2)___ ___.___
		3)___ ___.___

                                                                                       _____
                                                                                       Tech

4) Blood Pressure   Cuff Size _____ cm

                                                     r. zero
(Right Arm)   1) ____/   ____   ____
                              2) ____/   ____   ____

                                                     _____
                                                     Tech

Appendix E: Pubertal Development Scale for Females

Girls Development Scale – Elementary version

At your age, girls can begin to have many changes to their bodies. Please mark any changes you have had.

1. Have you noticed any skin changes like oily skin, pimples or acne?
____a - My skin has not yet started showing changes
____b - My skin has barely started showing changes
____c - My skin changes are definitely underway
____d - My skin changes seem finished

2. Girls your age may have a sudden increase in their height called a "growth spurt".
     Would you say your "growth spurt"...
____a - Has not yet started
____b - Has barely started
____c - Is definitely underway
____d - Seems finished

3. Have you noticed an increase in your weight over the last few months?
____a - I have not noticed an increase in weight
____b - I have barely noticed an increase in weight
____c - An increase in my body weight is definitely underway
____d - My body weight seems to have increased as much as it's going to

4. And how about the growth of underarm and pubic hair? Would you say it has...
____a – not started growing yet
____b - has barely started
____c - is definitely underway
____d - seems finished

5. Have your breasts begun to develop?
____a - Not yet started breast development
____b - Have barely started breast development
____c - Breast development is definitely underway
____d - Breast development seems finished

6. How old were you when you had your first menstrual period?
____a. - _____ years old
____b. - I have not started getting my monthly period yet.

YOU ARE FINISHED.
1- Fold this questionnaire and staple it closed.
2- Place it in the collection box provided.

Appendix F: Pubertal Development Scale for Males

Boys Development Scale-Elementary version

At your age, boys can begin to have many changes to their bodies. Please mark any changes you have had.

1. Have you noticed any skin changes like oily skin, pimples or acne?
_____a - My skin has not yet started showing changes
_____b - My skin has barely started showing changes
_____c - My skin changes are definitely underway
_____d - My skin changes are finished

2. Boys your age may have a sudden increase in their height called a "growth spurt" (getting   taller faster than usual). Would you say your "growth spurt"...
_____a - Has not yet started
_____b - Has barely started
_____c - Is definitely underway
_____d - Seems finished

3. Have you noticed a big change in your voice?
_____a - My voice has not yet started changing
_____b - My voice has barely started changing
_____c - My voice change is definitely underway
_____d - My voice change is finished

4. Do you have any hair growing where it didn't used to grow? (under your arms or
     in your private area) Would you say this hair...
_____a - has not started growing yet
_____b - has barely started
_____c - is definitely underway
_____d - seems finished

5. Have you noticed an increase in your weight over the last few months?
_____a - I have not noticed an increase in weight
_____b - I have barely noticed an increase in weight
_____c - An increase in my weight is definitely underway
_____d - My weight seems to have increased as much as it's going to

6.   Have you begun to grow hair on your face?
_____a - I have not yet started growing hair on my face
_____b - I have barely started growing hair on my face
_____c - My facial hair growth is definitely underway
_____d - My facial hair growth seems finished

YOU ARE FINISHED,
1- Fold this questionnaire and staple it closed.
2- Place it in the collection box provided.

Appendix G: US Census Category Data Collection Form

Please answer the following:

24. What is your birthdate? (example: January 10 1990)

_____   _____

_____

MONTH   DATE

YEAR

25.   What grade are you in now? (choose one)

_____a. 3rd grade

_____b. 4th grade

_____c. 5th grade

26. Are you **Hispanic** (family from Mexico, Puerto Rico, Central or South America, or any other Spanish culture regardless of race)?

_____a. Yes

_____b. No

27. Which of these best describes your race? (choose one)

_____a. **Asian or Pacific Islander** - (Chinese, Japanese, Korean,).

_____b. **Black** (African American).

_____c. **Native American** (American Indian).

_____d. **White** (Caucasian).

_____e. **Other** (specify_____)

28. Do you have any brothers or sisters who are also in this study?

_____a. NO

_____b. YES. Write their names here:

_____

Appendix H: Description of Risk Categories for Risk Factor Variables

Variable	Risk Category Definition
LDL-C Cholesterol[1]	Acceptable (< 110 mg/dl), Borderline (110-129), and High ($\geq$ 130)
HDL-C Cholesterol[1]	Normal (> 35 mg/dl) and Low ($\leq$ 35 mg/dl)
Triglycerides[1]	Normal (<150 mg/dl) and High ($\geq$150 mg/dl)
Insulin	Hyperinsulinemia defined as fasting insulin values > the 75th percentile, specific for age, race and gender for subjects in the study cohorts.
Glucose[2]	Normal (<100 mg/dl), Impaired Glucose Tolerance (100-126 mg/dl), and Diabetic (>126 mg/dl)
Systolic Blood Pressure [3]	Normal (< 90th percentile), Pre-Hypertensive ($\geq$ 90 - < 95th percentile), and Hypertensive ($\geq$95th percentile) (All percentiles specific to age and gender)
Diastolic Blood Pressure [3]	Normal (< 90th percentile), Pre-Hypertensive ($\geq$ 90 - < 95th percentile), and Hypertensive ($\geq$95th percentile) (All percentiles specific to age and gender)
BMI[4]	Not at risk (<85th percentile), At risk of overweight ($\geq$85th–< 95th percentile), and Overweight ($\geq$95th percentile) (All percentiles specific to age and gender)
Sum of Skin folds	N/A
Waist Circumference[5]	Normal (< 90th percentile), High ($\geq$ 90th percentile). (All percentiles specific to age, gender and race)
VO$_2$	Risk categories will be based on analysis of the VO$_2$ data. The upper tertile of VO$_2$ values will be considered a high level of fitness and the lower tertile will be low fitness
Pubertal Level	N/A
Age, Gender, Race	N/A

[1]Risk categories for LDL-C, HDL-C and triglycerides are based on guidelines of the 1991 National Cholesterol Education Program (NCEP) Expert Panel on Blood Cholesterol Levels in Children and Adolescents (NCEP, 2004).

[2]Risk category classification for glucose is based on current recommendations by the American Diabetes Association (ADA, 2006).

[3]Blood pressure risk categories are based on the 2004 Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

[4]BMI risk categories based on Centers for Disease Control Classification (CDC, 2003).

[5]Waist circumference classification based on percentiles from Fernandez et al. (2004).

References

Abbasi, F., Chu, J. W., Lamendola, C., McLaughlin, T., Hayden, J., & Reaven, G. M., et al. (2004). Discrimination between obesity and insulin resistance in the relationship with adiponectin. *Diabetes, 53*(3), 585-590.

ADA (2000). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care 23*, S4.

ADA (2006). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 29*, s43-s48.

Ailhaud, G., Grimaldi, P., & Negrel, R. (1992). Cellular and molecular aspects of adipose tissue development *Annual Review of Nutrition, 12*, 207-233.

Allard, P., Delvin, E. E., Paradis, G., Hanley, J. A., O'Loughlin, J., & Lavallee, C., et al. (2003). Distribution of fasting plasma insulin, free fatty acids, and glucose concentrations and of homeostasis model assessment of insulin resistance in a representative sample of Quebec children and adolescents. *Clinical Chemistry, 49*(4), 644-649.

American Academy of Pediatrics (1992). National Cholesterol Education Program: Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents. *Pediatrics, 89*(3 Pt 2), 525-584.

Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., & Miyagawa, J., et al. (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and Biophysical Research Communications, 257*(1), 79-83.

Arslanian, S., & Suprasongsin, C. (1996). Differences in the in vivo insulin secretion and sensitivity of healthy Black versus White adolescents. *The Journal of Pediatrics, 129*(3), 440-443.

