TRIGLYCERIDE/HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO AS A SCREENING TOOL FOR INSULIN RESISTANCE IN U.S. ADOLESCENTS, 12-19 YEARS OF AGE

by

CONSTANCE ELAINE CEPHUS

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON

DECEMBER 2014

Copyright © by Constance E Cephus 2014

All Rights Reserved

Acknowledgements

I want to thank my family (biological and adoptive) for their love, support, and understanding during this period of my life journey. To my mother, who always referred to me as “almost the doctor”, I did it, Mom. To my daughter, Nicole, who has endured my need for continuous learning for her entire life: Thank you, I love you beyond words.

I want to extend special thanks to my dissertation chair, Dr. Jennifer Gray. Your words of encouragement flowed from my initial investigation of doctoral work through the completion of this dissertation. I could not have done this without you. I am forever grateful. Thank you for your vision.

I want to thank my dissertation committee members, Dr. Wendy Barr and Dr. Barbara Raudonis. To Dr. Barr: You were there from the start, helping me navigate the road to dissertation. Thank you for your patience, time, and mentorship. To Dr. Raudonis: You let me see that the NIH was attainable. Thank you for changing my view of the world.

I want to thank my classmates and professors for giving me a sense of community and connectedness that was so necessary as a distance student.

November 12, 2014

Abstract

TRIGLYCERIDE/HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO,

AS A SCREENING TOOL FOR INSULIN RESISTANCE

IN U.S. ADOLESCENTS, 12-19 YEARS OF AGE

Constance E Cephus, PhD

The University of Texas at Arlington, 2014

Supervising Professor: Supervising Professor Jennifer R. Gray

Background: Insulin resistance appears to be a metabolic process that occurs prior to the onset of cardiovascular disease and type 2 diabetes. A screening tool for insulin resistance may help clinicians determine which adolescents need intensive lifestyle modification to decrease risk of cardiovascular disease and diabetes.

Purpose: The purpose of this study is to evaluate the performance characteristics of the TG/HDL ratio when used to assess risk of insulin resistance in U.S. adolescents.

Methods: A secondary analysis of 2011-2012 data for adolescents (12-19 years of age) from the National Health and Nutrition Examination Survey (NHANES) was conducted to evaluate the performance characteristics of the TG/HDL ratio.

Results: The performance characteristics of the TG/HDL: sensitivity14.8%, specificity 93.8%, positive predictive value 10.4%, negative predictive value 95.8%, overall accuracy 90.2%.

Conclusions: These findings indicate that the TG/HDL ratio would be useful to indicate low likelihood of insulin resistance.

Table of Contents

List of Illustrations

List of Tables

Chapter 1

Triglyceride/High Density Lipoprotein Cholesterol Ratio as a Screening Tool for Insulin Resistance in U.S. Adolescents

Primordial (prior to development of risk factors) and primary (modifying existing risk factors) prevention of cardiovascular disease and type II diabetes in adolescents is hindered by the lack of reliable, readily available screening methods (Weintraub et al., 2011). The increases in obesity and emergence of type 2 diabetes in children and adolescents have garnered the attention of public and private sectors (Narasimhan & Weinstock, 2014). Development of insulin resistance occurs prior to development of cardiovascular disease and type 2 diabetes (Lee, Herman, Okumura, Gurney, & Davis, 2006; Shah, Dolan, Gao, Kimball, & Urbina, 2011; Tobisch, Blatniczky, & Barkai, 2013), but is often missed. A screening tool for insulin resistance may help clinicians determine which adolescents need more intensive testing and therapy to prevent cardiovascular disease and type 2 diabetes.

Screening tests are judged by the following criteria: safety, simplicity, speed of completion, expense and acceptability to the public and medical community (Friis & Sellers, 2009). The test should be easy to learn and perform. The direct and indirect costs of the test should be balanced by the benefit (Cochrane & Holland, 1971). Ideally, the test results should be sensitive and specific and carry minimal potential harm to the individual or public at large. The test results should be readily available. Health care providers and the public must be willing to participate in the testing (Friis & Sellers, 2009; Wilson & Jungner, 1968).

In the context of screening, there are four performance characteristics that must be assessed: sensitivity, specificity, positive predictive value and negative predictive value (Friis & Seller, 2009; Lang & Secic, 2006). The performance characteristics reflect

the accuracy and reliability of a test and are the basis of the safety profile (Lang & Secic, 2006). The purpose of this study was to evaluate the performance characteristics of the triglyceride/HDL cholesterol ratio when used to determine increased risk of insulin resistance in the U.S. adolescent population. A secondary analysis of 2011-2012 data for adolescents (12-19 years of age) from the National Health and Nutrition Examination Survey (NHANES) was conducted to evaluate the performance characteristics of this screening test.

Chronic, non-communicable disease is the foremost cause of poor health and disability in the U.S. (Bauer, Briss, Goodman, & Bowman, 2014). Insulin resistance is a common, early element of several chronic adult diseases, now found in the adolescent population. In this chapter, discussion encompasses the urgent need for health promotion and disease prevention as related to diabetes and cardiovascular disease in the pediatric population. The performance characteristics of the triglyceride/high density lipoprotein cholesterol ratio (TG/HDL ratio), as a screening tool for insulin resistance among U.S. adolescents were assessed.

Background and Significance

In 2009, cardiovascular disease accounted for significant morbidity and mortality and was the leading cause of death in the U.S. (Bambs & Reis, 2011; Go et al., 2013). Since recognition, atherosclerosis has been the most significant determinant of coronary artery related heart disease (Strong & McGill, 1962). In 2013, one of every six cardiovascular deaths was related to coronary artery disease (Go et al., 2013). Vascular disease (cardiac and cerebral) is the major cause of morbidity and mortality in adults with type 2 diabetes (Narasimhan & Weinstock, 2014). For every minute during 2013, two people experienced a coronary related-event from which one of them died (Go et al., 2013). Stroke occurred approximately every 40 seconds and a person died as the result

of stroke every four minutes (Go et al., 2013). The direct and indirect costs of cardiovascular disease totaled over $300 billion (Roehrig & Rousseau, 2010).

Pathologic evidence demonstrates that cardiovascular disease begins in childhood (Berenson, Srinivasan, Bao, Newman, et al., 1998; Strong, Malcom, Oalmann &Wissler, 1997). In a landmark report, Enos, Holmes, and Beyer (1953) described evidence of coronary artery disease in 77% of the autopsied hearts (n = 300) of soldiers killed during the Korean War. The average age of the soldiers was 22 years (Enos, Holmes, & Beyer, 1953). Strong and McGill (1969) reported that the first evidence of coronary atherosclerosis occurred in children 10-20 years of age. By 1980, risk factors for coronary artery disease in children were identified and included hypertension, abnormal lipid levels, increased skinfold thickness, and family history (Khoury, P. et al., 1980; Webber, Voors, Srinivasan, Frerichs, & Berenson, 1979). During the 1990s, hyperinsulinemia, a known adult cardiovascular risk factor, was added as a risk factor for cardiovascular disease in children (Bao, Srinivasan, & Berenson, 1996).

Clustering of risk factors is associated with early cardiovascular disease (DHHS, 2012). Data from two prospective epidemiologic studies, Bogalusa Heart Study and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY), confirmed a positive correlation between atherosclerotic changes and risk factors in children as young as 5 years old (Berenson, Srinivasan, & Bao, 1997; Malcom, Oalmann, & Strong, 1997). In the PDAY study, 15-19 year olds with hyperglycemia and dyslipidemia had significantly more coronary artery atherosclerotic lesions than their study peers (Malcom, Oalmann, & Strong, 1997). Smoak and colleagues (1987) found that obese participants (aged 5-24 years) were 3.1 times more likely to have abnormal lipid indices and elevated insulin levels than age-matched lean peers.

Modifiable risk factors for cardiovascular disease include hypertension, hyperinsulinemia, elevated body mass index (BMI), dyslipidemia, and visceral adiposity (Hayman et al., 2007). Jaquith, Harris, and Penprase (2013) found that more than 20% of adolescents present for well child visits with two modifiable risk factors. Despite this information, only two-thirds of primary care providers, who answered an electronic survey (n=364/547), performed screening for risk factors in children and adolescents (Dixon, Steffen, Kornblum & Steinberger, 2013). One reason for scarcity of screening in the pediatric population may be the lack of a quick, easy, reliable screening tool.

Increased adiposity is the most important risk factor associated with development of type 2 diabetes in adolescents (Narasimhan & Weinstock, 2014). Acquired dyslipidemia is often associated with obesity, but may be secondary to drug therapy, renal disease, hepatic disease, or an endocrine or metabolic disorder (U.S. Department of Health and Human Services [DHHS], 2012). Obesity and acquired dyslipidemia are associated with insulin resistance (Bacha, Gungor, Saad, & Arslanian, 2006). The linkages among acquired dyslipidemia, insulin resistance, and obesity are important to understand, because the number of overweight/obese children and adolescents has increased over the past 40 years. More than one-third of U.S. adolescents are overweight or obese (Data Resource Center for Child and Adolescent Health [DRC], 2013). Hurt and colleagues (2014) examined pediatric obesity rates during the Healthy Kids study, which evaluated data from over 10,000 children aged 2-19 years. The prevalence of obesity increased with age, from more than 16% in 2-5 year olds to greater than 25% in 12-19 year olds (Hurt, De Pinto, Watson, Grant, & Gielner, 2014). Logically, more adolescents will be at risk for insulin resistance as they age, and thus, type 2 diabetes and cardiovascular disease.

Diabetes is a major cause of poor health and disability. During the period from 1980 through 2011, the number of Americans diagnosed with diabetes nearly quadrupled (5.6 million, 20.9 million, respectively) (Centers for Disease Control and Prevention, 2014). In 2009, approximately 23,500 persons under 20 years had diabetes (CDC, 2014). In 2012, that figure rose to 208,000, with type 2 diabetes diagnosed in one-third of persons aged 10-19 years (CDC, 2014). The estimated costs of diabetes in 2012 were $176 billion (direct costs) and $69 billion (indirect costs) (CDC, 2014).

Findings from the Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study support intervention prior to the development of type 2 diabetes. Adolescents with type 2 diabetes have more difficulty maintaining glycemic control than adults with type 2 diabetes (Narasimhan & Weinstock, 2014). There are few treatment options and treatment is not effective for many adolescents (Narasimhan & Weinstock, 2014). In this study of nearly 700 adolescents with type 2 diabetes, nearly half of the group developed poor glycemic control within 1 year (Narasimhan & Weinstock, 2014). By year 4 of the study, 1/3 of participants developed hypertension and dyslipidemia, and experienced a decline in beta cell function (Narasimhan & Weinstock, 2014). Sixteen percent experienced a decline in kidney function (Narasimhan & Weinstock, 2014). The atherogenic profile (lipid levels and inflammatory markers [C - reactive protein]) of adolescents with type 2 diabetes was concerning for risk of premature cardiovascular disease (Narasimhan & Weinstock, 2014).

By 2030, more than 40% of the US population is projected to have some form of cardiovascular disease (Go et al., 2013). Key national organizations and institutions, the American Heart Association (AHA), National Heart, Lung and Blood Institute (NHLBI), and the American Academy of Pediatrics (AAP), responded to this projection with endorsements of healthcare guidelines aimed at achievement of improved cardiovascular

health through primary and secondary prevention for children and adolescents (DHHS, 2012). One of these healthcare guidelines is a recommendation for universal lipid screening of all children with elevated BMI, which would accomplish two goals: identification of children with genetic abnormalities of lipid metabolism and detection of children with acquired dyslipidemia (DHHS, 2012). Both groups are at risk for early cardiovascular events.

In conclusion, insulin resistance appears to be the common metabolic process underlying cardiovascular disease, type 2 diabetes, hypertension, and non-alcoholic fatty liver disease (Boden, 2011; Samuel, Petersen, & Shulman, 2010). In order to detect risk of future cardiovascular events, screening for insulin resistance seems prudent. However, in current pediatric practice, screening for insulin resistance is uncommon because the gold standard test, the glucose clamp test, is expensive and involves intravenous infusion of glucose and insulin. Screening for risk of insulin resistance with the Homeostasis Model (HOMA-IR) closely approximates findings from glucose clamp studies, but use in clinical practice is hampered by patient safety issues. Another potential screening test, the triglyceride/ high-density lipid cholesterol ratio (TG/HDL ratio), can be calculated from routine serum laboratory results. What is not known is whether the TG/HDL ratio predicts insulin resistance with sufficient sensitivity and specificity.

Theoretical Framework

Insulin, a major anabolic hormone, is essential for growth and development and the regulation of carbohydrate and fat metabolism (Sesti, 2006). Insulin resistance is the state in which target organs demonstrate decreased responsiveness to normal circulating levels of insulin, and insulin stimulation of many metabolic pathways is diminished (Boden, 2011; Sesti, 2006). Higher than normal levels of insulin are required to achieve

normal biological effects (Mercurio, Carlomagno, Fazio & Fazio, 2012). Insulin resistance plays a crucial role (Figure1.1) in the pathogenesis of type 2 diabetes mellitus, non-alcoholic fatty liver disease, atherogenic dyslipidemia that leads to cardiovascular disease, hypertension, and hematologic abnormalities related to coagulation and fibrinolysis (Boden, 2011; Samuel, Petersen, & Shulman, 2010). In addition, insulin resistance appears to be the pathological process linking obesity (increased adiposity) to dyslipidemia (Kahn, Hull, & Utzschneider, 2006). The metabolic processes related to insulin resistance are not well understood. In Figure 1.1, insulin resistance is depicted as playing a central role in the development of atherosclerotic heart disease and diabetes. Increased adiposity (obesity) is closely linked to altered lipid metabolism and subsequent insulin resistance. The feedback mechanism between increased adiposity and insulin resistance is poorly understood. The TG/HDL ratio may indicate when intensive intervention is necessary.

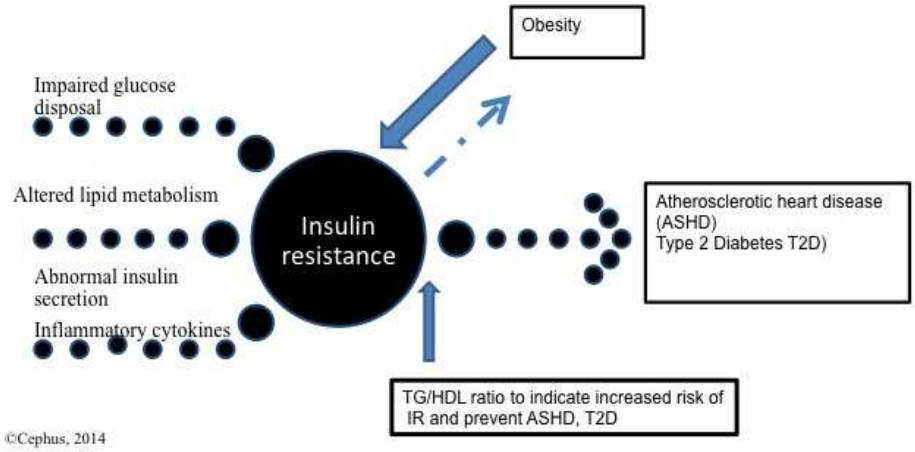

Figure 1.1 Altered metabolism of insulin resistance, link to obesity, ASHD and T2D

The Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in children and adolescents recommends dyslipidemia screening for children,

2-21 years with elevated BMI (DHHS, 2012). Analysis of the fasting blood sample yields quantification of total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol), and triglyceride levels. Elevated total cholesterol and LDL-cholesterol levels indicate increased risk for cardiovascular disease. Additional information may be gleaned from the analysis of this blood sample, at no additional cost.

$$\text{TG/HDL ratio} = \frac{\text{triglycerides (mg/dL)}}{\text{HDL cholesterol (mg/dL)}}$$

In the normal metabolic state, the normal ratio of triglyceride to HDL cholesterol in adolescents varies between 1:1 and 2:1 (NHLBI, 2012). During dysregulation of lipid metabolism, the ratio of triglyceride to HDL cholesterol may reach 20:1. Elevated triglyceride and low HDL-cholesterol levels are related to insulin resistance. The proposition examined in this study was that a TG/HDL-cholesterol ratio greater than a determined level would indicate increased risk for insulin resistance.

The study concepts are defined in the table below (Table 1.1).

Table 1.1 Study concepts

Study concept	Conceptual definitions
Obesity	Increased adiposity
Dyslipidemia	Abnormal lipoprotein (lipid) metabolism associated with elevated triglyceride and low HDL cholesterol levels (DHHS, 2012)
Insulin resistance	Physiological state in which target organs have decreased responsiveness to normal levels of insulin, normoglycemia is maintained with increased circulating insulin (Sesti, 2006)
Cardiovascular disease also known as atherosclerotic heart disease	Heart disease caused by atherosclerotic dyslipidemia (abnormal lipoprotein [lipid] metabolism associated with elevated total cholesterol and low density lipoprotein levels) (DHHS, 2012)
Type 2 diabetes	State of insulin resistance in which the body is no longer able to maintain normoglycemia, hyperglycemia exists despite the presence of insulin (Lieberman, 2013)

Conceptual Map

Insulin resistance occurs decades before evidence of type II diabetes and cardiovascular disease is clear. The mechanisms involved in development of insulin resistance are not well understood. There appears to be a relationship between fat storage, often manifested as overweight or obese status, and a form of dyslipidemia that is characteristic of insulin resistance (Kahn, Hull, & Utzschneider, 2006). Altered regulation of lipid metabolism results in elevated triglyceride levels and diminished HDL cholesterol levels (dyslipidemia). Hence, screening for elevated TG/HDL ratio might predict risk of insulin resistance and allow for intervention prior to development of diabetes and cardiovascular disease (Figure 1.2).