Asayama, K., Hayashibe, H., Dobashi, K., Uchida, N., Nakane, T., & Kodera, K., et al. (2003). Decrease in serum adiponectin level due to obesity and visceral fat accumulation in children. *Obesity Research, 11*(9), 1072-1079.

Bacha, F., Saad, R., Gungor, N., & Arslanian, S. A. (2005). Does adiponectin explain the lower insulin sensitivity and hyperinsulinemia of african-american children? *Pediatric Diabetes, 6*(2), 100-102.

Bacha, F., Saad, R., Gungor, N., & Arslanian, S. A. (2004). Adiponectin in youth: Relationship to visceral adiposity, insulin sensitivity, and beta-cell function. *Diabetes Care, 27*(2), 547-552.

Balagopal, P., George, D., Patton, N., Yarandi, H., Roberts, W. L., & Bayne, E., et al. (2005). Lifestyle-only intervention attenuates the inflammatory state associated with obesity: A randomized controlled study in adolescents. *The Journal of Pediatrics, 146*(3), 342-348.

Balagopal, P., George, D., Yarandi, H., Funanage, V., & Bayne, E. (2005). Reversal of obesity-related hypoadiponectinemia by lifestyle intervention: A controlled, randomized study in obese adolescents. *The Journal of Clinical Endocrinology and Metabolism, 90*(11), 6192-6197.

Baratta, R., Amato, S., Degano, C., Farina, M. G., Patane, G., & Vigneri, R., et al. (2004). Adiponectin relationship with lipid metabolism is independent of body fat mass: Evidence from both cross-sectional and intervention studies. *The Journal of Clinical Endocrinology and Metabolism, 89*(6), 2665-2671.

Baron, R. M., & Kenny, D. A. (1986). The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology, 51*(6), 1173-1182.

Beck-Nielson, H., Groop, L.C. (1994). Metabolic and genetic characterization of prediabetic states. Journal of Clinical Investigation, 94, 1714 - 1721.

Berberoglu, M. (2001). Evaluation of the correlation between serum tumor necrosis factor-alpha and relative body mass index (RBMI) in childhood. *Journal of Pediatric Endocrinology & Metabolism, 14*(5), 543-547.

Berg, A. H., & Scherer, P. E. (2005). Adipose tissue, inflammation, and cardiovascular disease. *Circulation Research, 96*(9), 939-949.

Berner, H. S., Lyngstadaas, S. P., Spahr, A., Monjo, M., Thommesen, L., & Drevon, C. A., et al. (2004). Adiponectin and its receptors are expressed in bone-forming cells. *Bone, 35*(4), 842-849.

Bluher, M., Bullen, J. W.,Jr, Lee, J. H., Kralisch, S., Fasshauer, M., & Kloting, N., et al. (2006). Circulating adiponectin and expression of adiponectin receptors in human skeletal muscle: Associations with metabolic parameters and insulin resistance and regulation by physical training. *The Journal of Clinical Endocrinology and Metabolism, 91*(6), 2310-2316.

Bottner, A., Kratzsch, J., Muller, G., Kapellen, T. M., Bluher, S., & Keller, E., et al. (2004). Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *The Journal of Clinical Endocrinology and Metabolism, 89*(8), 4053-4061.

Brancati, F. L., Whelton, P. K., Kuller, L. H., & Klag, M. J. (1996). Diabetes mellitus, race, and socioeconomic status. A population-based study. *Annals of Epidemiology, 6*(1), 67-73.

Brooks-Gunn, J. & Warren, M.P. (1988). The psychological significance of secondary sexual characteristics in nine-to-eleven-year-old girls. *Child Development, 59*, 1061-1069.

Bruce, C. R., Mertz, V. A., Heigenhauser, G. J., & Dyck, D. J. (2005). The stimulatory effect of globular adiponectin on insulin-stimulated glucose uptake and fatty acid oxidation is impaired in skeletal muscle from obese subjects. *Diabetes, 54*(11), 3154-3160.

Bruun, J. M., Lihn, A. S., Verdich, C., Pedersen, S. B., Toubro, S., & Astrup, A., et al. (2003). Regulation of adiponectin by adipose tissue-derived cytokines: In vivo and in vitro investigations in humans. *American Journal of Physiology, Endocrinology and Metabolism, 285*(3), E527-33.

Bush, N. C., Darnell, B. E., Oster, R. A., Goran, M. I., & Gower, B. A. (2005). Adiponectin is lower among African Americans and is independently related to insulin sensitivity in children and adolescents. *Diabetes, 54*(9), 2772-2778.

Butte, N. F., Comuzzie, A. G., Cai, G., Cole, S. A., Mehta, N. R., & Bacino, C. A. (2005). Genetic and environmental factors influencing fasting serum adiponectin in hispanic children. *The Journal of Clinical Endocrinology and Metabolism, 90*(7), 4170-4176.

Campbell, M. K., Thomson, S., Ramsay, C. R., MacLennan, G. S., & Grimshaw, J. M. (2004). Sample size calculator for cluster randomized trials. *Computers in Biology and Medicine, 34*(2), 113-125.

Carmena, R., Duriez, P., & Fruchart, J. C. (2004). Atherogenic lipoprotein particles in atherosclerosis. *Circulation, 109*(23 Suppl 1), III2-7.

Carroll, S., & Dudfield, M. (2004). What is the relationship between exercise and metabolic abnormalities? A review of the metabolic syndrome. *Sports Medicine, 34*(6), 371-418.

Castro-Rodriguez, J. A., Holberg, C. J., Morgan, W. J., Wright, A. L., & Martinez, F. D. (2001). Increased incidence of asthma like symptoms in girls who become overweight or obese during the school years. *American Journal of Respiratory and Critical Care Medicine, 163*(6), 1344-1349.

Centers for Disease Control (2003). BMI for children and teens. Retrieved from http://www.cdc.gov/nccdphp/dnpa/BMI/BMI-for-age.htm.

Centers for Disease Control (2006). National Health and Nutrition Examination Surveys: Z-score data file. Retrieved from: http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/zscore/zscore.htm.

Chandran, M., Phillips, S. A., Ciaraldi, T., & Henry, R. R. (2003). Adiponectin: More than just another fat cell hormone? *Diabetes Care, 26*(8), 2442-2450.

Charriere, G., Cousin, B., Arnaud, E., Andre, M., Bacou, F., & Penicaud, L., et al. (2003). Pre-adipocyte conversion to macrophage. evidence of plasticity. *Journal of Biological Chemistry, 278*(11), 9850-9855.

Chen, M. B., McAinch, A. J., Macaulay, S. L., Castelli, L. A., O'brien, P. E., & Dixon, J. B., et al. (2005). Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle of obese type 2 diabetics. *The Journal of Clinical Endocrinology and Metabolism, 90*(6), 3665-3672.

Chinetti, G., Zawadski, C., Fruchart, J. C., & Staels, B. (2004). Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPARalpha, PPARgamma, and LXR. *Biochemical and Biophysical Research Communications, 314*(1), 151-158.

Chu, N. F., Shen, M. H., Wu, D. M., & Lai, C. J. (2005). Relationship between plasma adiponectin levels and metabolic risk profiles in taiwanese children. *Obesity Research, 13*(11), 2014-2020.

Church, T. S., Finley, C. E., Earnest, C. P., Kampert, J. B., Gibbons, L. W., & Blair, S. N. (2002). Relative associations of fitness and fatness to fibrinogen, White blood cell count, uric acid and metabolic syndrome. *International Journal of Obesity and Related Metabolic Disorders, 26*(6), 805-813.

Cianflone, K., Lu, H., Smith, J., Yu, W., & Wang, H. (2005). Adiponectin, acylation stimulating protein and complement C3 are altered in obesity in very young children. *Clinical Endocrinology, 62*(5), 567-572.

Civitarese, A. E., Jenkinson, C. P., Richardson, D., Bajaj, M., Cusi, K., & Kashyap, S., et al. (2004). Adiponectin receptors gene expression and insulin sensitivity in non-diabetic mexican americans with or without a family history of type 2 diabetes. *Diabetologia, 47*(5), 816-820.

Clarke, W. R., & Lauer, R. M. (1993). Does childhood obesity track into adulthood? *Critical Reviews in Food Science and Nutrition, 33*(4-5), 423-430.

Cnop, M., Havel, P. J., Utzschneider, K. M., Carr, D. B., Sinha, M. K., & Boyko, E. J., et al. (2003). Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: Evidence for independent roles of age and sex. *Diabetologia, 46*(4), 459-469.

Combs, T. P., Berg, A. H., Obici, S., Scherer, P. E., & Rossetti, L. (2001). Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *The Journal of Clinical Investigation, 108*(12), 1875-1881.

Comuzzie, A. G., Funahashi, T., Sonnenberg, G., Martin, L. J., Jacob, H. J., & Black, A. E., et al. (2001). The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *The Journal of Clinical Endocrinology and Metabolism, 86*(9), 4321-4325.

Cone, J. B. (2001). Inflammation. *American Journal of Surgery, 182*(6), 558-562.

Conwell, L. S., Trost, S. G., Brown, W. J., & Batch, J. A. (2004). Indexes of insulin resistance and secretion in obese children and adolescents: A validation study. *Diabetes Care, 27*(2), 314-319.

Cook, S., Weitzman, M., Auinger, P., Nguyen, M., & Dietz, W. H. (2003). Prevalence of a metabolic syndrome phenotype in adolescents: Findings from the third national health and nutrition examination survey, 1988-1994. *Archives of Pediatrics & Adolescent Medicine, 157*(8), 821-827.

Cote, M., Mauriege, P., Bergeron, J., Almeras, N., Tremblay, A., & Lemieux, I., et al. (2005). Adiponectinemia in visceral obesity: Impact on glucose tolerance and plasma lipoprotein and lipid levels in men. *The Journal of Clinical Endocrinology and Metabolism, 90*(3), 1434-1439.

Cruz, M., Garcia-Macedo, R., Garcia-Valerio, Y., Gutierrez, M., Medina-Navarro, R., & Duran, G., et al. (2004). Low adiponectin levels predict type 2 diabetes in mexican children. *Diabetes Care, 27*(6), 1451-1453.

Davison, K. K., & Birch, L. L. (2001). Weight status, parent reaction, and self-concept in five-year-old girls. *Pediatrics, 107*(1), 46-53.

Degawa-Yamauchi, M., Dilts, J. R., Bovenkerk, J. E., Saha, C., Pratt, J. H., & Considine, R. V. (2003). Lower serum adiponectin levels in african-american boys. *Obesity Research, 11*(11), 1384-1390.

Delves, P. J., & Roitt, I. M. (2000). The immune system. First of two parts. *The New England Journal of Medicine, 343*(1), 37-49.

Duncan, B. B., Schmidt, M. I., Pankow, J. S., Ballantyne, C. M., Couper, D., & Vigo, A., et al. (2003). Low-grade systemic inflammation and the development of type 2 diabetes: The atherosclerosis risk in communities study. *Diabetes, 52*(7), 1799-1805.

Esposito, K., Pontillo, A., Di Palo, C., Giugliano, G., Masella, M., & Marfella, R., et al. (2003). Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: A randomized trial. *The Journal of the American Medical Association, 289*(14), 1799-1804.

Fagot-Campagna, A., Pettitt, D. J., Engelgau, M. M., Burrows, N. R., Geiss, L. S., & Valdez, R., et al. (2000). Type 2 diabetes among north american children and adolescents: An epidemiologic review and a public health perspective. *The Journal of Pediatrics, 136*(5), 664-672.

Fain, J. N., Bahouth, S. W., & Madan, A. K. (2005). Involvement of multiple signaling pathways in the post-bariatric induction of IL-6 and IL-8 mRNA and release in human visceral adipose tissue. *Biochemical Pharmacology, 69*(9), 1315-1324.

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Fain, J. N., Cheema, P. S., Bahouth, S. W., & Lloyd Hiler, M. (2003). Resistin release by human adipose tissue explants in primary culture. *Biochemical and Biophysical Research Communications, 300*(3), 674-678.

Fasshauer, M., Kralisch, S., Klier, M., Lossner, U., Bluher, M., & Klein, J., et al. (2003). Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications, 301*(4), 1045-1050.

Fasshauer, M., & Paschke, R. (2003). Regulation of adipocytokines and insulin resistance. *Diabetologia, 46*(12), 1594-1603.

Faulkner, F. & Tanner, J.M. (1986). Human Growth: A Comprehensive Treatise. (2nd Ed.). New York: Plenum Press.

Fernandez, J. R., Redden, D. T., Pietrobelli, A., & Allison, D. B. (2004). Waist circumference percentiles in nationally representative samples of african-american, european-american, and mexican-american children and adolescents. *The Journal of Pediatrics, 145*(4), 439-444.

Fernandez-Real, J. M., Botas-Cervero, P., Lopez-Bermano, A., Casamitjana, R., Funahashi, T., & Delgado, E., et al. (2004). Adiponectin is independently associated with glycosylated haemoglobin. *European Journal ofEndocrinology, 150*(2), 201-205.

Fernandez-Real, J. M., Lopez-Bermejo, A., Casamitjana, R., & Ricart, W. (2003). Novel interactions of adiponectin with the endocrine system and inflammatory parameters. *The Journal of Clinical Endocrinology and Metabolism, 88*(6), 2714-2718.

Ferris, W. F., Naran, N. H., Crowther, N. J., Rheeder, P., van der Merwe, L., & Chetty, N. (2005). The relationship between insulin sensitivity and serum adiponectin levels in three population groups. *Hormone and Metabolic Research, 37*(11), 695-701.

Frayn, K. N. (2003). Metabolic Regulation: A Human Perspective. (2nd Ed.). Oxford, UK: Blackwell Science Ltd.

Frayn, K. N., Karpe, F., Fielding, B. A., Macdonald, I. A., & Coppack, S. W. (2003). Integrative physiology of human adipose tissue. *International Journal of Obesity and Related Metabolic Disorders, 27*(8), 875-888.

Freedman, D. S., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (1999). The relation of overweight to cardiovascular risk factors among children and adolescents: The Bogalusa heart study. *Pediatrics, 103*(6 Pt 1), 1175-1182.

Freedman, D. S., Khan, L. K., Serdula, M. K., Ogden, C. L., & Dietz, W. H. (2006). Racial and ethnic differences in secular trends for childhood BMI, weight, and height. *Obesity (Silver Spring, Md.), 14*(2), 301-308.

Fried, S. K., Bunkin, D. A., & Greenberg, A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: Depot difference and regulation by glucocorticoid. *The Journal of Clinical Endocrinology and Metabolism, 83*(3), 847-850.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry, 18*(6), 499-502.

Fruebis, J., Tsao, T. S., Javorschi, S., Ebbets-Reed, D., Erickson, M. R., & Yen, F. T., et al. (2001). Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proceedings of the National Academy of Sciences of the United States of America, 98*(4), 2005-2010.

Furukawa, K., Hori, M., Ouchi, N., Kihara, S., Funahashi, T., & Matsuzawa, Y., et al. (2004). Adiponectin down-regulates acyl-coenzyme A:Cholesterol acyltransferase-1 in cultured human monocyte-derived macrophages. *Biochemical and Biophysical Research Communications, 317*(3), 831-836.

Gabay, C., & Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *The New England Journal of Medicine, 340*(6), 448-454.