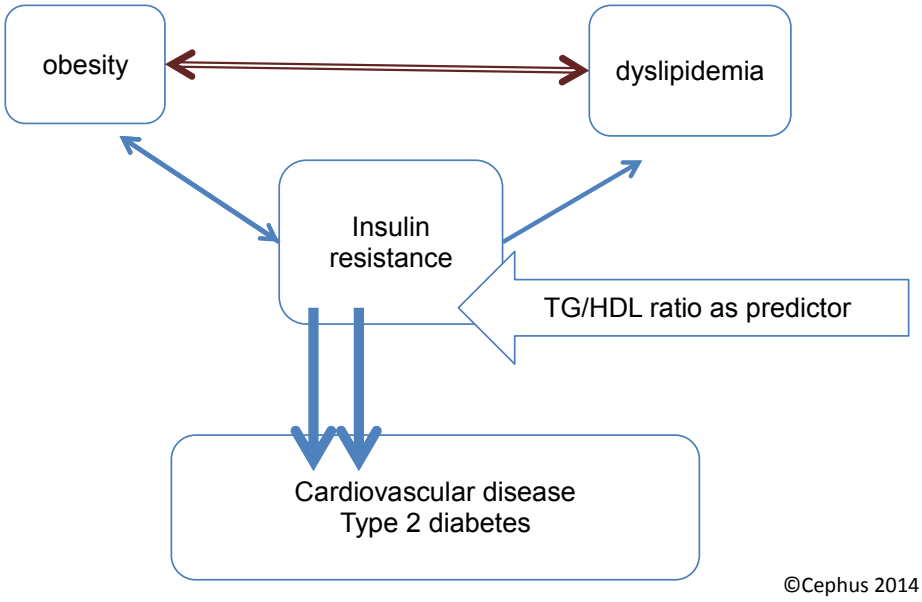

Figure 1.2 TG/HDL ratio as predictor in insulin resistance (IR)

Study Purpose

The purpose of this study was to evaluate the performance characteristics (sensitivity, specificity, positive predictive value and negative predictive value) of the

triglyceride/HDL cholesterol ratio when used as a screening tool to detect increased risk of insulin resistance in the U.S. adolescent population. The study questions were:

1. What was the cut-off value that indicates increased risk of insulin resistance by TG/HDL ratio in U.S. adolescents?
2. Did the cut-off value vary by race/ethnicity, gender, BMI percentile category, early (12-15 yrs.) vs. late (16-19 yrs.) adolescence?
3. What were the performance characteristics (measures of validity) of the TG/HDL ratio as a predictor of risk of insulin resistance?
4. How did performance characteristics of the TG/HDL ratio compare to the performance characteristics of the HOMA-IR model?
5. Did the TG/HDL ratio appear to be an appropriate tool for screening U.S. adolescents for insulin resistance?

Assumptions

The assumptions related to this study were:

1. Data, available from National Health and Nutrition Examination Survey (NHANES) 2011-2012, were correct and accurately reflected individual-level data.
2. The appropriate cut-off value to discriminate between low and high risk for insulin resistance in the adolescent population could be determined.

Summary

Insulin resistance is associated with comorbid conditions, including cardiovascular disease and type 2 diabetes. Obesity appears to increase the risk of developing insulin resistance. Some overweight/obese adolescents are not more insulin resistant than their normal weight peers are. Therefore, increased risk of insulin resistance cannot be presumed based on physical size.

Development of insulin resistance occurs years before diagnosis of type 2 diabetes or cardiovascular disease. Early intervention (lifestyle modification) to decrease insulin resistance may delay or decrease the incidence of type 2 diabetes and cardiovascular disease. Determination of which adolescents are at risk for insulin resistance would assist primary care providers in planning appropriate care. Currently, a screening test for insulin resistance is not in use in pediatric practice. The TG/HDL ratio can be calculated from routine serum laboratory results. What was not known was whether the TG/HDL ratio predicted insulin resistance with sufficient sensitivity and specificity to be useful in the clinical setting.

Chapter 2

Review of Relevant Literature

Primordial and primary prevention of cardiovascular disease and type II diabetes in adolescents is hindered by the lack of reliable, readily available screening methods. Development of insulin resistance occurs prior to development of cardiovascular disease and type 2 diabetes (Lee, Herman, Okumura, Gurney, & Davis, 2006; Shah, Dolan, Gao, Kimball, & Urbina, 2011; Tobisch, Blatniczky, & Barkai, 2013). A screening tool for insulin resistance may help clinicians determine which adolescents need therapy that is more intensive in order to decrease risk of cardiovascular disease and type 2 diabetes. The focus of this chapter was to review screening tool characteristics, current understanding of pathophysiology of insulin resistance, and measurement of insulin resistance. Research studies related to insulin resistance in adults and children are discussed. Two screening methods, used in the pediatric research, are compared to the TG/HDL ratio.

Prevention

Cardiovascular disease and diabetes are leading causes of morbidity and mortality in the U.S. (Weintraub et al., 2011). Primordial prevention of cardiovascular disease in children, as a goal, was introduced by Strausser in 1978 at the World Health Organization in Geneva. He advocated for a strategy to prevent epidemics of the onset of risk factors in whole societies (Strausser, 1980). Under his guidance, the WHO Expert Committee made the following recommendations: reduce salt intake, increase physical activity, avoid obesity, and eliminate smoking (WHO Expert Committee, 1990). Over the years, the American Heart Association, the American Academy of Pediatrics and the National Heart, Lung and Blood Institute have supported primordial and primary prevention of cardiovascular risk factors in children and adolescents (Labarthe, Dai, Day,

Fulton, & Grunbaum, 2009). In the 2012 guidelines, the Expert Committee recommended universal screening of all 9- and 10- year old children for elevated lipid levels (DHHS, 2012). In addition, they recommended screening of all children with elevated BMI for elevated lipid levels (DHHS, 2012).

A lipid panel includes measurement of triglycerides (TG), HDL (high-density lipoprotein) cholesterol, LDL (low-density lipoprotein) cholesterol, and total cholesterol. Additional indices vary with institutional choice. Elevation of the TG/HDL ratio has been proposed as a marker for increased risk of insulin resistance (Quijada, et al., 2008; Weiss, et al., 2011). The Expert Committee's recommendations represent a unique opportunity to screen for insulin resistance, as well as hypercholesterolemia and dyslipidemia.

General Screening Tool Criteria

A screening tool should meet certain criteria: a) address an important health problem, b) address a condition for which the natural history is adequately understood, c) address a condition for which a pre-symptomatic period exists, and d) address a condition for which effective intervention for primary prevention is available (Cochrane & Holland, 1971; Friis & Sellers, 2009; Somerville, Kumaran & Anderson, 2012). Research has supported developing a means of early detection of type 2 diabetes (Harris & Eastman, 2000). Insulin resistance appears to be a condition that develops prior to type 2 diabetes, which is a major cause of morbidity and mortality in the U.S. People with diabetes are at increased risk for cardiovascular events (Kashyap & Defronzo, 2007; Srikanth & Deedwania, 2011). The lifetime risk of cardiovascular disease is 4-6-fold higher in people with preventable risk factors (Berry et al., 2012). Prevention of type 2 diabetes, through intervention at the point of recognition of insulin resistance, has the potential to reduce future cardiovascular events (Srikanth & Deedwania, 2011).

Screening

The purpose of screening is to identify apparently healthy members of the population who may be at increased risk or actually have a clinical condition or disease (Somerville, Kumaran & Anderson, 2012). Ideally, the screening test should be very sensitive, so that persons who have the condition will be discovered. In addition, the screening test should be very specific, so that only persons with the condition will test positive. However, in reality, this is rarely the case. (Gordis, 2009; Somerville, Kumaran & Anderson, 2012). The distribution of test results between healthy and diseased people will overlap, as in figure 2.1.

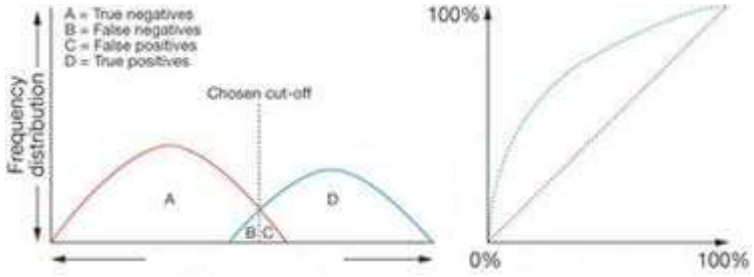

Figure 2.1 Distribution of test results (Adapted with permission. Kulasingam & Diamandis, 2008)

When testing for a condition that has a continuous variable as the result, like TD/HDL ratio, the distribution of results between healthy people and individuals with a specified condition overlap (Gordis, 2009). For any given test threshold, some people who have disease will test positive (true positives) and some people will test negative (false negatives) (Metz, 1978). Some people without the disease will test positive (false positives) and the remainder will test negative (true negatives) (Metz, 1978).

Sensitivity is defined as the ability to identify individuals with disease using a specific test (Friis & Sellers, 2009). The test should be sensitive enough to detect most individuals with disease (Table 2.1). The sensitivity of the test should be high when the

burden of disease is high, for example, cancer. Hofvind and colleagues (2012) reported that mammography has a sensitivity of ~92%. Specificity is defined as the ability to identify individuals without disease using the same test (Friis &Sellers, 2009).

The performance characteristics of a test (sensitivity, specificity, positive and negative predictive values) must be balanced to give the best result for the clinical condition or disease (Friis & Sellers, 2009; Somerville, Kumaran & Anderson, 2012). The positive predictive value of a test is the proportion of true positives among all positive tests in a study population (Somerville, Kumaran & Anderson, 2012). The negative predictive value of a test is the proportion of true negatives among all negative tests (Somerville, Kumaran & Anderson).

The predictive value depends on the prevalence of disease/condition in the population being tested (Grimes & Schulz, 2002). When the prevalence of a condition is low, the negative predictive value is high and the positive predictive value is low (Metz, 1978). In other words, an individual with a negative result is highly likely to be disease-free. An individual with a positive result may or may not have disease.

Table 2.1 Meaning of test characteristic

Test characteristic	Meaning of result
Sensitivity	Ability of the test to identify persons who actually have the condition of interest (Lang & Secic, 2006)
Specificity	Ability of the test to correctly identify persons who do not have the condition of interest (Lang & Secic, 2006)
Positive predictive value	Indicates if a person has a positive test, the probability of actually having the condition of interest (Friis & Sellers, 2009)
Negative predictive value	Indicates if a person has a negative test, the probability of not having the condition of interest (Friis & Sellers, 2009)
Accuracy	The degree of agreement between the screening test and the gold standard (Friis & Sellers, 2009)

For the individual clinician, knowledge of the prevalence of condition in the population served will assist in appropriate interpretation of test results (Table 2.1)

(Grimes & Schulz, 2002; Leeflang, et al., 2013). In a study of rapid antigen screening for streptococcal pharyngeal infections, the positive predictive value was 90.3% for children (0-9 years of age) and 70.4% for adults (Gurol et al., 2010). The positive predictive value is poor when the prevalence of the condition in the population is low. Even a test with high specificity will have more false positive results than true positive results when the prevalence of disease is less than 10 % (Grimes & Schulz, 2002).

Glucose and Lipid Metabolism

Energy homeostasis is a coordinated system of processes that is necessary for survival: a) fuel intake and storage, and b) fuel mobilization and expenditure (Wang & Fotsch, 2006). These metabolic processes depend on the coordinated control of hormones (Lieberman, Marks & Peet, 2013; Wang & Fotsch, 2006). Insulin is involved in glucose and fatty acid metabolism. In general, when insulin connects with cellular insulin receptors, glucose, amino acids and free fatty acids are taken into the cell (Saltiel & Kahn, 2001). Insulin promotes synthesis of lipids, proteins and glycogen (Saltiel & Kahn, 2001).

In the fed state, skeletal muscles metabolize glucose for immediate use as fuel, or for storage as glycogen (Saltiel & Kahn, 2001; Sesti, 2006). The Randle cycle theory postulates that in the presence of insulin, muscle glycogen synthesis is a major pathway for glucose metabolism (Randle, Garland, Hales, & Newsholme, 1963; Shulman, 2000). Plasma glucose enters the cell via glucose transporters and is phosphorylated by hexokinase II to form glucose-6-phospate (Beck-Nielsen, 2012; Shulman, 2000). Glucose-6-phosphate interacts with uridine diphosphate and glycogen synthase to form glycogen (Shulman, 2000). During exercise, muscle cells quickly deplete local glycogen stores and rely on fatty acids from the blood (Lieberman et al., 2013). In the fasting state, lipolysis provides skeletal muscle, the liver and other organs with fatty acids for fuel (Hue

& Taegtmeyer, 2009). Glucose is reserved for the brain, which is completely dependent upon glucose for cellular respiration (Hue & Taegtmeyer, 2009).

Fatty acid oxidation is a major source of energy for skeletal muscles (Lieberman et al., 2013). Triacylglycerols (triglycerides) and cholesterol, the major dietary lipids, are digested in the intestinal lumen, packaged in chylomicrons, and secreted into the blood stream via the lymph system (Klop, Elte, & Cabezas, 2013; Lieberman et al., 2013). The liver synthesizes glycogen and triglycerides from glucose (Lieberman et al., 2013). In an environment that is insulin-rich, the hepatocyte, through a series of phosphorylations, converts glucose to pyruvate (Saltiel & Kahn, 2001). Pyruvate enters the mitochondria and is converted to acetyl coenzyme A (acetyl-CoA) and oxaloacetate (Lieberman et al., 2013). In order to exit the mitochondria, acetyl-CoA molecules merge with oxaloacetate to form citrate (Lieberman et al., 2013). In the cytosol, acetyl-CoA is separated from oxaloacetate and used for fatty acid and cholesterol synthesis through pathways activated by insulin (Lieberman et al., 2013). Newly formed fatty acids are converted to triglycerols (triglycerides), which are packaged inside very low-density lipoproteins (VLDL) and secreted into the blood stream (Franssen, Monajemi, Lieberman et al., 2013; Stroes & Kastelein, 2011). Chylomicrons and VLDL transport free fatty acids to skeletal muscle, heart and adipose tissue (Klop et al., 2013). Insulin up-regulates the action of lipoprotein lipase, an endothelial cell enzyme, which results in release of triglycerides and breakdown to fatty acids (Klop et al., 2013). Free fatty acids are available as an energy source, or are taken up by adipose tissue, where they are stored as triglycerides (Hue & Taegtmeyer, 2009; Lieberman et al., 2013).

Insulin stimulates the transport of glucose into adipose tissue where adipocytes utilize glucose for energy and as a source of the glycerol component necessary for triglyceride formation (Lieberman et al., 2013). In addition, insulin stimulates production

and secretion of lipoprotein lipase in adipocytes, to enhance metabolism of triglycerides to fatty acids (Klop et al., 2013; Lieberman et al., 2013). Insulin is the most important regulator of fatty acid mobilization from adipose tissue in the fasting state (Klop et al., 2013). In a normal metabolic state, insulin mediates utilization and storage of glucose and lipids.

Insulin resistance has a major impact on metabolism of free fatty acids, chylomicrons, VLDL, and glucose (Hue & Taegtmeyer, 2009; Klop et al., 2013). In skeletal muscles, insulin resistance correlates with decreased insulin-stimulated glucose uptake (Capurso & Capurso, 2012). In the presence of insulin resistance, hepatocyte glucose production is dysregulated, and insulin-mediated adipocyte lipolysis is diminished (Capurso & Capurso, 2012). The pathogenesis of insulin resistance is complex and not well understood.

Theoretical Mechanisms of Insulin Resistance

Insulin resistance can be defined as a state in which target organs display reduced responsiveness to normal levels of circulating insulin (Lieberman et al., 2013). Traditionally, insulin resistance is defined as an insulin-glucose interaction, a glucocentric paradigm (Savage, Petersen & Shulman, 2005). Over the past 15 years, the role of insulin-fatty acid interaction in development of insulin resistance, a lipocentric process, has been recognized (Samuel, Petersen, &Shulman, 2010). Experimental research supports both mechanisms, but neither completely explains the pathogenesis of insulin resistance.

The Glucocentric Paradigm

Fructose and Insulin Resistance

High fructose corn syrup (HFCS) and sucrose have been the predominant sweeteners in most drinks and foods in the U.S. since the 1970s (Johnson & Murray,

2010). Prior to the development of the sugar industry, human consumption of fructose was limited (Johnson & Murray, 2010). In 1850, the average American consumed twenty pounds of sugar per year (Bray, 2012). By 2001, the average American consumed more than 160 pounds of sugar per year (Bray, 2012). Consumption of fructose, in the form of HFCS and sucrose, has been associated with altered lipid metabolism as demonstrated by increased hepatic lipid synthesis, reduced lipid clearance, and increased volume of intraabdominal fat depots (Stanhope, Griffen et al., 2008). Biomarkers of increased risk of cardiovascular disease (apolipoprotein B, LDL cholesterol, and small, dense LDL cholesterol) were also increased (Stanhope, Griffen et al., 2008).

Epidemiological studies have demonstrated a relationship between total fructose intake and higher rates of obesity, hypertension, kidney disease, metabolic syndrome and cardiovascular disease in adults (Bray, 2012; Elliott, Keim, Stern, Teff & Havel, 2002; Johnson, Segal et al., 2007). Consumption of sugar-sweetened drinks was associated with increased insulin resistance by the Homeostasis Assessment Model – Insulin Resistance (HOMA-IR) in participants in the Framingham Offspring Cohort study (Yoshida et al., 2007). Clinical studies have demonstrated that fructose dietary supplementation results in dyslipidemia, hypertension, and insulin resistance in adults (Stanhope, Schwarz et al., 2009). In a prospective, observational study of school age children, Ludwig and colleagues (2001) found the odds of normal-weight children becoming obese increased 1.6 times for each sugar-sweetened drink (can/glass) consumed per day.

The metabolism of fructose is different from other sugars. Fructose is phosphorylated rapidly, causing depletion of hepatic cellular adenosine triphosphate (ATP) (Johnson & Murray, 2010). In contrast to glucose metabolism, hepatic metabolism of fructose continues despite the absence of ATP, resulting in increased lipogenesis and

elevated triglyceride levels (Stanhope, Schwarz et al., 2009). Additional sequelae include decreased hepatic insulin sensitivity, increased hepatic glucose production, and increased insulin secretion (Stanhope, Schwarz et al., 2009). Laboratory data demonstrate characteristics of insulin resistance: impaired glucose tolerance, increased fasting glucose and increased fasting insulin (Stanhope, Schwarz et al., 2009). Fructose consumption appears to have a role in the development of obesity and insulin resistance.

Adiposity and Insulin Resistance

Obesity is associated with insulin resistance, impaired glucose metabolism, atherogenic dyslipidemia and other pathophysiological conditions (Capurso & Capurso, 2012, Kashyap & DeFronzo, 2012). For decades, insulin resistance in skeletal muscle and liver has been recognized as a physiologic feature of type 2 diabetes, obesity, atherogenic dyslipidemia and other diseases associated with increased cardiovascular risk (Boden, 2011). In the past, insulin resistance was defined within a glucocentric paradigm, where elevated circulating insulin was needed for euglycemia (Eckel, Grundy, & Zimmet, 2005). However, insulin resistance in adipose tissue appears to be the most important consequence of increased adiposity (Capurso & Capurso, 2012). The role of adipose tissue was elucidated only recently, but the cellular mechanisms are not well understood (Har, Carey, & Hawkins, 2013; Tateya, Kim, & Tamori, 2013).

The Glucose-Fatty Acid Cycle

In 1963, Randle and colleagues proposed an explanation of fatty-acid role in glucose metabolism – the glucose fatty-acid cycle. They theorized that fatty acid and ketone bodies are necessary for conservation of blood glucose and muscle glycogen during periods of starvation or dietary restriction of carbohydrates (Randle et al., 1963). They proposed that high rates of breakdown of glycerides in adipose tissue and muscle result in elevated plasma non-esterified fatty acids and accumulation of intracellular

glucose (Randle et al., 1963, Roden et al., 1996). The availability of fatty acids as a fuel source impairs glucose utilization through inhibition of glucose metabolism at specific enzymatic checkpoints (Randle et al., 1963; Samuel et al., 2010).

The Lipocentric Paradigm

The Inflammation Hypothesis

Adipose tissue is an organ that secretes biologically active substances that regulate insulin sensitivity in insulin-sensitive organs including the liver and skeletal muscles (Tateya et al., 2013). Coletta and Mandarino (2011) report sources of inflammation that result in insulin resistance in myocytes. Adipose tissue is a major source of cytokines that stimulate the inflammatory process in skeletal muscle (Coletta & Mandarino, 2011). Proinflammatory cytokines activate toll-like receptors in the myocytes cell wall that induce expression of proinflammatory genes (Coletta & Mandarino, 2011). In addition, elevated circulating free fatty acids increase inflammatory cytokines and activate proinflammatory signaling in myocytes (Coletta & Mandarino, 2011; Pan et al., 1997). Coletta and Mandarino (2011) propose a theoretical model that delineates the relationship between inflammation and insulin resistance in skeletal muscle: inflammation, mitochondrial changes, lipid accumulation, insulin-signaling defects, and finally insulin resistance.

Accumulation of subcutaneous fat is a normal physiologic activity that occurs when energy intake exceeds energy output (Goldenberg & Bathina, 2013). Once subcutaneous fat depots are full, fat is stored in visceral adipocytes (Goldenberg & Bathina, 2013). Extreme adipose hypertrophy and visceral fat storage result in adipocyte dysfunction (Capurso &Capurso, 2012; Goldenberg & Bathina, 2013). Hypertrophied adipocytes release pro-inflammatory cytokines, various interleukins, and tumor necrosis factor alpha (TNF-α) (Capurso & Capurso, 2012). TNF-α stimulates the release of

monocyte chemotactic protein-1 (MCP-1) from endothelial cells and pre-adipocytes located in adipose tissue. MCP-1 attracts macrophages to the site of hypertrophied adipocytes (Capurso & Capurso, 2012). Macrophages induce release of inflammatory cytokines, TNF-α, interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), which activate serine kinases (Har et al., 2013). Serine kinases promote serine phosphorylation of insulin receptor substrate -1 (IRS-1) instead of tyrosine kinase phosphorylation (Arner, 2003). The result is inability of the insulin receptor to bind with insulin and signaling defects that result in insulin resistance (Coletta & Mandarino, 2011). Chronic inflammation at the level of the adipocyte leads to insulin resistance (Goldenberg & Bathina, 2013; Xu et al., 2003). Interestingly, obese people express more than twice the TNF-α messenger ribonucleic acid (mRNA) and protein in adipose tissue than lean people express (Kashyap & DeFronzo, 2007; Schipper, Prakken, Kalkhoven, & Boes, 2012).

The Adipose Tissue Expandability Hypothesis

On a population level, there is a strong correlation between obesity and diabetes (Virtue & Vidal-Puig, 2008). Insulin resistance occurs about 10–20 years prior to the development of diabetes (D'Adamo & Caprio, 2011). Although obesity and insulin resistance are associated positively on a population level, on the individual level, the relationship remains uncertain (Kim et al., 2007; Virtue & Vidal-Puig, 2008).

Kim and colleagues (2007) noted that insulin resistance was a characteristic of lipodystrophic and lipoatrophic mouse models. The common feature of the mouse models, absence of functional adipocytes, resulted in deposition of triglycerides in skeletal muscle and the liver, dyslipidemia, hyperglycemia and hyperinsulinemia (Kim et al., 2007). Experimental findings mirrored the insulin resistance phenomenon observed in humans. Kim and colleagues (2007) postulated that insulin resistance could be avoided if excessive lipids did not accumulate in the liver or skeletal muscle. They

engineered a mouse model (transgenic ob/ob) that allowed for uninhibited adipose tissue growth (Kim et al., 2007). Transgenic ob/ob mice grew to nearly twice the size of their ob/ob littermates but had lower metabolic indices (Kim et al., 2007). Laboratory findings of transgenic ob/ob mice demonstrated improvement of inflammatory indices, and normal fasting glucose, insulin, triglycerides (TG) and free fatty acids (Kim et al., 2007). These findings supported the hypothesis that insulin resistance occurs because of lipid-induced toxicity after deposition of triglycerides and fatty acids in depots other than adipose tissue (Virtue & Vidal-Puig, 2008).

Uninhibited adipose tissue growth may be one key to avoiding metabolic changes associated with insulin resistance (Kim et al., 2007; Virtue & Vidal-Puig, 2008). On a clinical level, one-third of patients with severe obesity do not have metabolic derangements associated with insulin resistance (Bays et al., 2013). Cali and Caprio (2009) found that obese adolescents had a more favorable metabolic profile when they retained subcutaneous fat. Experimental human studies have shown significant interindividual variability in fat deposition during overfeeding (Alligier et al., 2013; Cali, Zern et al., 2007). The mechanism that controls location of fat deposition is not understood.

Insulin Resistance and Adolescence

Insulin resistance during puberty has been documented in cross-sectional studies. Amiel and colleagues (1986) were the first to demonstrate the association between puberty and insulin resistance in adolescents with insulin-dependent diabetes. Later studies have shown that insulin sensitivity decreases, in non-diabetic children, during puberty (Amiel, et al., 1991; Bloch, Clemmons, & Sperling, 1987; Caprio et al., 1989). Attempts to determine associations between sexual maturation (Tanner stage, see Table 2.2) and degree of insulin resistance have resulted in conflicting conclusions.

One study suggested that the puberty-related increase in insulin resistance occurs between Tanner stages I and II (Table 2.2) (Cook, Hoffman, Stene, & Hansen, 1993). In contrast, Travers, Jeffers, Bloch, Hill, and Eckel (1995) found vast variability between Tanner stage and insulin resistance. Instead, they suggested that body fatness, assessed as body mass index (BMI), correlates best with degree of insulin resistance (Travers et al., 1995).

Table 2.2 Tanner stages (Marshall & Tanner, 1969; 1970)

Tanner stage	Physical
I	Prepubertal
II	Enlargement of scrotum and testes, changes in skin texture Breast bud stage with enlargement of areola Sparse growth of hair at the base of the penis or along labia
III	Enlargement of the penis in length Further enlargement of breast and areola Darker, coarser, more curled hair covering pubis
IV	Increased penile size with development of glans, scrotal skin darkens, testes and scrotum larger Areola and papilla form secondary mound above the level of the breast Adult-type pubic hair, but covering smaller area than adult
V	Adult genitalia Recession of areola Full distribution of pubic hair

Moran and colleagues (1999) performed euglycemic clamp studies on 357 children, aged 10-14 years, to investigate the effect of BMI, gender, Tanner stage and ethnicity on insulin resistance. They found a curvilinear relationship between insulin resistance and Tanner stage. The highest level of insulin resistance was documented in children at Tanner stage III, regardless of gender (Moran et al., 1999). Insulin resistance was more than 25% higher in children who were in Tanner stage II, when compared to children in stage I (Moran et al., 1999). There was a strong correlation between BMI and insulin resistance at all Tanner stages (Moran et al., 1999).

Longitudinal studies confirmed a curvilinear relationship between insulin resistance and Tanner stages, and a significant change in insulin resistance between Tanner stage I and stage II (Ball et al., 2005; Goran & Gower, 2001). An increase in insulin secretion results in maintenance of glucose homeostasis (Ball et al., 2005). Hannon and colleagues (2006) found that there was a 125% increase in the ratio of fatty acid to glucose oxidation, supporting the Randle cycle theory. The findings of the above-mentioned studies are difficult to generalize secondary to small sample sizes, lack of diversity in the participants, and few attempts to reproduce findings. In addition, physical changes associated with puberty are the result of hypothalamic-pituitary interaction, which occurs before physical signs of puberty appear (Ganong, 2005). Determining the onset of puberty by Tanner staging may introduce an erroneous assumption.

Jeffery and colleagues (2012) conducted a prospective, longitudinal cohort study, which included 307 age-matched healthy children from random schools and varied socioeconomic backgrounds. Using luteinizing hormone (LH) as an indicator of metabolic onset of puberty, they found evidence of insulin resistance 2-3 years before the onset of puberty (Jeffery et al., 2012). Adiposity accounted for 25% and 30% of the variance in insulin resistance in boys and girls, respectively (Jeffery et al., 2012).

Insulin resistance has been associated with elevated visceral and ectopic adiposity in prepubertal obese children and adolescents (Bennett, 2012; Cali & Caprio, 2009). Children and adolescents with the highest proportion of visceral fat were greater than 5 times more likely to have metabolic syndrome than their peers with lower visceral fat (Cali & Caprio, 2009). In a retrospective cohort study involving 441 pre- and early pubertal children, intramyocellular lipid deposition was associated with markers of insulin resistance: elevated serum triglyceride level and triglyceride/high density lipoprotein-

cholesterol ratio (Brumbaugh, Crume, Nadeau, Scherzinger, & Dabelea, 2012). Assessment of intramyocellular lipid deposition is not feasible in the clinical setting.

In summary, insulin resistance results in dysregulated hepatic gluconeogenesis and adipocyte lipolysis, which increases circulating triglycerides and cholesterol. Insulin resistance appears to be the link between obesity and dyslipidemia. Other disease conditions such as non-alcoholic fatty liver disease, hypertension, type 2 diabetes mellitus, and cardiovascular disease appear to be outcomes of this interaction. The pathways of development of insulin resistance remain unclear.

Measurement of Insulin Resistance

Detection of insulin resistance, a risk factor for development of cardiovascular disease and type 2 diabetes, appears pivotal for prevention of morbidity and mortality. The gold standard methods for determination of insulin sensitivity, the hyperinsulinemic-euglycemic clamp technique (glucose clamp test) and the frequently sampled intravenous glucose tolerance test are impractical for use during routine pediatric visits (Giannini et al., 2011). Researchers have worked to develop invasive and non-invasive methods for determination of insulin resistance.

The Hypertriglyceridemic Waist

Abdominal fat is thought to contribute to risk for cardiovascular disease (Janssen, Katzmarzyk, & Ross, 2004). Abdominal fat may represent subcutaneous adiposity or visceral adiposity. Visceral adipose tissue is believed to be metabolically active in the process of insulin resistance (Despres, 2006, Capurso & Capurso, 2012; Goldenberg & Bathina, 2013).

Lemieux and colleagues (2000) proposed a screening method to identify men with increased risk for coronary artery disease secondary to the presence of characteristics associated with metabolic syndrome. The model, named

Hypertriglyceridemic Waist, focused on increased waist circumference and elevated triglyceride levels (Lemieux, Pascot et al., 2000). Their study findings suggested that obese men with elevated triglyceride levels and waist circumference > 90 cm were at increased risk for coronary artery disease (odds ratio, $p<0.03$) (Lemieux, Pascot et al., 2000). Over the next 10 years, several studies supported the use of the Hypertriglyceridemic Waist model to identify at-risk men and women (Blackburn et al., 2012; Khan & Valdez, 2003; LaMonte et al., 2003; Lemieux, Poirier et al., 2007; Tanko et al., 2005). None of the researchers, however, applied this model to children and adolescents.

Development of percentile curves

Fernandez, Redden, Pietrobelli and Allison (2004) investigated the distribution of waist circumference in children and adolescents. They used the Third National Health and Nutrition Examination Survey (NHANES III) data to determine age-, gender-, and ethnicity-specific waist circumference percentiles (Fernandez et al., 2004). Data from nearly 1000 children, aged 2-18 years were included in this study (Fernandez et al., 2004). Fernandez and colleagues (2004) recommended increased practitioner attention to children/adolescents with waist circumference greater than the 75th percentile for age.

Limitations of this study include the use of data that may have had limited clinical relevancy. NHANES III was conducted from 1988-1994 (Center for Disease Control and Prevention (CDC), 2013). The prevalence of obesity was 10% during the NHANES III data collection period (Ogden & Carroll, 2010). By 2004, the prevalence of obesity was 17.1% (Ogden & Carroll, 2010). The inclusion of only three ethnic groups (Hispanic, non-Hispanic White, non-Hispanic Black) represents another limitation, as many clinical practices serve clients that are of Middle-Eastern, Asian, American Indian, or Pacific descent.

Cole and colleagues (2000) constructed international centile curves for body mass index (BMI) using data from six countries. The centile curves were linked to adult reference points: 25 kg/m2 (overweight) and 30 kg/m2 (obese) (Cole, Bellizzi, Flegal & Dietz, 2000). In an attempt to make BMI centile curves more useful to predict health risk in children, Katzmarzyk and colleagues (2004) used data from the Bogalusa Heart study to construct waist circumference and BMI cutoff values for children 5-18 years of age. Katzmarzyk centile curves appeared to be more sensitive, but less specific than Fernandez and Cole curves, when used to evaluate cardiovascular health in children (Katzmarzyk et al., 2004).

More recently, Cook, Auinger and Huang (2009) developed percentile curves for waist circumference and lipid indices after combining data from major U.S. childhood studies and surveys. Researchers found that BMI and waist circumference were closely correlated, suggesting that measuring waist circumference in the clinical setting is more confirmatory than diagnostic for insulin resistance (Cook, Auinger, & Huang, 2009, Khoury, M. et al., 2012). Of note, percentile curves for lipid indices did not differ significantly from those developed with NHANES III, supporting continued use of NHANES III standards (Cook, Auinger, & Huang, 2009).

Use of waist circumference in studies

The utility of waist circumference percentiles in clinical practice is unclear. Data from 1647 adolescents (12-18 years) from the NHANES 1999-2002 study were analyzed for the purpose of using waist circumference to predict insulin resistance (Lee, Davis, Woolford, & Gurney, 2009). When used to identify adolescents with insulin resistance, Fernandez waist percentiles discriminated at the 75th and 90th percentiles (Lee et al., 2009). Importantly, study results suggested that no difference existed between

ethnicities. Therefore, different waist circumference cutoffs, based on ethnicity, were unnecessary.

Spolidoro and colleagues (2013) investigated the importance of waist circumference in monitoring children for metabolic syndrome. Metabolic syndrome was defined by de Ferranti criteria: 1) triglycerides > 100 mg/dL, 2) HDL cholesterol <50 mg/dL (female), <45 mg/dL (male), 3) fasting serum glucose > 110mg/dL, 4) waist circumference > 75th percentile for age and gender, and 5) systolic blood pressure > 90th percentile for age, gender and height (de Ferranti et al., 2004, Spolidoro, 2013). Waist circumference cutoff points were defined by Katzmarzyk curves. In study participants who were diagnosed with metabolic syndrome (n=14/159), waist circumference correlated with diagnosis more than BMI correlated (Spolidoro et al., 2013). The relationship between waist circumference and individual criteria for metabolic syndrome was not reported.

Waist circumference percentiles may be useful for epidemiological studies, but the value of waist circumference measurement in day-to-day clinical practice may be limited. Visceral adiposity appears to be associated with insulin resistance (Weiss, Dufour et al., 2003). Waist circumference measurement does not discriminate between subcutaneous and visceral adiposity.