Garson, G.D. (2007). Multiple regression. Retrieved from: http://www2.chass.ncsu.edu/garson/PA765/regress.htm#leverage.

Gidding, S. S., Nehgme, R., Heise, C., Muscar, C., Linton, A., & Hassink, S. (2004). Severe obesity associated with cardiovascular deconditioning, high prevalence of cardiovascular risk factors, diabetes mellitus/hyperinsulinemia, and respiratory compromise. *The Journal of Pediatrics, 144*(6), 766-769.

Gilardini, L., McTernan, P. G., Girola, A., da Silva, N. F., Alberti, L., & Kumar, S., et al. (2006). Adiponectin is a candidate marker of metabolic syndrome in obese children and adolescents. *Atherosclerosis,*

Gil-Campos, M., Canete, R., & Gil, A. (2004). Hormones regulating lipid metabolism and plasma lipids in childhood obesity. *International Journal of Obesity and Related Metabolic Disorders, 28 Suppl 3*, S75-80.

Goodman, E., Daniels, S. R., Morrison, J. A., Huang, B., & Dolan, L. M. (2004). Contrasting prevalence of and demographic disparities in the world health organization and national cholesterol education program adult treatment panel III definitions of metabolic syndrome among adolescents. *The Journal of Pediatrics, 145*(4), 445-451.

Goran, M. I., & Gower, B. A. (2001). Longitudinal study on pubertal insulin resistance. *Diabetes, 50*(11), 2444-2450.

Gower, B. A., Nagy, T. R., & Goran, M. I. (1999). Visceral fat, insulin sensitivity, and lipids in prepubertal children. *Diabetes, 48*(8), 1515-1521.

Gregoire, F. M. (2001). Adipocyte differentiation: From fibroblast to endocrine cell. *Experimental Biology and Medicine, 226*(11), 997-1002.

Gungor, N., Saad, R., Janosky, J., & Arslanian, S. (2004). Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. *The Journal of Pediatrics, 144*(1), 47-55.

Haffner, S. M., D'Agostino, R.,Jr, Goff, D., Howard, B., Festa, A., & Saad, M. F., et al. (1999). LDL size in Blacks, hispanics, and non-hispanic Whites : The insulin resistance atherosclerosis study. *Arteriosclerosis, Thrombosis, and Vascular Biology, 19*(9), 2234-2240.

Haffner, S. M., Miettinen, H., & Stern, M. P. (1997). The homeostasis model in the San Antonio heart study. *Diabetes Care, 20*(7), 1087-1092.

Hammill, B.G. & Preisser, J.S. (2006). A SAS/IML software program for GEE and regression diagnostics. *Computational Statistics & Data Analysis, 51,* 1197-1212.

Hanley, A. J., Connelly, P. W., Harris, S. B., & Zinman, B. (2003). Adiponectin in a native canadian population experiencing rapid epidemiological transition. *Diabetes Care, 26*(12), 3219-3225.

Hanley, J. A., Negassa, A., Edwardes, M. D., & Forrester, J. E. (2003). Statistical analysis of correlated data using generalized estimating equations: An orientation. *American Journal of Epidemiology, 157*(4), 364-375.

Hardin, J.W. & Hilbe, J.M. (2003). Generalized Estimating Equations. New York: Chapman & Hall/CRC, a CRC Press Company.

Hattori, Y., Suzuki, M., Hattori, S., & Kasai, K. (2003). Globular adiponectin upregulates nitric oxide production in vascular endothelial cells. *Diabetologia, 46*(11), 1543-1549.

Hausman, D. B., DiGirolamo, M., Bartness, T. J., Hausman, G. J., & Martin, R. J. (2001). The biology of White adipocyte proliferation. *Obesity Reviews, 2*(4), 239-254.

Havel, P. J. (2004). Update on adipocyte hormones: Regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes, 53 Suppl 1*, S143-51.

Hill, A. B. (1965). The environment and disease: association or causation? P*roceedings of the Royal Society of Medicine, 58*, 295-300.

Hotamisligil, G. S. (2003). Inflammatory pathways and insulin action. *International Journal of Obesity and Related Metabolic Disorders, 27 Suppl 3*, S53-5.

Hotta, K., Funahashi, T., Arita, Y., Takahashi, M., Matsuda, M., & Okamoto, Y., et al. (2000). Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arteriosclerosis, Thrombosis, and Vascular Biology, 20*(6), 1595-1599.

Hotta, K., Funahashi, T., Bodkin, N. L., Ortmeyer, H. K., Arita, Y., & Hansen, B. C., et al. (2001). Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes, 50*(5), 1126-1133.

Hu, E., Liang, P., & Spiegelman, B. M. (1996). AdipoQ is a novel adipose-specific gene dysregulated in obesity. *Journal of Biological Chemistry, 271*(18), 10697-10703.

Huang, K. C., Chen, C. L., Chuang, L. M., Ho, S. R., Tai, T. Y., & Yang, W. S. (2003). Plasma adiponectin levels and blood pressures in nondiabetic adolescent females. *The Journal of Clinical Endocrinology and Metabolism, 88*(9), 4130-4134.

Huang, K. C., Lue, B. H., Yen, R. F., Shen, C. G., Ho, S. R., & Tai, T. Y., et al. (2004). Plasma adiponectin levels and metabolic factors in nondiabetic adolescents. *Obesity Research, 12*(1), 119-124.

Hulthe, J., Hulten, L. M., & Fagerberg, B. (2003). Low adipocyte-derived plasma protein adiponectin concentrations are associated with the metabolic syndrome and small dense low-density lipoprotein particles: Atherosclerosis and insulin resistance study. *Metabolism: Clinical and Experimental, 52*(12), 1612-1614.

Hulver, M. W., Saleh, O., MacDonald, K. G., Pories, W. J., & Barakat, H. A. (2004). Ethnic differences in adiponectin levels. *Metabolism: Clinical and Experimental, 53*(1), 1-3.

Hundal, R. S., Petersen, K. F., Mayerson, A. B., Randhawa, P. S., Inzucchi, S., & Shoelson, S. E., et al. (2002). Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *The Journal of Clinical Investigation, 109*(10), 1321-1326.

Iwashima, Y., Katsuya, T., Ishikawa, K., Ouchi, N., Ohishi, M., & Sugimoto, K., et al. (2004). Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension, 43*(6), 1318-1323.

Jago, R., Harrell, J. S., McMurray, R. G., Edelstein, S., El Ghormli, L., & Bassin, S. (2006). Prevalence of abnormal lipid and blood pressure values among an ethnically diverse population of eighth-grade adolescents and screening implications. *Pediatrics, 117*(6), 2065-2073.

Janssen, I., Katzmarzyk, P. T., Srinivasan, S. R., Chen, W., Malina, R. M., & Bouchard, C., et al. (2005). Combined influence of body mass index and waist circumference on coronary artery disease risk factors among children and adolescents. *Pediatrics, 115*(6), 1623-1630.

Kantartzis, K., Fritsche, A., Tschritter, O., Thamer, C., Haap, M., & Schafer, S., et al. (2005). The association between plasma adiponectin and insulin sensitivity in humans depends on obesity. *Obesity Research, 13*(10), 1683-1691.

Kantartzis, K., Rittig, K., Balletshofer, B., Machann, J., Schick, F., & Porubska, K., et al. (2006). The relationships of plasma adiponectin with a favorable lipid profile, decreased inflammation, and less ectopic fat accumulation depend on adiposity. *Clinical Chemistry, 13*, 1683-91.

Katsiougiannis, S., Kapsogeorgou, E. K., Manoussakis, M. N., & Skopouli, F. N. (2006). Salivary gland epithelial cells: A new source of the immunoregulatory hormone adiponectin. *Arthritis and Rheumatism, 54*(7), 2295-2299.