Hypertriglyceridemic Waist in children

M. da Conceicao-Machado and colleagues (2013) investigated the prevalence of The Hypertriglyceridemic Waist phenotype in Brazilian children. Approximately 7% of participants (77/1076) had data that were characteristic of The Hypertriglyceridemic Waist phenotype (da Conceicao et al., 2013). Participants with The Hypertriglyceridemic Waist phenotype were more likely to have low HDL cholesterol levels (OR = 2.7, 95% CI: 1.5-4.8) (da Conceicao et al., 2013).

In a study conducted by Hobkirk and colleagues (2013), the triglyceride level, but not The Hypertriglyceridemic Waist, was an independent predictor of favorable change in risk for insulin resistance after weight loss. Cali, Zern, and colleagues (2007) found support for triglyceride level as a predictor of fatty liver disease in a study of insulin resistant adolescents. No known pediatric studies have supported the use of The Hypertriglyceridemic Waist to predict insulin resistance in children and adolescents.

Invasive techniques

Glucose Clamp Technique

The glucose clamp technique, developed by DeFronzo, Tobin and Andres, is the gold standard for determining insulin resistance in humans (Muniyappa, Lee, Chen & Quon, 2007). The glucose clamp technique is a direct measurement of beta-cell sensitivity to glucose (DeFronzo, Tobin, & Andres, 1979). In brief, the glucose clamp technique involves an intravenous infusion of glucose to maintain steady-state serum glucose (DeFronzo, Tobin & Andres, 1979). Serum insulin is acutely raised and maintained by continuous intravenous infusion, so that a state of euglycemia occurs (DeFronzo, Tobin & Andres, 1979).

When hepatic glucose production is suppressed, then the glucose infusion rate is equal to total body glucose disposal (DeFronzo, et al., 1979). An insulin sensitivity index is determined from the following equation: SIclamp = M/(G X I), where glucose disposal (M) (normalized for body weight or fat-free mass) is divided by steady state glucose concentration (G) multiplied by the change in insulin concentration (I) (fasting and steady state) (Muniyappa et al., 2007). Multiple serum samples are necessary to complete this direct measure of insulin sensitivity (DeFronzo, Tobin & Andres, 1979). Insulin resistant persons require higher insulin infusion rates than persons with normal insulin sensitivity (Muniyappa et al., 2007).

The glucose clamp technique is not practical for clinical use. It is expensive because of the requirement for intravenous lines, fluids, blood samples and laboratory analyses. It is time, labor, and knowledge intensive, as an experienced technician would be required to ensure patient safety and accurate test performance (Muniyappa et al., 2007). In addition, the patient would be subjected to risk and pain associated with multiple venipunctures, arterial line placement, or large-bore venous line placement. Nonetheless, the glucose clamp technique remains the standard by which other assessments of insulin resistance are judged.

Homeostasis Assessment Model (HOMA)

Hosker and colleagues (1985) developed a computer-derived model of insulin/glucose interaction during continuous infusion of glucose (CIGMA). They used this model to predict mean glucose and insulin levels for different degrees of insulin resistance and beta cell function (Hosker et al., 1985). The model estimation of insulin resistance correlated with glucose clamp estimates in normal subjects ($Rs = 0.75$, $p<0.02$), diabetic participants ($Rs = 0.82$, $p<0.005$) and both groups ($Rs = 0.78$, $p<0.0001$) (Hosker et al., 1985).

CIGMA required a one-hour infusion of glucose (Hosker et al., 1985). Serum glucose and insulin levels were measured three times during the last 15 minutes of the glucose infusion (Hosker et al., 1985). Mean insulin and glucose levels were compared with a standardized chart of values that could be expected in varying degrees of insulin resistance (Hosker et al., 1985). Although test results were comparable to the glucose clamp technique, CIGMA was time intensive and expensive.

Matthews and colleagues (1985) sought to determine if participant fasting plasma glucose and insulin levels predicted insulin resistance using the CIGMA generated chart – homeostasis model assessment (HOMA). Insulin resistance was calculated as the

result of the following equation: fasting serum insulin (μU/ml) X fasting plasma glucose (mmol/L) divided by a constant 22.5 (Matthews et al., 1985). The constant 22.5 is the product of normal fasting glucose and insulin in a healthy individual (4.5mmol/L X 5 μU/ml) (Muniyappa et al., 2007). Hence, an individual with normal insulin sensitivity would be expected to have a HOMA-IR = 1 (Matthews, et al., 1985). HOMA estimate of insulin resistance (HOMA-IR) correlated with the glucose clamp technique in normal and diabetic participants ($Rs = 0.88$, $p<0.0001$) (Matthews et al., 1985).

HOMA-IR is a simple and inexpensive alternative to glucose clamp technique, but it was developed with a small sample size. Bonora and colleagues (2000) investigated the utility of HOMA-IR in large-scale studies. There was a strong correlation between glucose clamp technique designation of insulin resistance and HOMA-IR scores of insulin resistance in adult participants (Bonora et al., 2000). They concluded that HOMA-IR could be reliably used in large-scale studies, but the study sample size was 115 adults (Bonora et al., 2000). Abbasi and Reaven (2011) confirmed the findings in a study of nearly 500 adults: HOMA-IR was a surrogate for insulin action.

Haffner, Miettinen and Stern (1997) suggested that HOMA-IR scores should be considered relative rather than absolute. In a group of middle-aged, obese, non-Hispanic whites and Mexican Americans, they found the normal HOMA-IR to be 2.1 and 2.7, respectively (Haffner, Miettinen, & Stern, 1997). Further, Muniyappa and colleagues (2007) suggested that in the presence of poor or absent β-cell function, or other insulin related conditions, HOMA-IR might not give appropriate results.

HOMA-IR in pediatrics

Investigations of insulin resistance in pediatric studies are scarce. Gungor, Saad, Janosky and Arslanian (2004) compared insulin sensitivity in 156 bi-racial children (age 10 – 19 years) using glucose clamp and simple estimates. HOMA insulin sensitivity

(HOMA-IS), the reciprocal of HOMA-IR, was highly correlated with glucose clamp studies ($r2 = 0.82$, $p<0.001$). Conwell, Brown, Trost and Batch (2004) and Schwartz and colleagues (2008) obtained similar findings with sample sizes of 18 and 323 early adolescents, respectively.

In contrast, Uwaifo and colleagues (2002) reported moderate correlation ($r = -51$, $p<0.05$) between HOMA-IR score and clamp studies completed in 31 bi-racial children (age 6-11 years). Brandou, Brun and Mercier (2005) concluded that HOMA-IR might have limited value for screening in youth with predisposition for diabetes. Yeckel and colleagues (2004) found that HOMA-IR was well correlated with clamp studies, ($r = -0.57$, $p = <0.005$) in an obese group of normal and impaired glucose tolerant youths (age 8-18 years).

Nguyen and colleagues (2010) investigated the utility of HOMA-IR for prediction of pre-diabetes and diabetes in the community-based study known as the Bogalusa Heart Study. Over 1100 bi-racial participants were included in this retrospective study of five cross-sectional surveys from 1981-2000 (Nguyen et al., 2010). Fasting glucose, insulin and HOMA-IR were examined to determine persistence in the highest level of the distribution over the 17-year period (Nguyen et al., 2010). Participants were divided into10 groups, according to blood levels. Children who remained in the top 10% through the 17-year period were 5.8 times more likely to develop diabetes than peers with lower blood levels of glucose and insulin ($p < 0.0001$) (Nguyen et al., 2010). Children in the top 10% who did not develop diabetes were 2.5 times more likely to develop pre-diabetes than peers in the lower percentiles ($p<0.05$) (Nguyen et al., 2010). Morrison and colleagues (2009) found elevated HOMA-IR at 9-10 years of age persisted at 18-19 years of age. These study findings support screening for insulin resistance in children.

Triglyceride/High-Density Lipoprotein Cholesterol Ratio

Yip, Facchini and Reaven (1998) identified insulin resistance as a predictor of cardiovascular disease in one of five healthy, non-obese adults. The National Cholesterol Education Program (NCEP) (2001) designated clinical criteria for the diagnosis of metabolic syndrome (insulin resistance syndrome) which were linked to cardiovascular disease in adults. NCEP's Adult Treatment Panel III (ATP III) included three or more of the following conditions: waist circumference >102 cm in men and >88cm in women, blood pressure > 130/85 or controlled hypertension, fasting glucose >110mg/dL, fasting triglyceride level > 150mg/dL, fasting high density lipoprotein-cholesterol <40mg/dL in men and <50 in women (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults, 2001). McLaughlin and colleagues (2003) identified the triglyceride/high density lipoprotein-cholesterol ratio (TG/HDL ratio) as a metabolic marker for insulin resistance in adults.

Overweight participants with a TG/HDL ratio > 3 were two times more likely to be insulin resistant than their overweight peers with TG/HDL ratio <3 (McLaughlin et al., 2003). TG/HDL ratio was the best surrogate of insulin resistance in a retrospective cohort study of more than three thousand adult participants (Kannel et al., 2008). TG/HDL ratio and the log (TG/HDL ratio) have been correlated with increased coronary risk (Hermans, Ahn & Rousseau, 2012; Marotta, Russo, & Ferrara, 2010).

Ethnicity and TG/HDL ratio

Standard criteria for determination of insulin resistance using the TG/HDL ratio remain controversial. In a group of mostly overweight (98/125) African American adults, TG/HDL ratio was a poor discriminator of insulin resistance (Sumner, Finley, Genovese, Criqui, & Boston, 2005). Fasting insulin was a better predictor of insulin resistance than ATP III or TG/HDL ratio (Sumner, Finley, et al., 2005). Kim-Dorner and colleagues

(2010) suggested that lipid criteria appeared inadequate for use in predicting insulin resistance in African Americans. Analyses from the Jackson Heart Study suggested that the TG/HDL ratio should not be used to screen African American women for insulin resistance because of poor sensitivity and specificity (Sumner, Harman et al., 2010).

In the Northern Manhattan Study, the TG/HDL ratio was considerably higher in Hispanics (3.9) than non-Hispanic Whites (3.3) and Blacks (2.6) (Willey et al., 2011). Hadaegh and colleagues (2009) found that the prevalence of insulin resistance in Iranian men was 63% if the TG/HDL ratio >6.9 and 15% if the TG/HDL <4.4. Chiang, Lai, Chang, and Koo (2011) were unable to determine an appropriate TG/HDL ratio cut-off value for insulin resistance during their study of 800 Taiwanese adults. Gasevic, Frohlich, Mancini and Lear (2012) found that the TG/HDL ratio cut-off values were lower for Aboriginals (0.9), Chinese (1.1) and European (1.1) participants than reported for other ethnicities (2 or 3). They suggested that use of TG/HDL ratio for indication of insulin resistance in South Asians should be avoided (Gasevic et al., 2012).

TG/HDL ratio in pediatric studies

McLaughlin and colleagues (2003) determined the optimal TG/HDL ratio cut-point of 3 was a predictor of insulin resistance in adults. Results from early studies, conducted in adolescents, confirmed that overweight or obese children were more likely to have TG/HDL ratio >3 (Hannon, Bacha, Lee, Janosky, & Arslanian, 2006; Quijada et al., 2008). Further, a TG/HDL ratio approximating 2.5, measured in late adolescence, predicted an atherogenic lipid profile more than 10 years later (Weiss, Otvos, Sinnreich, Miserez, & Kark, 2011). Despite these findings, universal criteria for TG/HDL ratio have not been established.

In a study of over 900 Argentinian adolescents (age 11-14 years), overweight/obese participants had TG/HDL ratios that were significantly higher than non-

obese/overweight participants (Musso et al., 2011). Those adolescents with insulin resistance had TG/HDL ratios that were higher than insulin sensitive adolescents regardless of weight (Musso, et al., 2011). Researchers were unable to establish a cut-point for TG/HDL ratio (Musso, et al., 2011).

Giannini and colleagues (2011) suggested that cut-points should be determined by ethnicity. When they divided study participants' data by ethnicity, the top tertile by TG/HDL ratio in each group had the most pro-insulin resistant profiles (Giannini et al., 2011). Although there was a clear association between the TG/HDL ratio and insulin resistance, statistical significance was reached in the White males and females only (Giannini et al., 2011). Of note, in this study, the TG/HDL ratio was more highly correlated with glucose clamp studies (gold standard) than HOMA-IR (Giannini, et al., 2011). The TG/HDL ratio has been correlated with HOMA-IR, BMI, increased atherogenic particles, arterial wall stiffness, changes in right ventricle wall thickness and left ventricular mass in children (Di Bonito et al., 2012; Olson, Hendricks & Murdock, 2012; Urbina et al., 2013).

Despite the apparent usefulness of this index, no universal criteria for TG/HDL ratio have been established in pediatrics. Small sample size and lack of heterogeneity in BMI, as well as ethnicity, have limited generalization to the general pediatric population. Further research is needed to establish the usefulness of the TG/HDL ratio as a clinical tool in a busy pediatric practice.

Advantages of TG/HDL ratio for screening

The TG/HDL ratio has many advantages when compared to other methods used for risk assessment in insulin resistance (Table 2.2.). The TG/HDL ratio is a simple calculation. In the U.S., laboratory measurement of triglycerides and HDL cholesterol is standardized to the same reference system (Warnick, Kimberly, Waymack, Leary, &

Myers, 2008). Both indices are reported in milligrams/deciliter, which allows for use of the numerical information without concern for units of measurement. In contrast, the HOMA-IR involves different units of measurement and thus requires complex calculations.

The product of basal plasma insulin (microunits per liter) and basal plasma glucose (millimoles per liter) divided by a constant (22.5) is the basis of the computer generated basal homoeostasis assessment model (HOMA) (Matthews et al., 1985). Researchers have demonstrated confusion about the units of measurement for insulin. Several research groups calculated HOMA-IR by multiplying insulin (microunits per milliliter) and glucose (millimoles per liter) and dividing by 22.5 (Geloneze et al., 2009; Lee et al., 2006; Tresaco et al., 2005; Yeckel et al., 2004). Di Bonito and colleagues (2012) divided the product of fasting insulin (units per liter) and glucose (millimoles per liter) by 22.5. Neither group used the original unit of measure for insulin.

In the U.S., the common unit of measurement for glucose is milligrams per deciliter. In order to use the HOMA-IR model, conversion of the constant from molar units to mass units is necessary and results in a revised constant of 405. At the clinical practice level, variation in units of measurement may result in inaccurate calculations and inappropriate therapy, which compromises patient safety.

Screening for dyslipidemia in children is recommended by NHLBI, AHA, and AAP (DHHS, 2012). Assessment of individual lipid values, genetic forms of hyperlipidemia, and risk for insulin resistance can be accomplished with one test, a lipid panel. In contrast, fasting glucose, fasting insulin and HOMA-IR assess risk for insulin resistance only. The Hypertriglyceridemic (HTG) Waist requires a waist measurement, in addition to a triglyceride level (Hobkirk et al., 2013). Each of the above-mentioned screening tests involves patient discomfort from venipuncture (Table 2.3). What is not known is how

waist measurement affects adolescents' self-esteem during a life period when concern for body image is typical.

Table 2.3 TG/HDL ratio adds no additional cost or testing

	HOMA-IR	TG/HDL ratio	HTG waist
Cost	Serum glucose Serum insulin	Lipid panel	Disposable tape measure Lipid panel
Information gleaned	Risk for insulin resistance	Risk for insulin resistance, genetic forms of hyperlipidemia, risk for atherosclerotic heart disease	Waist measurement Risk for insulin resistance
Potential for error	Complex calculation	Simple calculation	Simple calculation
Client comfort	Discomfort related to blood draw	Discomfort related to blood draw	Discomfort related to blood draw and body image

The serum analysis for lipid values is necessary for the TG/HDL ratio and the HTG Waist. The cost of the lipid panel is incurred. The cost of a disposable measuring tape is added to complete the calculation for insulin resistance in the HTG Waist model. HOMA-IR involves 2 separate tests and an increased cost. In summary, the TG/HDL ratio has many characteristics of a good screening tool: safety, simplicity, speed of completion, expense and acceptability to the public and medical community (Friis & Sellers, 2009). What is not known is whether the TG/HDL ratio predicts insulin resistance with sufficient sensitivity and specificity to be useful in the pediatric clinical setting.

Chapter 3

Methods

Cardiovascular disease is associated with the highest mortality and morbidity in the United States (CDC, 2013). Insulin resistance is thought to be the underlying link between dyslipidemia, type 2 diabetes, hypertension and cardiovascular disease (Capurso & Capurso, 2012). Obesity is associated with insulin resistance, impaired glucose metabolism, and atherogenic dyslipidemia (Capurso & Capurso, 2012). However, all obese persons are not insulin resistant, nor are all persons with insulin resistance obese (d'Annunzio et al., 2009). A screening tool that is minimally invasive, inexpensive, and useful in determining which patients are at increased risk for insulin resistance would be helpful to outpatient providers.

Purpose

The purpose of this study was to evaluate the performance characteristics of the triglyceride/high density lipoprotein cholesterol ratio (TG/HDL) when used as a screening tool to detect increased risk of insulin resistance in the U.S. adolescent population. Performance characteristics of a screening test include sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the screening test (Lang & Secic, 2006). Positive and negative predictive values are affected by the prevalence of disease (Friis & Sellers, 2009). Table 2.1includes the meaning of these terms.

The TG/HDL ratio as a screening test was known to be minimally invasive and inexpensive. The usefulness of this measure, however, for indicating increased risk of insulin resistance was unknown. In addition, whether results from TG/HDL ratio were comparable to other measures of insulin resistance, when used in this population, was unknown.

The glucose (euglycemic) clamp technique is the gold standard for determining degree of insulin resistance (Muniyappa et al., 2007). Intravenous fluids, multiple blood samples and analyses are required during the performance of the glucose clamp study (Muniyappa et al., 2007). Despite its usefulness in research protocols, this test is not practical for clinical use.

The homeostasis assessment model for insulin resistance (HOMA-IR) is often used instead of the glucose clamp method. In the HOMA-IR model, the product of fasting serum glucose and fasting serum insulin is divided by a constant to approximate the degree of insulin resistance (Matthews et al., 1985). The HOMA-IR model correlated well with the glucose clamp method ($r = 0.88$) (Matthews et al., 1985). Another possible indicator of insulin resistance is the TG/HDL ratio, which is the result of dividing the fasting triglyceride level by the HDL cholesterol level. In this study, performance characteristics of the TG/HDL ratio and HOMA-IR were compared to approximate how the TG/HDL ratio might compare to the gold standard, glucose clamp model.

Study Design

This descriptive study used a cross-sectional, secondary data analysis design to examine the performance characteristics of the TG/HDL ratio in screening for risk of insulin resistance. Publically accessible files from the 2011-2012 National Health and Nutrition Examination Survey (NHANES) were used for this study. Demographic, anthropometric and laboratory data were analyzed to examine performance characteristics of TG/HDL ratio in a nationally representative sample of non-institutionalized U.S. adolescents.

Sample

The National Center for Health Statistics (NCHS), under the guidance of the Center for Disease Control (CDC), conducts the NHANES program of studies. The

NHANES involves continuous data collection that is designed to provide ongoing assessment of the health and nutritional status of the U.S. population (CDC, 2014). A complex, multistage probability sampling strategy is used to obtain a representative survey group of non-institutionalized, civilian, U.S. residents (Johnson, Paulose-Ram, et al., 2013). During the 2011-2012 survey, Hispanic persons, non-Hispanic Black persons, non-Hispanic Asian persons, non-Hispanic White, and Other persons living at or below 130% federal poverty level, were oversampled (Johnson, Paulose-Ram, et al., 2013; Mirel et al., 2013). Complete details on the NHANES interview are available on the website: http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/questexam09_10.htm. This study examined the weighted subpopulation of adolescents (12-19 years of age) who participated in the interview, physical examination and laboratory sections of the NHANES 2011-2012 survey.

Inclusion/Exclusion Criteria

Adolescents (greater than or equal to 12 to less than 20 years of age) who participated in the interview, physical examination, and laboratory sections of the NHANES 2011-2012 survey were eligible for inclusion in this study. Availability of the following laboratory data was necessary for inclusion in the study: HDL-cholesterol, triglycerides, glucose, insulin and oral glucose tolerance test results. In addition, certain demographic and anthropometric data were required: age, gender, race/ethnicity, and body mass index (BMI).

Variables and Operational Definitions

Demographic Variables

Demographic data were gathered during the NHANES interview. Table 3.1 is a list of operational information for this study. Age, gender, and race/ethnicity were used in this study to identify subsets within the data set used in this study. Gender and

race/ethnicity were self-reported. The relationship between race/ethnicity and insulin resistance in adolescents was not well understood. When Giannini and colleagues (2011) studied nearly 1500 multi-ethnic obese youths, they were unable to determine an optimal cutoff value for TG/HDL ratio for all youths. Specifically, an optimal cutoff value could not be determined for Hispanic and African American adolescents (Giannini et al., 2011). The impact of gender on risk of insulin resistance had not been established.

Table 3.1 Demographic and anthropometric variables

Variable	Conceptual Definition	Operational Definition	Code
Age	Years since birth	Age at exam (years)	RIDEXAGY 12-19
Ethnicity/Race	Race - ancestry or group that participant culturally and socially identifies with Ethnicity – Hispanic (H)or non-Hispanic (nonH)	Self-reported race/ethnicity	RIDRETH3 1=Mexican American 2=other Hispanic 3=nonH White 4=nonH Black 6= nonH Asian 7=other including multi-racial
Gender	Sexual biological or physiological characteristics	Self-reported male/female	RIAGENDR 1=M, 2=F
Height	Standing measurement from top of head to bottom of feet	Standing length in centimeters/meters from top of head to floor	BMXHT centimeters
Weight	Heaviness of a person or relative mass resulting in a downward force	Number obtained when participant stands on a scale used for weighing	BMXWT Weight (kilograms)
Body mass index	Indicator of body fat based on height, weight, age and gender	Calculated number based on height, weight, age, and gender	BMXBMI Kg/m**2
Body mass index percentile category	Relative position of a participant's BMI among children of the same age and sex	BMI category based on BMI for age and sex charts	BMDBMIC 1=Underweight (<5th) 2= Healthy weight (5th- 84th) 3= Overweight (=≥85th -94th) 4 = Obese (≥95th)

Age was calculated based on date of birth and the age at the time of screening in whole years (Table 3.1). Study findings suggest that insulin sensitivity decreases, in non-diabetic children, during puberty (Amiel et al., 1991; Bloch, Clemmons, & Sperling, 1987; Caprio et al., 1989). Controversy exists about the relationship between Tanner stage and insulin resistance. Tanner staging was not performed on the participants in the 2011-2012 NHANES survey. Hence, the Tanner stage of participants was unknown. The study cohort was divided into two groups, early adolescence (12-15 years) and late adolescence (16-19 years), to examine the relationship between TG/HDL ratio and adolescence.

Anthropometric Variables

Height is a measurement of maximal vertical size. During the NHANES medical examination, standing height was measured using a stadiometer with a fixed vertical backboard and an adjustable headpiece (CDC, 2009a). The stadiometer was calibrated with an 80-centimeter metal rod. The participant was asked to stand with heels together, and toes apart. In addition, the participant was asked to make contact with the backboard at the level of the head, shoulder blades, buttocks and heels. The head was aligned so that a horizontal line between the ear canal and the lower border of the eye orbit was parallel to the floor and perpendicular to the backboard of the stadiometer. The headpiece was firmly rested on the participant's head. He/she was asked to take a deep breath and hold the position while the measurement was taken. Height was recorded in centimeters (CDC, 2009a)

Weight was measured in kilograms using a digital weight scale or portable scales. The digital scale was installed in the floor of the medical examination unit. The scales were calibrated with 10kg calibration weights daily. Participants were asked to wear a standard examination outfit: disposable shirt, pants and slippers. Each participant

was asked to stand in the center of the digital scale with hands hanging at his/her sides and look straight ahead. The weight was recorded. If the participant's weight was greater than 440 pounds, two portable scales were used to obtain the weight. The participant was asked to stand with one foot on each scale. The displayed weights were added to obtain the participant's weight (CDC, 2009a).

Body mass index (BMI) was used to categorize participants. BMI values were calculated using participant's height in meters and weight in kilograms.

$$BMI = \frac{\text{weight (kg)}}{\text{height } (m^2)}$$

Weight categories for adults, using BMI criteria established by the National Institutes of Health (1998) include: underweight (<18.5), normal/desirable weight (18.5-24.9), overweight (25.0-29.9), obese-Class I (30-34.9), obese-Class II (35.0-39.9), extremely obese (>40). These categories cannot be used for children because the amount of body fat changes with age and the amount of body fat differs for boys and girls. In order to determine a child's BMI, the calculated BMI must be plotted on a BMI-for-age chart for boys or girls, as appropriate for age (DHHS, 2002). The BMI-for-age was reported as a BMI percentile, and referred to the relative position of a child's BMI when compared to other boys or girls of the same age. Four BMI percentile categories were used to describe the sample: underweight (less than 5th percentile), healthy weight (5th percentile to less than the 85th percentile), overweight (85th percentile to less than the 95th percentile), obese (greater than or equal to the 95th percentile) (DHHS, 2002a).

Adiposity, as measured by BMI, was included in the study because the study of insulin resistance in pediatric populations was limited. Evidence suggesed that a positive correlation exists between adiposity and insulin resistance. Adiposity accounted for more than 25% of the variance in insulin resistance in boys and girls, (5-14 years) who participated in a prospective, longitudinal cohort study (Jeffery et al., 2012). Murdock and

colleagues (2006) found insulin resistance present in nearly half of the obese children who had a mean age of 7.5 years. However, the caveat was that the universal criteria for insulin resistance in children and adolescents have not been established.

Laboratory Variables

NHANES laboratory files contain results of blood tests performed during the course of the study. The NHANES laboratory manual details the procedural requirements for testing, processing, and analyzing blood samples. Specific information related to the study variables is discussed in the following section (Table 3.2).

Table 3.2 Laboratory variables

Variable	Conceptual Definition	Operational Definition	Code
Triglycerides	Hepatic product of stored fatty acids and glucose	Serum triglyceride level after nine-hour or longer period of fasting	LBXTR (mg/dL)
HDL cholesterol	High density lipoprotein that functions in the transfer of cholesterol from peripheral tissues to the liver	Serum HDL cholesterol level after nine-hour or longer period of fasting	LBDHDD (mg/dL)
Insulin	Major anabolic hormone that regulates fuel mobilization and storage to ensure that cells have a constant source of glucose, fatty acids, and amino acids for ATP generation and cellular activity	Serum insulin level after nine-hour or longer period of fasting	LBXIN (µU/mL)
Glucose	Monosaccharide that is the predominant sugar in human blood; major biosynthetic precursor in the body; major fuel source	Serum glucose after nine-hour or longer period of fasting	LBXGLU (mg/dL)
Oral glucose tolerance test (OGTT) –Two hour glucose	Test to detect impaired glucose tolerance, a risk factor for development of diabetes. >50% of persons with prediabetes are detected with this test only	Serum glucose 2 hours after ingestion of 75-grams of Trutol (OGTT solution)	LBXGLT (mg/dL) Positive = 2-hr serum glucose $\geq$ 140mg/dL Negative = 2-hr serum glucose <140mg/dL

Insulin resistance in muscle decreases cellular uptake of glucose (Ganong, 2005). In contrast, insulin resistance in the liver results in failure to suppress gluconeogenesis (Singh & Saxena, 2010). Both mechanisms of insulin resistance result in a clinical picture that includes elevated insulin level, impaired glucose tolerance, dyslipidemia (hypertriglyceridemia and decreased HDL cholesterol) (Singh & Saxena, 2010). Impaired glucose tolerance was evaluated by the NHANES researchers by conducting an oral glucose tolerance test (OGTT). A positive OGTT predicted an intermediate state of hyperglycemia that was characterized by higher than normal blood glucose level two hours after a 75-gram oral glucose load. The blood glucose was less than the diagnostic cut-off for diabetes, but indicated increased risk for diabetes (Ganong, 2005).

OGTT was included on the NHANES to allow estimation of the prevalence of impaired glucose tolerance in the U.S. population (CDC, 2009b). In this study, OGTT was used to determine the number and percentage of U.S. adolescents with impaired glucose tolerance, a clinical characteristic of insulin resistance. Participants with a positive OGTT were considered at increased risk for insulin resistance. Participants with a negative OGTT were considered at normal risk for insulin resistance. Serum glucose and insulin levels were used to calculate risk for insulin resistance by HOMA-IR method. HOMA-IR was calculated for each weighted participant using the following formula:

$$\text{HOMA-IR} = \frac{\text{Glucose (mg/dL)} \times \text{Insulin (µU/ml)}}{405}$$

Serum triglyceride and HDL cholesterol levels were used to calculate risk of insulin resistance by the TG/HDL ratio. The TG/HDL ratio was calculated for each weighted participant using the following formula:

$$\text{TG/HDL ratio} = \frac{\text{triglycerides (mg/dL)}}{\text{HDL cholesterol (mg/dL)}}$$

Dependent Variable

Risk of insulin resistance was the dependent variable. The main outcome measure was determination of performance characteristics of TG/HDL ratio as a screening test to indicate increased risk of insulin resistance in this population.

Procedure

Upon arrival to the NHANES study site for morning sessions, participants were asked to verify fasting status. Participants who fasted for nine hours were immediately eligible for glucose tolerance testing. In addition, participants who would complete the nine-hour fast with one hour and forty minutes left in the medical examination session were eligible (CDC, 2009b).

Immediately after initial phlebotomy, participants were instructed to drink a calibrated dose of the glucose solution (Trutol) (CDC, 2009c). The dose was determined by body weight (Table 3.3). The participant drank the entire dose within a 10-minute period.

Fasting state was maintained during the period before the second phlebotomy session. Anthropometric measurements and/or medical interviews were conducted during this time period. The second blood draw occurred 100 – 135 minutes after consumption of the glucose solution (CDC, 2009b).

Initial venipuncture resulted in enough blood for determination of triglyceride, HDL-cholesterol, glucose and insulin levels. Blood specimens were handled and stored according to standardized laboratory methods. Blood specimens were transported, processed and analyzed by NHANES Diabetes Laboratory, Fairview – University Medical Center, Minneapolis, MN (CDC, 2009b).

Table 3.3 Trutol calibrated dosage chart

Body weight		75 gram concentration	
Pounds	Kilograms	Ounces	Milliliters
94+	42.7+	10	295
90-93	40.9	9.5	283
85-89	38.6	9.0	267
80-84	36.4	8.5	251
75-79	34.1	8.0	235
70-74	31.8	7.4	220
65-69	29.5	6.9	204
60-64	27.3	6.4	188

Analyses to Describe the Sample

The demographic variables were described using frequencies, percentages, means, and standard deviations, as appropriate for each variable. Anthropometric and laboratory data were also analyzed using descriptive statistics as appropriate for the type of data.

Analysis to Address Research Questions

Statistical software packages, Statistical Analysis Systems (SAS) 9.3 and IBM SPSS 21, were used to conduct all analyses. The NHANES had a complex, multistage, probability cluster design. The code needed to specify sample design parameters in SAS included the SAS survey procedure, *proc survey*, the stratum statement, *sdmvstra*, and the cluster statement, *sdmvpsu*. The stratum statement was used to specify the strata, and account for the effects of stratification. The cluster statement was used to specify the primary sampling unit and account for the design effects of clustering (Delwiche & Slaughter, 2008). The oral glucose tolerance test two-year medical examination component subsample survey weight, *WTSOG2YR-OGTT*, was used during analysis (Johnson & et al., 2013). This weight was associated with the smallest subpopulation that included the variables of interest.

The ROC Curve

For any test, a point at which the test is considered positive must be determined (threshold or criterion value). Sensitivity and specificity are inversely related (Cochrane & Holland, 1971). If the test is highly sensitive, detection of true positives will be high. However, false positives will increase also. If the test is highly specific, detection of true negatives will be high. However, some people with disease (false negatives) will go undetected. The test threshold is set at a point where the fewest number of tolerable false positives and false negatives occur (Cochrane & Holland, 1971).

When using a quantitative test, it is possible to vary sensitivity and specificity by changing the level at which the test result is considered positive (Cochrane & Holland, 1971). The receiver operating characteristic (ROC) curve is a graph of all sensitivity and specificity points of a test (Hajian-Tilaki, 2013; Zweig & Campbell, 1993). The ROC curve is used to evaluate the ability to discriminant between positive (diseased) and negative (healthy) individuals when using a particular test (Hajian-Tilaki, 2013; Zweig & Campbell, 1993).

Initially used for radar operators, ROC analysis was applied to diagnostic radiology in 1960 by Lusted who analyzed data on detection of tuberculosis (Metz, 1978). Today, ROC analysis is used to assess the accuracy of many diagnostic tests (Hajian-Tilaki, 2013). To construct an ROC curve, a population containing healthy individuals and individuals known to have the disease of interest must be available. All individuals must have had the standard/gold standard test to establish positive or negative status (Metz. 1978).

The receiver operating characteristics curve is a graph of the sensitivity and specificity of a test. In determining the cut point for insulin resistance, a balance between the degree of sensitivity and specificity must be accepted. The best cut-off value is the

point on the curve closest to the upper left hand corner (Hajian-Tilaki, 2013; Lang & Secic, 2006). The best screening test is the one with the greatest area under the curve. ROC graphs can be constructed using parametric, nonparametric or semi-parametric methods (Colak, et al., 2012). The nonparametric method is preferred for continuous laboratory data because no data are discarded, no parametric assumptions are required and the plot goes through all points (Zweig & Campbell, 1993). All possible sensitivity/specificity pairs are graphed, providing a comprehensive picture of decision thresholds (Zweig & Campbell, 1993).

Determining the threshold point from results of the ROC analysis is common (Greiner, Sohr, & Gobel, 1995). To illustrate this point, consider the following. Timing of patient presentation for evaluation complicates the use of amylase and lipase levels to diagnose pancreatitis. Keim and colleagues (1998) conducted a study to determine the usefulness of lipase and amylase levels in patients presenting with acute abdominal pain. They found that lipase levels greater than twice normal on day 0-1 were diagnostic for pancreatitis. The sensitivity was 94%, and the specificity was 95%. The overall accuracy of the test was 95% (Keim, et al., 1998). In other words, 95 times out of 100, the patient had pancreatitis.

ROC analysis was performed to determine a threshold for the TG/HDL ratio in this study. All data for the TG/HDL ratio and the oral glucose tolerance test (OGTT) were entered in SPSS. OGTT results were used as the designation of whether the adolescent was positive or negative for disease. A table with sensitivity and specificity for each possible data point was produced. A visual of the relationship between sensitivity and specificity (ROC curve) across the data points was produced (Figure 4.1).

Question 1: What was the cut-off value that indicates increased risk of insulin resistance by the TG/HDL ratio in U.S. adolescents?

SPSS was used to perform receiver operating characteristic curve (ROC) analysis, a procedure designed to determine an appropriate cut-off value for risk of insulin resistance using the TG/HDL ratio. The oral glucose tolerance test result for each participant was evaluated to determine presence or absence of insulin resistance (poor glucose tolerance). OGTT result greater than or equal to 140 mg/dL was consistent with impaired glucose tolerance (Gavin, Alberti, Davidson, DeFronzo et al., 1997).The OGTT level was changed to a dichotomous variable, with absence indicated by the number 1 and presence indicated by the number 2. The TG/HDL ratio was calculated for each study participant.