Katzmarzyk, P. T., Srinivasan, S. R., Chen, W., Malina, R. M., Bouchard, C., & Berenson, G. S. (2004). Body mass index, waist circumference, and clustering of cardiovascular disease risk factors in a biracial sample of children and adolescents. *Pediatrics, 114*(2), e198-205.

Kawanami, D., Maemura, K., Takeda, N., Harada, T., Nojiri, T., & Imai, Y., et al. (2004). Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: A new insight into adipocytokine-endothelial cell interactions. *Biochemical and Biophysical Research Communications, 314*(2), 415-419.

Kazumi, T., Kawaguchi, A., Sakai, K., Hirano, T., & Yoshino, G. (2002). Young men with high-normal blood pressure have lower serum adiponectin, smaller LDL size, and higher elevated heart rate than those with optimal blood pressure. *Diabetes Care, 25*(6), 971-976.

Kern, P. A., Di Gregorio, G. B., Lu, T., Rassouli, N., & Ranganathan, G. (2003). Adiponectin expression from human adipose tissue: Relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. *Diabetes, 52*(7), 1779-1785.

Kershaw, E. E., & Flier, J. S. (2004). Adipose tissue as an endocrine organ. *The Journal of Clinical Endocrinology and Metabolism, 89*(6), 2548-2556.

Kharroubi, I., Rasschaert, J., Eizirik, D. L., & Cnop, M. (2003). Expression of adiponectin receptors in pancreatic beta cells. *Biochemical and Biophysical Research Communications, 312*(4), 1118-1122.

Kim, C. S., Park, J. S., Park, J., Nam, J. S., Kang, E. S., & Ahn, C. W., et al. (2006). Does birthweight predict adolescent adiponectin levels? *Clinical Endocrinology, 64*(2), 162-168.

Kishore, U., Gaboriaud, C., Waters, P., Shrive, A. K., Greenhough, T. J., & Reid, K. B., et al. (2004). C1q and tumor necrosis factor superfamily: Modularity and versatility. *Trends in Immunology, 25*(10), 551-561.

Kobayashi, H., Ouchi, N., Kihara, S., Walsh, K., Kumada, M., & Abe, Y., et al. (2004). Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circulation Research, 94*(4), e27-31.

Kondo, H., Shimomura, I., Matsukawa, Y., Kumada, M., Takahashi, M., & Matsuda, M., et al. (2002). Association of adiponectin mutation with type 2 diabetes: A candidate gene for the insulin resistance syndrome. *Diabetes, 51*(7), 2325-2328.

Korner, A., Wabitsch, M., Seidel, B., Fischer-Posovszky, P., Berthold, A., & Stumvoll, M., et al. (2005). Adiponectin expression in humans is dependent on differentiation of adipocytes and down-regulated by humoral serum components of high molecular weight. *Biochemical and Biophysical Research Communications, 337*(2), 540-550.

Kriketos, A. D., Gan, S. K., Poynten, A. M., Furler, S. M., Chisholm, D. J., & Campbell, L. V. (2004). Exercise increases adiponectin levels and insulin sensitivity in humans. *Diabetes Care, 27*(2), 629-630.

Kumada, M., Kihara, S., Ouchi, N., Kobayashi, H., Okamoto, Y., & Ohashi, K., et al. (2004). Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation, 109*(17), 2046-2049.

Kumada, M., Kihara, S., Sumitsuji, S., Kawamoto, T., Matsumoto, S., & Ouchi, N., et al. (2003). Association of hypoadiponectinemia with coronary artery disease in men. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(1), 85-89.

Lambert, M., Paradis, G., O'Loughlin, J., Delvin, E. E., Hanley, J. A., & Levy, E. (2004). Insulin resistance syndrome in a representative sample of children and adolescents from quebec, canada. *International Journal of Obesity and Related Metabolic, 28*(7), 833-841.

Lawlor, D. A., Davey Smith, G., Ebrahim, S., Thompson, C., & Sattar, N. (2005). Plasma adiponectin levels are associated with insulin resistance, but do not predict future risk of coronary heart disease in women. *The Journal of Clinical Endocrinology and Metabolism, 90*(10), 5677-5683.

Lee, S., Bacha, F., Gungor, N., & Arslanian, S. A. (2006). Racial differences in adiponectin in youth: Relationship to visceral fat and insulin sensitivity. *Diabetes Care, 29*(1), 51-56.

Levine, J. A., Jensen, M. D., Eberhardt, N. L., & O'Brien, T. (1998). Adipocyte macrophage colony-stimulating factor is a mediator of adipose tissue growth. *The Journal of Clinical Investigation; the Journal of Clinical Investigation, 101*(8), 1557-1564.

Li, A. C., & Glass, C. K. (2002). The macrophage foam cell as a target for therapeutic intervention. *Nature Medicine, 8*(11), 1235-1242.

Libby, P., & Aikawa, M. (2002). Stabilization of atherosclerotic plaques: New mechanisms and clinical targets. *Nature Medicine, 8*(11), 1257-1262.

Lihn, A. S., Ostergard, T., Nyholm, B., Pedersen, S. B., Richelsen, B., & Schmitz, O. (2003). Adiponectin expression in adipose tissue is reduced in first-degree relatives of type 2 diabetic patients. *American Journal of Physiology. Endocrinology and Metabolism, 284*(2), E443-8.

Linton, M. F., & Fazio, S. (2003). Macrophages, inflammation, and atherosclerosis. *International Journal of Obesity and Related Metabolic, 27 Suppl 3*, S35-40.

Lodish et al., (2004). *Molecular Cell Biology*. (5th Ed.). New York, NY: WH Freeman & Company.

Lutgendorf, S. K., & Costanzo, E. S. (2003). Psychoneuroimmunology and health psychology: An integrative model. *Brain, Behavior, and Immunity; Brain, Behavior, and Immunity, 17*(4), 225-232.

Lyon, C. J., Law, R. E., & Hsueh, W. A. (2003). Minireview: Adiposity, inflammation, and atherogenesis. *Endocrinology; Endocrinology, 144*(6), 2195-2200.

Ma, J., Hennekens, C. H., Ridker, P. M., & Stampfer, M. J. (1999). A prospective study of fibrinogen and risk of myocardial infarction in the physicians' health study. *Journal of the American College of Cardiology; Journal of the American College of Cardiology, 33*(5), 1347-1352.

Maeda, K., Okubo, K., Shimomura, I., Funahashi, T., Matsuzawa, Y., & Matsubara, K. (1996). cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose most abundant gene transcript 1). *Biochemical and Biophysical Research Communications, 221*(2), 286-289.

Martin, L. J., Woo, J. G., Daniels, S. R., Goodman, E., & Dolan, L. M. (2005). The relationships of adiponectin with insulin and lipids are strengthened with increasing adiposity. *The Journal of Clinical Endocrinology and Metabolism, 90*(7), 4255-4259.

McGill, H. C.,Jr, McMahan, C. A., Herderick, E. E., Malcom, G. T., Tracy, R. E., & Strong, J. P. (2000). Origin of atherosclerosis in childhood and adolescence. *The American Journal of Clinical Nutrition, 72*(5 Suppl), 1307S-1315S.

McMurray, R. G., Guion, W. K., Ainsworth, B. E., & Harrell, J. S. (1998). Predicting aerobic power in children. A comparison of two methods. *The Journal of Sports Medicine and Physical Fitness, 38*(3), 227-233.

Medzhitov, R., & Janeway, C.,Jr. (2000). Innate immunity. *The New England Journal of Medicine, 343*(5), 338-344.

Mei, Z., Grummer-Strawn, L. M., Pietrobelli, A., Goulding, A., Goran, M. I., & Dietz, W. H. (2002). Validity of body mass index compared with other body-composition screening indexes for the assessment of body fatness in children and adolescents. *The American Journal of Clinical Nutrition, 75*(6), 978-985.