The prevalence of impaired glucose tolerance (indicator of insulin resistance) in the study population was calculated. Point prevalence was determined by the following equation:

$$\frac{\text{Number of person with IGT at a point in time}}{\text{Total number in the group at the same point in time}}$$ (Friis & Sellers, 2009).

ROC analysis was conducted using as OGTT as criterion for insulin resistance. The cut-off value was selected from the table of sensitivities and specificities generated from ROC analysis.

Question 2: Did the cut-off value vary by race/ethnicity, gender, BMI percentile category, early (12-15 years) vs. late (16-19 years) adolescence?

ROC analysis was performed to determine if the cutoff value for TG/HDL ratio varied by race/ethnicity, gender, BMI percentile category, BMI, early vs late adolescence.

Question 3: What were the performance characteristics (measures of validity) of the TG/HDL ratio as a predictor of risk of insulin resistance?

Using SAS, a 2X2 table (Table 3.4) of sensitivity, specificity, positive predictive value and negative predictive values was constructed for the TG/HDL ratio.

Table 3.4 Determination of performance characteristics

Condition according to gold standard					
Test Result		Present	Absent	Total	
	Positive	A= true positives	B= false positives	A+B	Positive predictive value a/(a+b)
	Negative	C=false negatives	D= true negatives	C+D	Negative predictive value d/(c+d)
	Total	A+C	B+D	Grand Total	
		Sensitivity a/(a+c)	Specificity d/(b+d)		

Question 4: How did performance characteristics of the TG/HDL ratio compare to the performance characteristics of the HOMA-IR model?

Using SAS, a 2X2 table (Table 3.4) of sensitivity, specificity, positive predictive value and negative predictive values was constructed for the HOMA-IR model. Accuracy of the screening test (percentage of agreement between the screening test and the gold standard test) was determined by the following equation, (a+d)/(a+b+c+d). (Friis & Sellers, 2009).

Question 5: Did the TG/HDL ratio appear to be an appropriate tool for screening for increased risk of insulin resistance in U.S. adolescents?

The performance characteristics are used to determine the safety of the TG/HDL ratio. The qualities of the TG/HDL ratio were compared to the qualities of a good screening test.

Summary

Screening tests are performed on outwardly healthy, asymptomatic persons to identify risk, justify further testing, and initiate preventive measures (Lang & Secic, 2006). The purpose of this study was to evaluate the performance characteristics of the TG/HDL

ratio when used as a screening tool to detect increased risk for insulin resistance. Insulin resistance is a condition in which people appear healthy until the disease state progresses to the moderate to severe stage. Insulin resistance is a clinical feature of type 2 diabetes and is associated with cardiovascular disease. Early detection of increased risk of insulin resistance has the potential to decrease progression to severe disease.

Screening tests are validated using a prevalence of 50%, but the prevalence of a condition is rarely 50% (Haynes, 1981). In free-living environments, prevalence of a condition differs in primary care settings when compared to tertiary care settings (Haynes, 1981). These data were collected from individuals who could be seen in a primary care setting. Hence, calculating the predictive value of screening tests for this group, when prevalence of risk of insulin resistance was known, provided information about the usefulness of the TG/HDL ratio as a screening tool for insulin resistance in the primary care setting.

Chapter 4

Results

Introduction

The purpose of this chapter is to present the findings of a study conducted to assess the performance characteristics of the triglyceride/HDL cholesterol ratio when used to indicate the presence of increased risk of insulin resistance in U.S. adolescents, aged 12-19 years. This chapter contains the demographic, anthropometric, and metabolic characteristics of the study participants. Performance characteristics of the TG/HDL ratio are presented and compared to the Homeostatic Model Assessment of insulin resistance (HOMA-IR) performance characteristics.

Description of Sample

Participant data were obtained from the publically available, de-identified, 2011-2012 National Health and Nutrition Examination Survey (NHANES) data set. Survey sample weights accounted for the complex survey design, oversampling, and survey nonresponse. The total screened, unweighted sample of adolescents (age 12-19 years) for the NHANES 2011-2012 survey consisted of 1,617 persons. Of 1,273 interviewed sample participants (Figure 4.1), 41 study participants completed the interview portion only. The remainder, (N=1232), completed the interview and medical examination portions of the NHANES 2011-2012 survey. A subset of randomly assigned sample participants (N = 452) underwent two-hour oral glucose tolerance testing (OGTT). Calculation of performance characteristics of TG/HDL ratio and HOMA-IR were based on data from this subset.

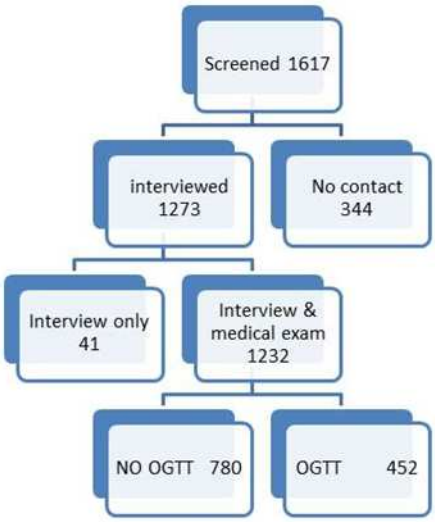

Figure 4.1 Study participant screening process

Data from adolescents, aged 12 – 19 years (n = 452), who underwent oral glucose tolerance testing (OGTT), were analyzed. These data represent over 33.4 million U.S. adolescents.

The mean age at the time of data collection was 15.42 years (95% CI 15.21-15.63). More 14 year-olds participated in the study than other ages (Table 4.1). Less 18 year-olds participated in the study than other age groups.

Table 4.1 Age at exam

Age at exam	Frequency	Weighted Frequency	Percent of weighted frequency
12	48	3,919,767	11.7348
13	65	3,677,192	11.0086
14	68	5,237,667	15.6802
15	53	4,591,900	13.7470
16	61	4,951,029	14.8221
17	59	4,297,708	12.8662
18	40	3,048,818	9.1274
19	58	3,678,929	11.0138
Total	452	33,403,011	100.0000

Early adolescence, defined as 12-15 years of age, (n = 234) represented more than half of the U.S. adolescents who participated in the study. Males (n = 235) represented 50.8% of participants (Figure 4.2).

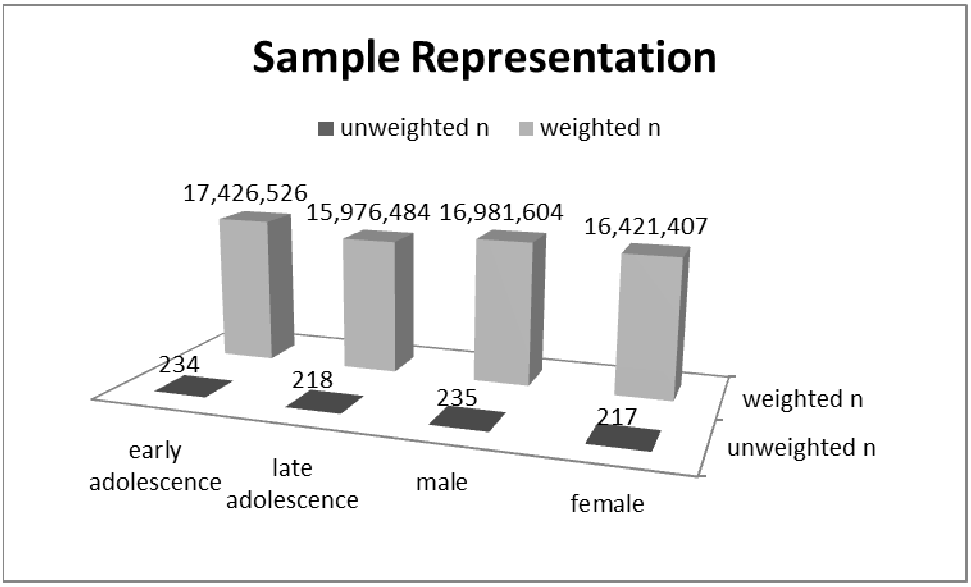

Figure 4.2 Subset relationship to U.S. adolescent population

Six ethnicity/race categories were defined and represented (Table 4.2): Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, non-Hispanic Asian, and other including Multi-Racial.

Table 4.2 Sample by ethnicity/race

Ethnicity/Race (E/R)	Unweighted sample size	Weighted sample size
Mexican American	79	5,135,528
Other Hispanic	40	2,174,294
Non-Hispanic White	111	18,433,562
Non-Hispanic Black	149	5,239,591
Non-Hispanic Asian	57	1,543,116
Other/Multiracial	16	876,920
Total	452	33,403,011

As can be seen in Figure 4.3, the unweighted sample composition did not reflect the general U.S. adolescent population composition. Non-Hispanic Blacks and Non-Hispanic Asians were oversampled.

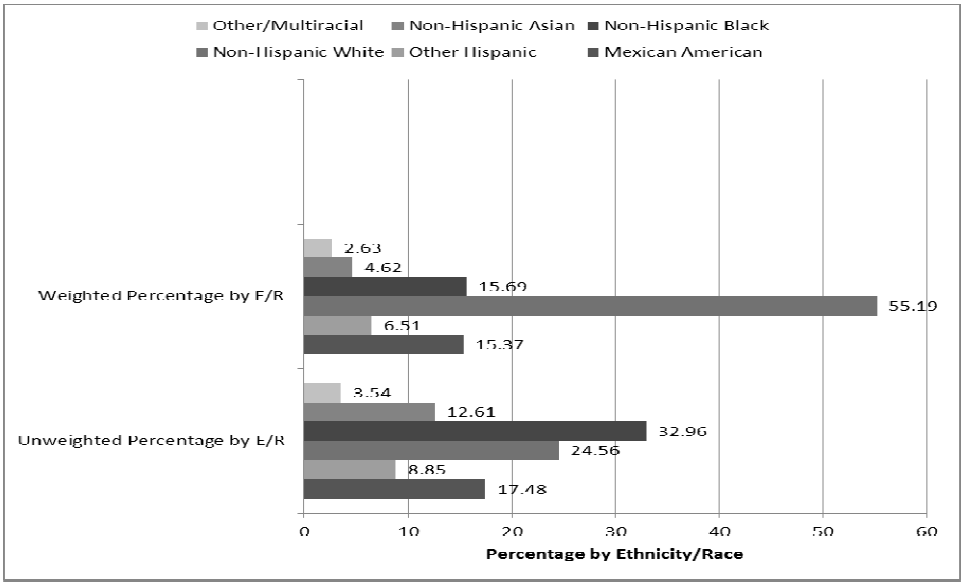

Figure 4.3 Comparison of weighted and unweighted sample sizes

The weight for study participants ranged from 30.7 to 180.6 kg and had a mean (SD) of 66.61kg (19.89 kg). The height ranged from 140.3 to 193.7 cm and had a mean (SD) 165.68cm (9.44 cm). The BMI was calculated from the height and weight. The mean (SD) BMI was 24.06 (6.06). Adolescents were classified by BMI into the following categories: underweight, healthy weight, overweight, and obese. Most participants were healthy weight (63.3%). Many participants were overweight\obese (33.9%), and 2.7% were underweight. Data were missing for 3 participants.

Table 4.3 Percentage in BMI category by weighted frequency

BMI category	Frequency	Weighted frequency	Percentage
Underweight	17	904,945	2.74
Healthy weight	267	20,934,398	63.34
Overweight	68	4,431,267	13.40
Obese	97	6,782,172	20.52

Statistical Analysis of Study Results

The purpose of this study was to evaluate the performance characteristics (sensitivity, specificity, positive predictive value and negative predictive value) of the triglyceride/high-density lipoprotein cholesterol ratio (TG/HDL ratio) when used as a screening tool to detect increased risk of insulin resistance in the U.S. adolescent population. Data were analyzed using SAS and SPSS statistical software programs, as appropriate. The sample size varied during analysis (Figure 4.4).

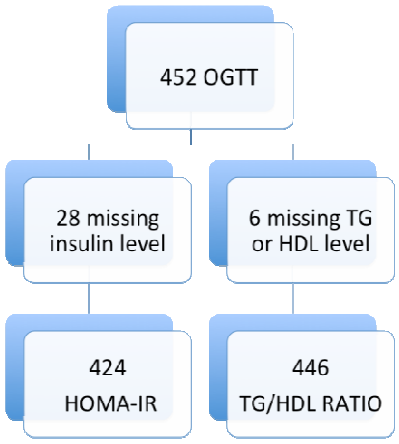

Figure 4.4 Sample size for analysis

Table 4.4 Descriptive statistics for laboratory variables for study sample

Variable	N	Mean	SD	Min	Max
Triglyceride	446	81.84	54.22	18	406
HDL cholesterol	447	51.57	10.82	24	96
Glucose	452	94.02	13.45	61	331
Insulin	424	14.78	11.11	2.8	92
Oral glucose tolerance test	452	99.51	30.91	44	501

The statistics for laboratory variables analyzed for the study sample varied slightly from the statistics from the population data (weighted data) (Table 4.5). The minimum and maximum values were identical and were not repeated in the table.

Table 4.5 Descriptive statistics for laboratory variables for U.S. adolescent population

Variable	N	Mean	SD
Triglyceride	33,163,493	83.68	52.14
HDL cholesterol	33,192,029	51.04	10.95
Glucose	33,403,011	93.92	11.43
Insulin	31,752,249	13.79	10.03
Oral glucose tolerance test	33,403,011	91.62	27.40

Question 1: What was the cut-off value that indicates increased risk of insulin resistance by the TG/HDL ratio in U.S. adolescents?

The first step: Dichotomous variable was created and prevalence calculated

The oral glucose tolerance test (OGTT) results were evaluated to create a dichotomous variable for insulin resistance. OGTT results, greater than 139 mg/dL, indicate impaired glucose tolerance (insulin resistance). OGTT was used as the gold standard for insulin resistance and the criterion for ROC analysis. In the unweighted sample, 25 participants were identified as insulin resistant based on OGTT results. The weighted frequency was 1,533,713. Due to missing data, one participant was excluded from further analysis (Figure 4.6). The prevalence of impaired glucose tolerance (insulin resistance) within the study group was 4.7%. The characteristics of the participants with insulin resistance were listed in Table 4.6 and Table 4.7.

Table 4.6 Characteristics of unweighted sample with insulin resistance

Variable	Subcategory	Total N=452	Insulin Resistant N=24
Adolescent group	Early	234	15
	Late	218	9
BMI category	Underweight	17	1
	Healthy weight	267	9
	Overweight	68	5
	Obese	97	9
Gender	Female	217	15
	Male	235	9

Table 4.7 Ethnicity/race of unweighted sample with insulin resistance

Ethnicity/Race	Total N =452	Insulin Resistant N=24
Mexican American	79	6
Other Hispanic	40	3
Non-Hispanic White	111	3
Non-Hispanic Black	149	6
Non-Hispanic Asian	57	5
Other/Multiracial	16	1

The second step: ROC analysis

Using SPSS, the TG/HDL ratio was calculated for each participant. ROC analysis of the TG/HDL ratios, using OGTT as criterion, resulted in a table of sensitivity and specificity for each point on the curve.

The third step: TG/HDL cut-point was determined

The cut-point value was determined for the TG/HDL ratio by 1) review of the sensitivity and specificity table produced by ROC curve analysis and 2) prevalence of insulin resistance in the study group. The cut-point for the TG/HDL ratio was set at ≥3.7 (Figure 4.5).

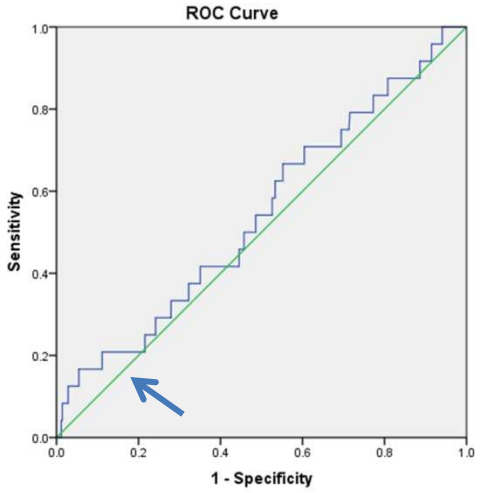

Figure 4.5 ROC curve for TG/HDL ratio, AUC = .54, *p* = 0.6

Question 2: Did the cut-off value vary by ethnicity/race, gender, BMI percentile category, or early (12-15 years) vs. late (16-19 years) adolescence?

ROC analysis was performed to determine if the cut-off value varied by ethnicity/race, gender, BMI percentile category, or early vs late adolescence. The TG/HDL ratio cut-off value was higher in the Non-Hispanic White and Non-Hispanic Asian participants than the cut-off value for the entire group of participants (Table 4.8). The TG/HDL ratio cut-off value was lower for the Non-Hispanic Black participants. The specificity was similar for all groups. The sensitivity was low for all groups. Two groups (other Hispanics and Other/Multiracial) had too few insulin resistant participants to perform analysis.

Table 4.8 TG/HDL ratio cut-off value by ethnicity/race

Ethnicity/Race	TG/HDL ratio cut-off value	Sensitivity	Specificity
Mexican American	3.7	40%	94.4%
Non-Hispanic White	3.9	0%	93.5%
Non-Hispanic Black	3.1	33%	93.6%
Non-Hispanic Asian	4.2	0%	92%

The TG/HDL ratio cut-off value was not different based on gender (Table 4.9).

Table 4.9 TG/HDL ratio cut-off value by gender

Gender	TG/HDL ratio cut-off value	Sensitivity	Specificity
Female	3.6	20%	94.9%
Male	3.7	11%	93.7%

Table 4.10 TG/HDL ratio cut-off by BMI percentile category

BMI percentile category	TG/HDL ratio cut-off value	Sensitivity	Specificity
Healthy weight	3.7	0.0%	96.4%
Overweight/Obese	4.3	23%	93.3%

The TG/HDL ratio cut-off value for overweight/obese adolescents was higher than the cut-off value for the healthy weight adolescents (Table 4.10). Similarly, the TG/HDL ratio cut-off was slightly higher for late vs early adolescence (Table 4.11).

Table 4.11 TG/HDL ratio cut-off by adolescence category

Adolescence (years)	TG/HDL ratio cut-off value	Sensitivity	Specificity
Early (12-15 years)	3.26	13.3%	93.2%
Late (16-19 years)	3.7	22%	93.6%

Question 3: What were the performance characteristics of the TG/HDL ratio as a predictor of risk of insulin resistance?

Using a TG/HDL ratio $\geq$ 3.7, 6.6% of the study participants were identified as at risk for insulin resistance. Performance characteristics were calculated using weighted data (Table 4.12). The sensitivity of the TG/HDL ratio was 14.8%. The specificity of the TG/HDL ratio was 93.8%. The positive predictive value was 10.4%. The negative predictive value was 95.8%.

Table 4.12 Performance characteristics of TG/HDL ratio

Oral Glucose Tolerance Test Result					
		Present	Absent	Total	
TG/HDL Ratio Result	Positive	226,342	1,949,271	2,175,613	Positive predictive value 10.4%
	Negative	1,307,371	29,680,510	30,987,881	Negative predictive value 95.8%
	Total	1,533,713	31,629,781	33,163,512	
		Sensitivity 14.8%	Specificity 93.8%	,	

Question 4: How did performance characteristics of the TG/HDL ratio compare to the performance characteristics of the HOMA-IR model?

The cut-point for insulin resistance by HOMA-IR was determined from pediatric studies (Lee et al., 2006; Quijada et al., 2008; Schwartz et al., 2008) and ROC analysis, which confirmed the cut-point from the literature.

The performance characteristics of the TG/HDL ratio and HOMA-IR model are displayed in Table 4.13. Neither test was particularly sensitive, but both had high specificity and negative predictive values. That is, if the result was negative, risk for insulin resistance was low and client was likely to be disease-free.

Table 4.13 Performance characteristics of the TG/HDL ratio and HOMA-IR model

Performance Characteristics	TG/HDL ratio	HOMA-IR
Sensitivity	14.8%	57.1%
Specificity	93.8%	87.9%
Positive Predictive Value	10.4%	19.4%
Negative Predictive Value	95.8%	97.6%

The observed agreement between the TG/HDL ratio and the HOMA-IR results was 85.1% (weighted data). The Kappa statistic was 0.81, which indicates excellent agreement beyond chance (Gordis, 2009). The accuracy of the TG/HDL ratio was higher than the HOMA-IR model, 90.2% vs. 86.4%, respectively.

Question 5:Did the TG/HDL ratio appear to be an appropriate tool for screening U.S. adolescents for insulin resistance?

Table 4.14 Evaluation of the TG/HDL ratio as a screening test

Qualities for Judging Screening Tests	Good Screening Test	TG/HDL ratio
Address major health problem	Yes	Yes
Expense of testing	Low	Low
Simplicity	Simple to perform	Simple to perform
Acceptability to the public and medical community	Yes	Unknown
Minimal potential harm/safety	Yes	Yes
High sensitivity	Yes	No
High specificity	Yes	Yes

The TG/HDL ratio appears to have many of the qualities of a good screening tool. Using a cut-off value ($\geq$3.7), the TG/HDL ratio had 90.2% accuracy.

In summary, the ROC curve analyses showed that the best cut-off value for the TG/HDL ratio was $\geq$3.7. The area under the ROC curve was 0.54. This suggested that the ability to detect insulin resistance using $\geq$3.7 as the cut-off value for the TG/HDL ratio would be low. However, the negative predictive value of the TG/HDL ratio when using $\geq$3.7 as the cut-off value was very high. This suggested that the TG/HDL ratio was an effective tool for screening out insulin resistance.

Summary

In this chapter, the findings related to assessment of the performance characteristics of the TG/HDL ratio to indicate increased risk of insulin resistance in the U.S. adolescent population were presented. The NHANES data set was used for analysis, and included demographic, anthropometric, and laboratory variables for 452 adolescents who participated in the study. Weighted (population) data were used for analysis when possible. ROC analysis provided a means to determine the cut-off value that would indicate increased risk for insulin resistance in an adolescent. The TG/HDL ratio performance characteristics were compared to a surrogate gold standard measure: the HOMA-IR model. Finally, an assessment of the TG/HDL ratio as a screening tool for insulin resistance in U.S. adolescents was completed.

Chapter 5

Discussion

Quantifying insulin resistance in adolescents is of great importance because insulin resistance appears to be the underlying mechanism and predictor of cardiovascular disease, diabetes and other conditions (Singh & Saxena, 2010). Furthermore, insulin resistance occurs years before the development of cardiovascular disease and diabetes. In this study, a screening tool for insulin resistance, the TG/HDL ratio, was investigated. It is important to remember that screening tests are not diagnostic, instead, screening tests indicate which individuals are more likely to have a condition (Lang & Secic, 2006).

Question 1: What was the cut-off value that indicates increased risk of insulin resistance by the TG/HDL ratio in U.S. adolescents?

The 2011-2012 NHANES data set used in this study included an oversampled non-Hispanic Asian population and non-Hispanic Black population. Thus, the unweighted sample contained a larger percentage of non-Hispanic Asian and non-Hispanic Black adolescents than in the U.S. population. ROC analysis could only be performed on the unweighted data set, as there is no known software for personal computing that is capable of performing this task on complex survey data. The TG/HDL ratio cut-off value was set at $\geq$3.7. When ROC analysis was performed on data from individual ethnic groups: Non-Hispanic Blacks had the lowest TG/HDL ratio cut-off value: 3.1. Non-Hispanic Asians had the highest TG/HDL ratio cut-off value: 4.2. The area under the curve for ROC analysis conducted on the study sample was 0.54, which suggested that the TG/HDL ratio was not better than 'flipping a coin' to indicate insulin resistance (Hajian-Tilaki, 2012).

In most studies of adult biracial populations (non-Hispanic White and non-Hispanic Black), a TG/HDL ratio > 3 has been associated with insulin resistance and increased risk for cardiovascular disease (da Luz, Favarato, Faria-Neto, Lemons, & Chagas, 2008; Kannel et al., 2008). Gasevic and colleagues (2012) found that the TG/HDL ratio > 4 correlated with insulin resistance in individuals of Chinese, Aboriginal, and South Asian origin. The composition of the data set may have affected the results of this analysis.

Question 2: Did the cut-off value vary by race/ethnicity, gender, BMI percentile category, early (12-15 years) vs. late (16-19 years) adolescence?

Research from Giannini and colleagues (2011) supported varying the cut-off value by ethnicity. In this study, the cut-off value for the TG/HDL ratio ranged from 3.1-4.2. ROC analysis produced the lowest TG/HDL ratio cut-off value in Non-Hispanic Blacks (3.1) and the highest TG/HDL ratio cut-off value in non-Hispanic Asians (4.2). The cut-off values for Mexican American and non-Hispanic Whites were similar, 3.7, 3.9, respectively. The negative predictive value was similar for all groups (92%-94.4%). The sample sizes for the Other Hispanic and Other/Multiracial groups were too small for analysis.

The TG/HDL ratio cut-off value was slightly lower for females than males (3.6, 3.7, respectively). This finding was in contrast to research that suggested that females have higher levels of insulin resistance because of higher body fatness (Travers et al, 1995). However, adolescents in the overweight/obese category had a higher TG/HDL ratio cut-off value (4.3) than their healthy weight peers (3.7), which supported Travers' research.

Several research groups have documented a change in insulin sensitivity during sexual maturation in human and animal studies (Amiel et al, 1991; Bloch, Clemmons, &

Sperling, 1987, Caprio et al, 1989; Virtue & Vidal-Puig, 2008). In this study, adolescents with insulin resistance based on results from the OGTT were more likely to be overweight/obese, early adolescent females. More adolescents in the early adolescence group were identified as insulin resistance by OGTT than adolescents in the late adolescence group (62.5% vs 37.5%). The early adolescence group had a lower TG/HDL ratio (3.26) than the late adolescence group (3.7). This finding would suggest that in order to identify insulin resistance in the early adolescence population, the TG/HDL cut-off value may need to be set lower than 3.7.

Question 3: What were the performance characteristics (measures of validity) of the TG/HDL ratio as a predictor of risk of insulin resistance?

The TG/HDL ratio cut-off value was set at $\geq$3.7, and the performance characteristics were calculated using the weighted data set. The TG/HDL ratio cut-off $\geq$3.7 resulted in a sensitivity of 16.7% and specificity of 94.3%. The sensitivity indicated that if a person was insulin resistant, there was a 16.7% chance that the TG/HDL ratio would be positive. The specificity indicated that if the individual was negative for insulin resistance, there was a 94.3% chance that the test would be negative. Even though, sensitivity and specificity are not mathematically dependent on disease prevalence, the accuracy of the test (area under the curve) can vary with prevalence (Leeflang, Bossuyt & Irwig, 2009; Leeflang, Rutjes, Reitsma, Hooft & Bossuyt, 2013). Since the prevalence of insulin resistance in the sample was low, the false positives were contained by setting a high threshold (Metz, 1978).

The prevalence of insulin resistance in the study population was 4.7%. Predictive values of a screening test are affected by prevalence of condition (Friis & Sellers, 2009). When the prevalence of a condition is low in the population, then the positive predictive value will be low, indicating low probability of having a condition when

the test is positive (Friis & Sellers, 2009, Haynes, 1981). The positive predictive value of the TG/HDL ratio was 10.4% in this study population. Thus, an individual with a positive test result had a 10.4% probability of having insulin resistance. A high negative predictive value is helpful when the prevalence of disease is low. The negative predictive value of the TG/HDL ratio was 95.8%. Thus, a person who tested negative had a 95.8% probability of freedom from disease.

Question 4: How did performance characteristics of the TG/HDL ratio compare to the performance characteristics of the HOMA-IR model?

The goal standard to determine insulin resistance is the hyperinsulinemic-euglycemic clamp test (glucose clamp test) (Eyzaguirre & Mericq, 2009). This test is impractical for use in clinical settings (Giannini et al., 2011). One well-correlated surrogate for the glucose clamp test is the homeostasis model assessment of insulin resistance (HOMA-IR), r = .81 (George et al, 2011; Nguyen et al, 2010; Schwartz et al., 2008). Giannini and colleagues (2011) demonstrated a higher correlation between the TG/HDL ratio and the glucose clamp than between the HOMA-IR and the glucose clamp in obese youths. In this study, a medium correlation was found between the TG/HDL ratio and HOMA-IR, $r = .27$, $p<.01$. In contrast, Quijada (2008) found no significant correlation between TG/HDL ratio and HOMA-IR in a study of obese children.

The TG/HDL ratio was more accurate than HOMA-IR when used to indicate insulin resistance in this study group, 90.2% vs. 86.4% respectively. Importantly, the negative predictive value was similar, 95.8% and 97.6%. Both tests could be used to screen out insulin resistance.

Question 5:Did the TG/HDL ratio appear to be an appropriate tool for screening U.S. adolescents for insulin resistance?

The TG/HDL ratio appeared to be an appropriate tool for initial screening of adolescents when there was concern for insulin resistance. The TG/HDL ratio had many qualities of a good screening test: 1) addresses two major health care diseases, 2) simple to perform, 3) no additional initial expense to client or payor, and 4) the performance characteristics are within an expected range. The most recent guidelines for pediatric cardiovascular care support cholesterol screening. Use of the TG/HDL ratio is limited by the inability to determine an absolute cut-off value to indicate increased risk of insulin resistance. However, an elevated TG/HDL ratio should promote a discussion of lifestyle modification.

Limitations/Bias

Two important factors limited the generalization of the findings from this study: 1) composition of the unweighted sample and 2) inability to perform ROC analysis on weighted data set. The 2011-2012 HANES is the first half of the 4-year study of nutrition and health in the U.S. For this 2-year study group, Asians were oversampled, which resulted in a decreased sample size in the Hispanic groups and non-Hispanic White group (Johnson, Dohrmann, Burt, & Mohadjer, 2014). In addition, the targeted number of Asian families was not achieved, resulting in an overall decreased screening rate (Johnson et al., 2014). In addition, non-Hispanic Blacks were oversampled. Hence, a better representation of insulin resistance in the U.S. adolescent population may be accomplished by conducting research on the entire 4-year study group.

Parametric and nonparametric methods have been developed to perform ROC analysis for simple random sampling, but not complex sampling designs (Yao, 2013). Sample weights were utilized for all measurements of interest during the 2011-2012

NHANES data collection. The sample weights estimate the number of people in the population that participants represent. Ignoring the sample weight could lead to inaccurate representation of the data. In this study, the composition of the sample, in terms of proportions of race/ethnicity, changed significantly between the weighted and unweighted sample (Figures 4.3).

One of the benefits of using a weighted sample is that the findings are generalizable to the entire population. In the study, 4.7% of the sample population (25/452) was insulin resistant by OGTT. The TG/HDL ratio cut-off value was based on analyses from this small sample. Hence, the findings of this study are not generalizable to the U.S. adolescent population. The absolute number for the TG/HDL ratio cut-off is not known.

Yao (2013) demonstrated that area under the curve (test accuracy) varied slightly with the use of weighted data. This variance altered the findings in his study (Yao, 2013). No known software has been developed to perform ROC analysis on complex survey data, therefore during this study ROC analysis was performed on unweighted data. The composition of the unweighted sample population did not mirror the composition of the U.S. adolescent population.

The TG/HDL ratio is not as sensitive a method to determine insulin resistance as glucose clamp or other surrogates (Matthews et al., 1985). The TG/HDL ratio is measured in the fasting state and the glucose clamp studies evaluate insulin resistance in the insulin-stimulated state (DeFronzo, Tobin, & Andres, 1979). However, the TG/HDL ratio has advantages and can be added to the clinician's tool chest for assessing risk of insulin resistance. Given that 1/3 of adolescents in the U.S. are overweight or obese, and that type 2 diabetes is diagnosed at an increasing rate in adolescents, it is prudent to develop methods of risk assessment for clinical use.

Implications of Study Findings

Implications for clinical practice

A screening test should meet certain criteria: a) address an important health problem, b) address a condition for which the natural history is adequately understood, c) address a condition for which a pre-symptomatic period exists, and d) address a condition for which effective intervention for primary prevention is available (Friis & Sellers, 2009; Somerville, Kumaran & Anderson, 2012). In this study, the TG/HDL ratio was used as a screening test for insulin resistance. Research has supported developing a means of early detection of type 2 diabetes (Harris & Eastman, 2000). Insulin resistance appears to be a condition that develops prior to type 2 diabetes, which is a major cause of morbidity and mortality in the U.S. People with diabetes are at increased risk for cardiovascular events (Kashyap & Defronzo, 2007; Srikanth & Deedwania, 2011). The lifetime risk of cardiovascular disease is 4-6-fold higher in people with preventable risk factors (Berry et al., 2012). Prevention of type 2 diabetes, through intervention at the point of recognition of insulin resistance, has the potential to reduce future cardiovascular events (Srikanth & Deedwania, 2011).

The advantages of the TG/HDL ratio as a screening tool include: a) no additional expense for testing, b) simple calculation, c) no additional client discomfort, and d) minimal training if performed by person other than the provider. The purpose of this study was to evaluate the performance characteristics of the TG/HDL ratio when used as a screening tool to detect increased risk of insulin resistance in the U.S. adolescent population. A potential screening scheme is depicted in Figure 5.1.

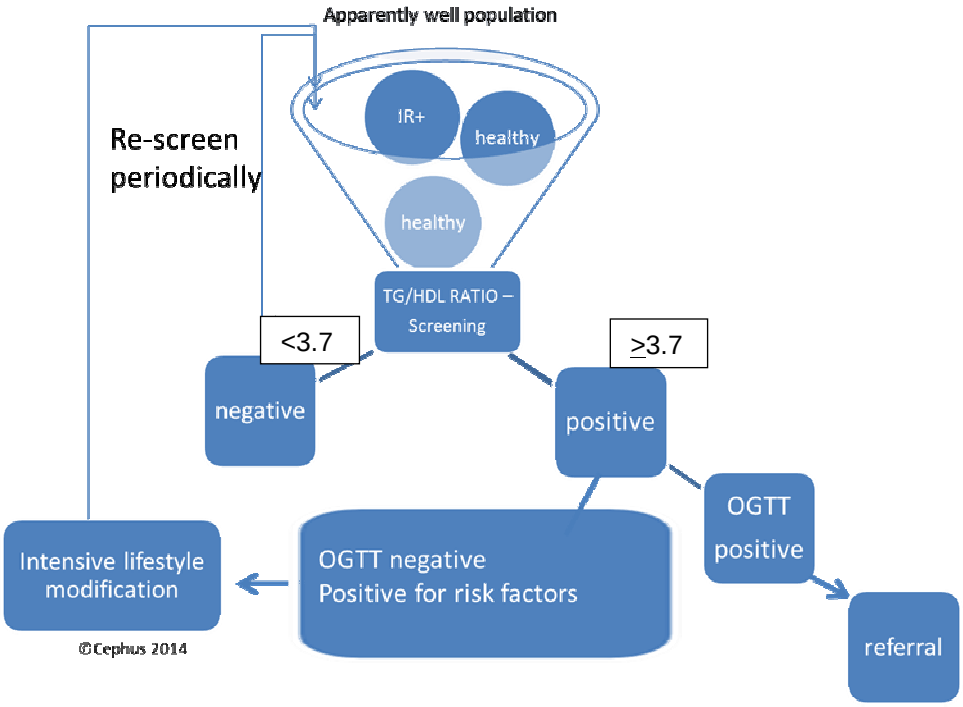

Figure 5.1 Screening for insulin resistance

The usefulness of the TG/ HDL ratio for detection of insulin resistance is dependent on test sensitivity, specificity and positive and negative predictive values. In this study, the sensitivity of the TG/HDL ratio was 14.8%, which indicated that the ability to detect insulin resistance was low. The specificity and negative predictive value were high (93.8%, 95.8% respectively) which suggested that the TG/HDL ratio may be useful for determining that individuals are unlikely to have insulin resistance (Florkowski, 2008). An individual who tests positive should receive additional evaluation. The positive predictive value was low (10.4%), indicating that an individual with a positive test result is unlikely to be truly positive. However, a clinician must be mindful that insulin resistance in children has a clinical spectrum that includes hormonal imbalance, elevation in inflammatory markers, and destruction of beta cells (Eyzaguirre & Mericq, 2009; Weiss,

2007). During the period prior to diagnosis, risk of micro and microvascular complications usually associated with diabetes is high (Harris & Eastman, 2000; Nichols, Arondekar, & Herman, 2008). Urbina and colleagues (2013) found that the TG/HDL ratio was an independent predictor of arterial stiffness in adolescents and young adults. This supports the work of Di Bonito and colleagues (2012) who found increased blood pressure in children with elevated TG/HDL ratios. Hence, further investigation, after positive results, is likely warranted (Figure 5.1).