Mensah, G. A., Mokdad, A. H., Ford, E. S., Greenlund, K. J., & Croft, J. B. (2005). State of disparities in cardiovascular health in the united states. *Circulation, 111*(10), 1233-1241.

Mocellin, R., Lindemann, H., Rutenfranz, J., & Sbresny, W. (1971). Determination of W 170 and maximal oxygen uptake in children by different methods. *Acta Paediatrica Scandinavica.Supplement, 217*, 13-17.

Monzillo, L. U., Hamdy, O., Horton, E. S., Ledbury, S., Mullooly, C., & Jarema, C., et al. (2003). Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance. *Obesity Research, 11*(9), 1048-1054.

Morales, A., Wasserfall, C., Brusko, T., Carter, C., Schatz, D., & Silverstein, J., et al. (2004). Adiponectin and leptin concentrations may aid in discriminating disease forms in children and adolescents with type 1 and type 2 diabetes. *Diabetes Care, 27*(8), 2010-2014.

Must, A., & Strauss, R. S. (1999). Risks and consequences of childhood and adolescent obesity. *International Journal of Obesity and Related Metabolic Disorders, 23 Suppl 2*, S2-11.

Nakano, Y., Tajima, S., Yoshimi, A., Akiyama, H., Tsushima, M., & Tanioka, T., et al. (2006). A novel enzyme-linked immunosorbent assay specific for high-molecular-weight adiponectin. *Journal of Lipid Research, 47*(7), 1572-1582.

Nakano, Y., Tobe, T., Choi-Miura, N. H., Mazda, T., & Tomita, M. (1996). Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *Journal of Biochemistry, 120*(4), 803-812.

Nassis, G. P., Papantakou, K., Skenderi, K., Triandafillopoulou, M., Kavouras, S. A., & Yannakoulia, M., et al. (2005). Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism: Clinical and Experimental, 54*(11), 1472-1479.

Nathan, C. F. (1987). Secretory products of macrophages. *The Journal of Clinical Investigation, 79*(2), 319-326.

National Center for Health Statistics (1987). Vital and health statistics, 11. Hyattsville, MD: Public Health Service.

National Heart, Lung & Blood Institute (1998). Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: The evidence report. *Obesity Research, 6 suppl 2*, 51S-209S.

National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. (2004). The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics, 114*(2 Suppl 4th Report), 555-576.

Nemet, D., Wang, P., Funahashi, T., Matsuzawa, Y., Tanaka, S., & Engelman, L., et al. (2003). Adipocytokines, body composition, and fitness in children. *Pediatric Research, 53*(1), 148-152.

Neovius, M., Linne, Y., & Rossner, S. (2005). BMI, waist-circumference and waist-hip-ratio as diagnostic tests for fatness in adolescents. *International Journal of Obesity (2005), 29*(2), 163-169.

Newby, A. C. (2005). Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. *Physiological Reviews, 85*(1), 1-31.

Norton, E. C., Bieler, G. S., Ennett, S. T., & Zarkin, G. A. (1996). Analysis of prevention program effectiveness with clustered data using generalized estimating equations. *Journal of Consulting and Clinical Psychology, 64*(5), 919-926.

Ogawa, Y., Kikuchi, T., Nagasaki, K., Hiura, M., Tanaka, Y., & Uchiyama, M. (2005). Usefulness of serum adiponectin level as a diagnostic marker of metabolic syndrome in obese japanese children. *Hypertension Research, 28*(1), 51-57.

Ogden, C. L., Carroll, M. D., Curtin, L. R., McDowell, M. A., Tabak, C. J., & Flegal, K. M. (2006). Prevalence of overweight and obesity in the united states, 1999-2004. *JAMA : The Journal of the American Medical Association, 295*(13), 1549-1555.

Ohashi, K., Ouchi, N., Kihara, S., Funahashi, T., Nakamura, T., & Sumitsuji, S., et al. (2004). Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. *Journal of the American College of Cardiology, 43*(7), 1195-1200.

Okada, T., Saito, E., Kuromori, Y., Miyashita, M., Iwata, F., & Hara, M., et al. (2005). Relationship between serum adiponectin level and lipid composition in each lipoprotein fraction in adolescent children. *Atherosclerosis,*

Ouchi, N., Kihara, S., Arita, Y., Nishida, M., Matsuyama, A., & Okamoto, Y., et al. (2001). Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation, 103*(8), 1057-1063.

Ouchi, N., Kihara, S., Arita, Y., Okamoto, Y., Maeda, K., & Kuriyama, H., et al. (2000). Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation, 102*(11), 1296-1301.

Ouchi, N., Kihara, S., Funahashi, T., Matsuzawa, Y., & Walsh, K. (2003). Obesity, adiponectin and vascular inflammatory disease. *Current Opinion in Lipidology, 14*(6), 561-566.

Pajvani, U. B., Du, X., Combs, T. P., Berg, A. H., Rajala, M. W., & Schulthess, T., et al. (2003). Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. implications fpr metabolic regulation and bioactivity. *Journal of Biological Chemistry, 278*(11), 9073-9085.

Pan, W. (2001). Akaike's information criterion in generalized estimating equations. *Biometrics*, 57, 120-125.

Park, K. G., Park, K. S., Kim, M. J., Kim, H. S., Suh, Y. S., & Ahn, J. D., et al. (2004). Relationship between serum adiponectin and leptin concentrations and body fat distribution. *Diabetes Research and Clinical Practice, 63*(2), 135-142.

Patel, D.A., Srinivasan, S.R., Xu, J. Chen, W. & Berenson, G.S. (2006). Adiponectin and its correlates of cardiovascular risk in young adults: the Bogalusa Heart Study. Metabolism, 55, 1551-1557.

Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon, R. O.,3rd, & Criqui, M., et al. (2003). Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the centers for disease control and prevention and the american heart association. *Circulation, 107*(3), 499-511.

Petersen, A.C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence, 17*, 117-133.

Pickup, J. C. (2004). Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care, 27*(3), 813-823.

Pickup, J. C., & Crook, M. A. (1998). Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia, 41*(10), 1241-1248.

Pilz, S., Horejsi, R., Moller, R., Almer, G., Scharnagl, H., & Stojakovic, T., et al. (2005). Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. *The Journal of Clinical Endocrinology and Metabolism,*

Pineiro, R., Iglesias, M. J., Gallego, R., Raghay, K., Eiras, S., & Rubio, J., et al. (2005). Adiponectin is synthesized and secreted by human and murine cardiomyocytes. *FEBS Letters, 579*(23), 5163-5169.

Pischon, T., Girman, C. J., Hotamisligil, G. S., Rifai, N., Hu, F. B., & Rimm, E. B. (2004). Plasma adiponectin levels and risk of myocardial infarction in men. *The Journal of the American Medical Association, 291*(14), 1730-1737.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *The Journal of the American Medical Association, 286*(3), 327-334.

Preisser, J. S., Young, M. L., Zaccaro, D. J., & Wolfson, M. (2003). An integrated population-averaged approach to the design, analysis and sample size determination of cluster-unit trials. *Statistics in Medicine, 22*(8), 1235-1254.

Prins, J. B., & O'Rahilly, S. (1997). Regulation of adipose cell number in man. *Clinical Science, 92*(1), 3-11.

Punthakee, Z., Delvin, E. E., O'loughlin, J., Paradis, G., Levy, E., & Platt, R. W., et al. (2006). Adiponectin, adiposity and insulin resistance in children and adolescents. *The Journal of Clinical Endocrinology and Metabolism,*

Rakatzi, I., Mueller, H., Ritzeler, O., Tennagels, N., & Eckel, J. (2004). Adiponectin counteracts cytokine- and fatty acid-induced apoptosis in the pancreatic beta-cell line INS-1. *Diabetologia, 47*(2), 249-258.

Rask-Madsen, C., Dominguez, H., Ihlemann, N., Hermann, T., Kober, L., & Torp-Pedersen, C. (2003). Tumor necrosis factor-alpha inhibits insulin's stimulating effect on glucose uptake and endothelium-dependent vasodilation in humans. *Circulation, 108*(15), 1815-1821.

Rasmussen, M. S., Lihn, A. S., Pedersen, S. B., Bruun, J. M., Rasmussen, M., & Richelsen, B. (2006). Adiponectin receptors in human adipose tissue: Effects of obesity, weight loss, and fat depots. *Obesity (Silver Spring, Md.), 14*(1), 28-35.

Reinehr, T., Roth, C., Menke, T., & Andler, W. (2004). Adiponectin before and after weight loss in obese children. *The Journal of Clinical Endocrinology and Metabolism, 89*(8), 3790-3794.

Retnakaran, R., Zinman, B., Connelly, P. W., Harris, S. B., & Hanley, A. J. (2006). Nontraditional cardiovascular risk factors in pediatric metabolic syndrome. *The Journal of Pediatrics, 148*(2), 176-182.

Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P., & Hennekens, C. H. (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *The New England Journal of Medicine, 336*(14), 973-979.

Ridker, P. M., Hennekens, C. H., Buring, J. E., & Rifai, N. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *The New England Journal of Medicine, 342*(12), 836-843.

Ridker, P. M., Rifai, N., Stampfer, M. J., & Hennekens, C. H. (2000). Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation, 101*(15), 1767-1772.

Ross, R. (1999). Atherosclerosis is an inflammatory disease. *American Heart Journal, 138*(5 Pt 2), S419-20.

Ross, R. (1993). The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature, 362*(6423), 801-809.

Ryan, A. S., Berman, D. M., Nicklas, B. J., Sinha, M., Gingerich, R. L., & Meneilly, G. S., et al. (2003). Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. *Diabetes Care, 26*(8), 2383-2388.

Ryan, A. S., Nicklas, B. J., Berman, D. M., & Elahi, D. (2003). Adiponectin levels do not change with moderate dietary induced weight loss and exercise in obese postmenopausal women. *International Journal of Obesity and Related Metabolic, 27*(9), 1066-1071.

Sbarbati, A., Osculati, F., Silvagni, D., Benati, D., Galie, M., & Camoglio, F. S., et al. (2006). Obesity and inflammation: Evidence for an elementary lesion. *Pediatrics, 117*(1), 220-223.

Scherer, P. E., Williams, S., Fogliano, M., Baldini, G., & Lodish, H. F. (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. *Journal of Biological Chemistry, 270*(45), 26746-26749.

Schulze, M. B., Rimm, E. B., Shai, I., Rifai, N., & Hu, F. B. (2004). Relationship between adiponectin and glycemic control, blood lipids, and inflammatory markers in men with type 2 diabetes. *Diabetes Care, 27*(7), 1680-1687.

Shepherd, P. R., & Kahn, B. B. (1999). Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. *The New England Journal of Medicine, 341*(4), 248-257.

Shoelson, S. E., Lee, J., & Yuan, M. (2003). Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. *International Journal of Obesity and Related Metabolic, 27 Suppl 3*, S49-52.

Singhal, A., Jamieson, N., Fewtrell, M., Deanfield, J., Lucas, A., & Sattar, N. (2005). Adiponectin predicts insulin resistance but not endothelial function in young healthy adolescents. *The Journal of Clinical Endocrinology and Metabolism, 90,* 4615-4621.

Sinha, R., Fisch, G., Teague, B., Tamborlane, W. V., Banyas, B., & Allen, K., et al. (2002). Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *The New England Journal of Medicine,346*(11), 802-810.

Sivan, E., Mazaki-Tovi, S., Pariente, C., Efraty, Y., Schiff, E., & Hemi, R., et al. (2003). Adiponectin in human cord blood: Relation to fetal birth weight and gender. *The Journal of Clinical Endocrinology and Metabolism, 88*(12), 5656-5660.

Slaughter, M. H., Lohman, T. G., Boileau, R. A., Horswill, C. A., Stillman, R. J., & Van Loan, M. D., et al. (1988). Skinfold equations for estimation of body fatness in children and youth. *Human Biology, 60*(5), 709-723.

Snehalatha, C., Mukesh, B., Simon, M., Viswanathan, V., Haffner, S. M., & Ramachandran, A. (2003). Plasma adiponectin is an independent predictor of type 2 diabetes in asian indians. *Diabetes Care, 26*(12), 3226-3229.

Spranger, J., Kroke, A., Mohlig, M., Bergmann, M. M., Ristow, M., & Boeing, H., et al. (2003). Adiponectin and protection against type 2 diabetes mellitus. *Lancet, 361*(9353), 226-228.

Srinivasan, S. R., Myers, L., & Berenson, G. S. (2002). Predictability of childhood adiposity and insulin for developing insulin resistance syndrome (syndrome X) in young adulthood: The bogalusa heart study. *Diabetes, 51*(1), 204-209.

Steeber, D. A., Venturi, G. M., & Tedder, T. F. (2005). A new twist to the leukocyte adhesion cascade: Intimate cooperation is key. *Trends in Immunology, 26*(1), 9-12.

Stefan, N., Bunt, J. C., Salbe, A. D., Funahashi, T., Matsuzawa, Y., & Tataranni, P. A. (2002). Plasma adiponectin concentrations in children: Relationships with obesity and insulinemia. *The Journal of Clinical Endocrinology and Metabolism, 87*(10), 4652-4656.

Stefan, N., Stumvoll, M., Vozarova, B., Weyer, C., Funahashi, T., & Matsuzawa, Y., et al. (2003). Plasma adiponectin and endogenous glucose production in humans. *Diabetes Care, 26*(12), 3315-3319.

Stefan, N., Vozarova, B., Funahashi, T., Matsuzawa, Y., Weyer, C., & Lindsay, R. S., et al. (2002). Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. *Diabetes, 51*(6), 1884-1888.

Steffes, M. W., Gross, M. D., Schreiner, P. J., Yu, X., Hilner, J. E., & Gingerich, R., et al. (2004). Serum adiponectin in young adults--interactions with central adiposity, circulating levels of glucose, and insulin resistance: The CARDIA study. *Annals of Epidemiology, 14*(7), 492-498.

Steinberger, J., Moran, A., Hong, C. P., Jacobs, D. R.,Jr, & Sinaiko, A. R. (2001). Adiposity in childhood predicts obesity and insulin resistance in young adulthood. *The Journal of Pediatrics, 138*(4), 469-473.

Strauss, R. S. (2000). Childhood obesity and self-esteem. *Pediatrics, 105*(1), e15.

Svec, F., Nastasi, K., Hilton, C., Bao, W., Srinivasan, S. R., & Berenson, G. S. (1992). Black-White contrasts in insulin levels during pubertal development. the bogalusa heart study. *Diabetes; Diabetes, 41*(3), 313-317.

Trayhurn, P., & Beattie, J. H. (2001). Physiological role of adipose tissue: White adipose tissue as an endocrine and secretory organ. *The Proceedings of the Nutrition Society; the Proceedings of the Nutrition Society, 60*(3), 329-339.

Trujillo, M. E., & Scherer, P. E. (2005). Adiponectin--journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *Journal of Internal Medicine, 257*(2), 167-175.

Tsao, T. S., Tomas, E., Murrey, H. E., Hug, C., Lee, D. H., & Ruderman, N. B., et al. (2003). Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. different oligomers activate different signal transduction pathways. *Journal of Biological Chemistry, 278*(50), 50810-50817.

Tsou, P. L., Jiang, Y. D., Chang, C. C., Wei, J. N., Sung, F. C., & Lin, C. C., et al. (2004). Sex-related differences between adiponectin and insulin resistance in schoolchildren. *Diabetes Care, 27*(2), 308-313.

Ukkola, O., & Santaniemi, M. (2002). Adiponectin: A link between excess adiposity and associated comorbidities? *Journal of Molecular Medicine, 80*(11), 696-702.

Valsamakis, G., McTernan, P. G., Chetty, R., Al Daghri, N., Field, A., & Hanif, W., et al. (2004). Modest weight loss and reduction in waist circumference after medical treatment are associated with favorable changes in serum adipocytokines. *Metabolism: Clinical and Experimental, 53*(4), 430-434.

Verges, B., Petit, J. M., Duvillard, L., Dautin, G., Florentin, E., & Galland, F., et al. (2006). Adiponectin is an important determinant of apoA-I catabolism. *Arteriosclerosis, Thrombosis, and Vascular Biology, 26*(6), 1364-1369.

Vikram, N. K., Misra, A., Pandey, R. M., Dwivedi, M., & Luthra, K. (2004). Adiponectin, insulin resistance, and C-reactive protein in postpubertal asian indian adolescents. *Metabolism: Clinical and Experimental, 53*(10), 1336-1341.

Viner, R. M., Segal, T. Y., Lichtarowicz-Krynska, E., & Hindmarsh, P. (2005). Prevalence of the insulin resistance syndrome in obesity. *Archives of Disease in Childhood, 90*(1), 10-14.

Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., & Harris, T. B. (2001). Low-grade systemic inflammation in overweight children. *Pediatrics, 107*(1), E13.

Vozarova, B., Weyer, C., Hanson, K., Tataranni, P. A., Bogardus, C., & Pratley, R. E. (2001). Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obesity Research, 9*(7), 414-417.

Wang, Y. (2004). Epidemiology of childhood obesity--methodological aspects and guidelines: What is new? *International Journal of Obesity and Related Metabolic, 28 Suppl 3*, S21-8.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W.,Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *The Journal of Clinical Investigation, 112*(12), 1796-1808.

Weiss, R., Dufour, S., Groszmann, A., Petersen, K., Dziura, J., & Taksali, S. E., et al. (2003). Low adiponectin levels in adolescent obesity: A marker of increased intramyocellular lipid accumulation. *The Journal of Clinical Endocrinology and Metabolism, 88*(5), 2014-2018.

Weiss, R., Dziura, J., Burgert, T. S., Tamborlane, W. V., Taksali, S. E., & Yeckel, C. W., et al. (2004). Obesity and the metabolic syndrome in children and adolescents. *The New England Journal of Medicine, 350*(23), 2362-2374.

Weiss, R., Taksali, S. E., Dufour, S., Yeckel, C. W., Papademetris, X., & Cline, G., et al. (2005). The "obese insulin-sensitive" adolescent: Importance of adiponectin and lipid partitioning. *The Journal of Clinical Endocrinology and Metabolism, 90*(6), 3731-3737.

Wells, J. C., & Fewtrell, M. S. (2006). Measuring body composition. *Archives of Disease in Childhood, 91*(7), 612-617.

Weyer, C., Funahashi, T., Tanaka, S., Hotta, K., Matsuzawa, Y., & Pratley, R. E., et al. (2001). Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. *The Journal of Clinical Endocrinology and Metabolism, 86*(5), 1930-1935.

Whitaker, R. C., Wright, J. A., Pepe, M. S., Seidel, K. D., & Dietz, W. H. (1997). Predicting obesity in young adulthood from childhood and parental obesity. *The New England Journal of Medicine, 337*(13), 869-873.

Whitehead, J. P., Richards, A. A., Hickman, I. J., Macdonald, G. A., & Prins, J. B. (2006). Adiponectin--a key adipokine in the metabolic syndrome*Diabetes, Obesity & Metabolism, 8*(3), 264-280.

Williams, C. L., Hayman, L. L., Daniels, S. R., Robinson, T. N., Steinberger, J., & Paridon, S., et al. (2002). Cardiovascular health in childhood: A statement for health professionals from the committee on atherosclerosis, hypertension, and obesity in the young (AHOY) of the council on cardiovascular disease in the young, american heart association. *Circulation, 106*(1), 143-160.

Winkleby, M. A., Robinson, T. N., Sundquist, J., & Kraemer, H. C. (1999). Ethnic variation in cardiovascular disease risk factors among children and young adults: Findings from the third national health and nutrition examination survey, 1988-1994. *The Journal of the American Medical Association, 281*(11), 1006-1013.

Winzer, C., Wagner, O., Festa, A., Schneider, B., Roden, M., & Bancher-Todesca, D., et al. (2004). Plasma adiponectin, insulin sensitivity, and subclinical inflammation in women with prior gestational diabetes mellitus. *Diabetes Care, 27*(7), 1721-1727.

Woo, J. G., Dolan, L. M., Daniels, S. R., Goodman, E., & Martin, L. J. (2005). Adolescent sex differences in adiponectin are conditional on pubertal development and adiposity. *Obesity Research, 13*(12), 2095-2101.

Wu, T., Mendola, P., & Buck, G. M. (2002). Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: The third national health and nutrition examination survey, 1988-1994. *Pediatrics, 110*(4), 752-757.

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., & Chou, C. J., et al. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *The Journal of Clinical Investigation, 112*(12), 1821-1830.

Xydakis, A. M., Case, C. C., Jones, P. H., Hoogeveen, R. C., Liu, M. Y., & Smith, E. O., et al. (2004). Adiponectin, inflammation, and the expression of the metabolic syndrome in obese individuals: The impact of rapid weight loss through caloric restriction. *The Journal of Clinical Endocrinology and Metabolism, 89*(6), 2697-2703.

Yamamoto, Y., Hirose, H., Saito, I., Nishikai, K., & Saruta, T. (2004). Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: Two-year follow-up study in japanese population. *The Journal of Clinical Endocrinology and Metabolism, 89*(1), 87-90.

Yamauchi, T., Kamon, J., Ito, Y., Tsuchida, A., Yokomizo, T., & Kita, S., et al. (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature, 423*(6941), 762-769.

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., & Uchida, S., et al. (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine, 8*(11), 1288-1295.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., & Hara, K., et al. (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Medicine, 7*(8), 941-946.

Yang, W. S., Lee, W. J., Funahashi, T., Tanaka, S., Matsuzawa, Y., & Chao, C. L., et al. (2001). Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *The Journal of Clinical Endocrinology and Metabolism, 86*(8), 3815-3819.

Yki-Jarvinen, H. (2004). Thiazolidinediones. *The New England Journal of Medicine, 351*(11), 1106-1118.

Yokota, T., Oritani, K., Takahashi, I., Ishikawa, J., Matsuyama, A., & Ouchi, N., et al. (2000). Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood, 96*(5), 1723-1732.

Yokoyama, H., Emoto, M., Mori, K., Araki, T., Teramura, M., & Koyama, H., et al. (2006). Plasma adiponectin level is associated with insulin-stimulated nonoxidative glucose disposal. *The Journal of Clinical Endocrinology and Metabolism, 91*(1), 290-294.

Young-Hyman, D., Schlundt, D. G., Herman, L., De Luca, F., & Counts, D. (2001). Evaluation of the insulin resistance syndrome in 5- to 10-year-old overweight/obese african-american children. *Diabetes Care, 24*(8), 1359-1364.

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z. W., & Karin, M., et al. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of ikkbeta. *Science, 293*(5535), 1673-1677.

Ziccardi, P., Nappo, F., Giugliano, G., Esposito, K., Marfella, R., & Cioffi, M., et al. (2002). Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation; Circulation, 105*(7), 804-809.