The TG/HDL cholesterol has many advantages for use in clinical practice. It addresses two major health problems in the time-period prior to the onset of symptoms. Therefore, it is useful for primordial and primary prevention of diabetes and cardiovascular disease. The performance characteristics of the TG/HDL ratio suggested that a negative test, in the absence of other risk factors, would support usual healthy lifestyle recommendations. A positive test, in the presence of other risk factors, would support intensive therapy to improve lifestyle, further diagnostic testing, and possible medical therapy. Full use of the TG/HDL ratio is hampered by inability to determine a universal cut-off value to indicate increase risk of insulin resistance in all populations. In addition, the lack of a universal definition of insulin resistance in adolescents and children continues to impede momentum in addressing this problem.

Implications for nursing education and practice

Prevention of disease and promotion of health are pillars of nursing practice. Interventions to prevent cardiovascular disease and diabetes should begin with healthy lifestyle education. Cholesterol education should be incorporated into the pathophysiology and nutrition curricula for nursing students. Education of bedside nursing providers could promote discussion of healthy lifestyle behaviors with patients and families.

Most of the population, including health care providers, are unaware of the implications of lipid results, particularly the HDL cholesterol and triglyceride levels. Universal screening for lipid disorders should result in lipid tests for many adolescents. The TG/HDL ratio would be an excellent tool to add to the every nurse's toolkit. Discussion of the TG/HDL ratio result would be an excellent place for the nurse provider to educate patients and families about cholesterol, and the importance of healthy lifestyle habits throughout the lifespan.

Implications for research

In this study, the TG/HDL ratio was shown to be useful for ruling out increased risk of insulin resistance in U.S. adolescents. However, the absolute cut-off value for the TG/HDL ratio remains uncertain because of the inability to use ROC analysis on weighted data. Efforts should be made to develop these statistical/computer capabilities to maximize the usefulness of weighted national samples such as the NHANES. Future studies should focus on discovering the absolute cut-off value for the TG/HDL ratio to detect increased risk of insulin resistance. With an established cut-off value for the TG/HDL ratio, researchers could conduct longitudinal studies with large samples to validate the effectiveness of the screening tool.

Summary

Insulin resistance develops years before the diagnosis of type 2 diabetes or cardiovascular disease becomes evident. Early intervention, in the form of primordial and primary prevention, is needed to decrease insulin resistance. Data from the NHANES survey were used to determine the performance characteristics of the TG/HDL ratio as an early indicator of risk for insulin resistance among adolescents seen in pediatric practices. The performance characteristics of the TG/HDL ratio compared favorably with

a surrogate goal standard test. The TG/HDL ratio had a strong negative predictive value with sufficient sensitivity and specificity to be useful in the clinical setting.

Appendix A

Supplemental Material

Institutional Review Board Study Approval Letter

UNIVERSITY OF TEXAS ARLINGTON | OFFICE OF RESEARCH ADMINISTRATION
REGULATORY SERVICES

Institutional Review Board
Notification of Exemption

October 6, 2014

Constance Cephus
Dr. Jennifer Gray
College of Nursing

Protocol Number: 2015-0017

Protocol Title: *TG/HDL ratio as a screening tool for insulin resistance in U.S. adolescents 12-19 years of age*

EXEMPTION DETERMINATION

The UT Arlington Institutional Review Board (IRB) Chair, or designee, has reviewed the above referenced study and found that it qualified for exemption under the federal guidelines for the protection of human subjects as referenced at Title 45CFR Part 46.101(b)(4).

- (4). Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available **or** if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

You are therefore authorized to begin the research as of **October 1, 2014**.

Pursuant to Title 45 CFR 46.103(b)(4)(iii), investigators are required to, "promptly report to the IRB ***any*** proposed changes in the research activity, and to ensure that such changes in approved research, during the period for which IRB approval has already been given, are **not initiated without prior IRB review and approval** except when necessary to eliminate apparent immediate hazards to the subject." Please be advised that as the principal investigator, you are required to report local adverse (unanticipated) events to the Office of Research Administration; Regulatory Services within 24 hours of the occurrence or upon acknowledgement of the occurrence. All investigators and key personnel identified in the protocol must have documented Human Subject Protection (HSP) Training on file with this office. Completion certificates are valid for 2 years from completion date.

The UT Arlington Office of Research Administration; Regulatory Services appreciates your continuing commitment to the protection of human subjects in research. Should you have questions, or need to report completion of study procedures, please contact Robin Dickey at 817-272-9329 or robind@uta.edu. You may also contact Regulatory Services at 817-272-3723 or regulatoryservices@uta.edu.

REGULATORY SERVICES
SERVICES

The University of Texas at Arlington, Center for Innovation
202 E. Border Street, Ste. 201, Arlington, Texas 76010, Box#19188
(T) 817-272-3723 (F) 817-272-5808 (E) regulatoryservices@uta.edu (W) www.uta.edu/rs

Permission to Adapt Figure

Subject: RE: permission to adapt figure

From: Diamandis, Dr. Eleftherios (ediamandis@mtsinai.on.ca)

To: cecephus@att.net;

Date: Sunday, October 19, 2014 7:46 AM

Permission granted

Good luck with your thesis

Yours sincerely,
Eleftherios P. Diamandis, MD, PhD, FRCP(C),FRSC

Hold'em for Life Chair in Prostate Cancer Biomarkers
Head of Clinical Biochemistry,
Mount Sinai Hospital and University Health Network
Professor & Head, Division of Clinical Biochemistry,
Dept. of Laboratory Medicine & Pathobiology, University of Toronto

Mailing Address
Department of Pathology and Laboratory Medicine, Mount Sinai Hospital
6th Floor, Room 6-201,Box 32,60 Murray Street,Toronto,Ontario,Canada M5T 3L9
Tel:(416) 586-8443;Mobile:(416) 505-2844;Fax:(416)619-5521
Mail to:ediamandis@mtsinai.on.ca
Please visit our website:http://www.acdclab.org

From: Constance Cephus [mailto:cecephus@att.net]
Sent: October 18, 2014 12:37 PM
To: Diamandis, Dr. Eleftherios
Subject: permission to adapt figure

Dear Dr. Diamandis,

I am finishing my PhD dissertation and would like to adapt the figure in Box1 of the 2008 article in Nature Clinical Practice Oncology. Of course, I would give credit as usual. I need to take out the words 'tumor marker'. I would not use the wording that is above the figure in the box. I would like to have your permission to adapt this figure.

Respectfully,

Constance Cephus

PhD Candidate

University of Texas at Arlington

Arlington, Texas, USA

References

Abbasi, F., & Reaven, G.M. (2011). Comparison of two methods using plasma triglyceride concentration as a surrogate estimate of insulin action in nondiabetic subjects: triglycerides x glucose versus triglyceride/high-density lipoprotein cholesterol. *Metabolism Clinical and Experimental, 60*, 1673-1676. doi: 10.1016/j.metabol.2011.04.006

Alligier, M., Gabert, L., Meugnier, E., Lambert-Porcheron, S., Chanseaume, E., Pilleul, F., … Laville, M. (2013). Visceral fat accumulation during lipid overfeeding is related to subcutaneous adipose tissue characteristics in healthy men. *Journal of Clinical Endocrinology & Metabolism, 98*(2), 802-810. doi: 10.1210/jc.2012-3289

Amiel, S.A., Caprio, S., Sherwin, R.S., Plewe, G., Haymond, M.W., & Tamborlane, W.V. (1991). Insulin resistance of puberty: A defect restricted to peripheral glucose metabolism. *Journal of Clinical Endocrinology and Metabolism, 72*(2), 277-282. doi:10.1210/jcem-72-2-277

Amiel, S.A., Sherwin, R.S., Simonson, D.C., Lauritano, A.A., & Tamborlane, W.V. (1986). Impaired insulin action in puberty: a contributing factor to poor glycemic control in adolescents with diabetes. *New England Journal of Medicine, 315*(4), 215-219. doi: 10.1056/NEJM198607243150402

Arner, P. (2003). The adipocyte in insulin resistance: Key molecules and the impact of the thiazolidinediones. *Trends in Endocrinology and Metabolism, 14*(3), 137-145. doi:10.1016/S1043-2760(03)00024-9

Bacha, F., Gungor, N., Saad, R., & Arslanian, S. (2006). Are obesity-related metabolic risk factors modulated by the degree of insulin resistance in adolescents*? Diabetes Care, 29*(7), 1599-1604. doi: 10.2337/dc06-0581

Ball, G.D., Huang, T.T.K., Gower, B.A., Cruz, M.L., Shaibi, G.Q., Weigensberg, M.J, & Goran, M.I. (2006). Longitudinal changes in insulin sensitivity, insulin secretion, and β-cell function during puberty. *Journal of Pediatrics, 148*, 16-22. doi: 10.1016/j.peds.2005.08.059

Bambs, C. & Reis, S.E. (2011). Embracing primordial prevention for ideal cardiovascular health. *Future Cardiology, 7*(4), 447-450. doi:10.2217/fca.11.38

Bao, W., Srinivasan, S.R, & Berenson, G.S. (1996). Persistent elevation of plasma insulin levels is associated with increased cardiovascular risk in children and young adults: The Bogalusa Heart Study. *Circulation, 93*, 54-59. doi: 10.1161/01.CIR.93.1.54

Bauer, U.E., Briss, P.A., Goodman, R.A., & Bowman, B.A. (2014). The health of Americans1: Prevention of chronic disease in the 21st century: Elimination of the leading preventable causes of premature death and disability in the USA. *Lancet, 384*, 45-52. doi: 10.1016/S0140-6736(14)60648-6

Bays, H.E., Toth, P.P., Kris-Etherton, P.M., Abate, N., Aronne, L.J., Brown, W.V., …Samuel, V.T. (2013). Obesity, adiposity, and dyslipidemia: A consensus statement from the National Lipid Association. *Journal of Clinical Lipidology, 7*(4), 304-383. doi: 10.1016/j.jacl.2013.04.001

Beck-Nielsen, H. (2012). The role of glycogen synthase in the development of hyperglycemia in type 2 diabetes – 'To store or not to store glucose, that's the question'. *Diabetes/Metabolism Research and Reviews, 28*, 635-644. doi: 10.1002/dmrr.2337

Bennett, B., Larson-Meyer, D.E., Ravussin, E., Volaufova, J., Soros, A., Cefalu, W.T. …Sothern, M. (2012). Impaired insulin sensitivity and elevated ectopic fat in

healthy obese vs. nonobese prepubertal children. *Obesity, 20*(2), 371-375. doi: 10.1038/oby.2011.264

Berenson, G.S., Srinivasan, S.R., & Bao, W. (1997). Precursors of cardiovascular risk in young adults from a biracial (Black-White) population: The Bogalusa Heart Study. *Annals of the New York Academy of Sciences, 811*, 189-198. doi: 10.1111/j.1749-6632.1997.tb48206.x

Berenson, G.S., Srinivasan, S.R., Bao, W., Newman, III, W.P., Tracy, R.E., & Wattigney, W.A. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults: The Bogalusa Heart Study. *The New England Journal of Medicine, 338*, 1650-1656. doi:10.1056/NEJM199806043382302

Berry, J.D., Dyer, A., Cai, S., Garside, D.B., Ning, H., Thomas, A., …Lloyd-Jones, D.M. (2012). Lifetime risks of cardiovascular disease. *The New England Journal of Medicine, 366*(4), 321-329. doi: 10.1056/NEJMoa1012848

Blackburn, P., Lemieux, I., Lamarche, B., Bergeron, J., Perron, P., Tremblay, G., …Despres, J. (2012). Hypertriglyceridemic waist: a simple clinical phenotype associated with coronary artery disease in women. *Metabolism Clinical and Experimental, 61*, 56-64. doi: 10.1016/j.metabol.2011.05.017

Bloch, C.A., Clemons, P., & Sperling, M.A. (1987). Puberty decreases insulin sensitivity. *Journal of Pediatrics, 110*, 481-487. Retrieved from www.jpeds.com

Boden, G. (2011). Obesity, insulin resistance, and free fatty acids. *Current Opinion in Endocrinology, Diabetes & Obesity, 18*,139-143. doi: 10.1097/MED.0b013e3283444b09

Bonora,E., Saggiani, F., Targher, G., Zenere, M.B., Alberiche, M., Monauni, T., …Muggeo, M. (2000). Homeostasis Model Assessment closely mirrors the

Glucose Clamp Technique in the assessment of insulin sensitivity: Studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care, 23*(1), 57-63. doi: 10.2337/diacare.23.1.57

Brandou, F., Brun, J-F. & Mercier, J. (2005). Limited accuracy of surrogates of insulin resistance during puberty in obese and lean children at risk for altered glucoregulation. *The Journal of Clinical Endocrinology & Metabolism, 90*(2), 761-767. Retrieved from http://www.endo-society.org

Bray, G.A. (2012). Fructose and risk of cardiometabolic disease. *Current Atherosclerosis Reports, 14*, 570-578. doi: 10.1007/s11883-012-0276-6

Brumbaugh, D.E., Crume, T.L., Nadeau, K., Scherzinger, A., & Dabelea, D. (2012). Intramyocellular lipid is associated with visceral adiposity, markers of insulin resistance, and cardiovascular risk in prepubertal children: The EPOCH study. *Journal of Clinical Endocrinology & Metabolism, 97*(7), E1099-E1105. doi:10.1210/jc.2011-3243

Cali, A.M., & Caprio, S. (2009). Ectopic fat deposition and the metabolic syndrome in obese children and adolescents. *Hormone Research in Paediatrics, 71*(suppl 1), 2-7. doi: 10.1159/000178028

Cali, A.M., Zern, T.L., Taksali, S.E., de Oliveira, A.M., Dufour, S., Otvos, J.D. Caprio, S. (2007). Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents. *Diabetes Care, 30*(12), 30093-3098. doi: 10.2337/dc07-1088

Caprio, S., Plewe, G., Diamond, M.P., Simonson, D.C., Boulware, S.D., Sherwin, R.S., & Tamborlane, W.V. (1989). Increased insulin secretion in puberty: a compensatory response to reductions in insulin sensitivity. *Journal of Pediatrics, 114*, 963-967. Retrieved from www.jpeds.com

Capurso, C., & Capurso, A. (2012). From excess adiposity to insulin resistance: The role of free fatty acids. *Vascular Pharmacology, 57*, 91-97. doi: 10.1016/j.vph.2012.05.003

Centers for Disease Control and Prevention, National Center for Health Statistics. (2013) Department of Health and Human Services, *National Health and Nutrition Examination Survey Data, 2011-2012*. Retrieved from www.cdc.gov/nchs/nhanes.htm

Centers for Disease Control and Prevention (2002). *2000 CDC Growth Charts for the United States: Methods and Development*, Vitals and Health Statistics. National Center for Health Statistics, Series 11, Number 246. Retrieved from www.cdc.gov/nchs/nhanes.htm

Centers for Disease Control and Prevention, Department of Health and Human Services, (2009a). *Anthropometry Procedure Manual.* Retrieved from www.cdc.gov/nchs/nhanes.htm

Centers for Disease Control and Prevention, Department of Health and Human Services, (2009b). *Laboratory Procedure Manual*. Retrieved from www.cdc.gov/nchs/nhanes.htm

Centers for Disease Control and Prevention, Department of Health and Human Services, (2009c). *OGTT: Trutol Administration*. Retrieved from www.cdc.gov/nchs/nhanes.htm

Centers for Disease Control and Prevention, Department of Health and Human Services, (2014). *National Diabetes Statistics Report,* Retrieved from http://www.cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web.pdf

Chiang, J-K., Lai, N-S., Chang, J-K., & Koo, M. (2011). Predicting insulin resistance using the triglyceride-to-high-density lipoprotein cholesterol ratio in Taiwanese adults.

Cardiovascular Diabetology, 10, 93. Retrieved from http://www.cardiab.com/content/10/1/93

Cochrane, A.L., & Holland, W.W. (1971). Validation of screening procedures. *British Medical Bulletin, 27*(1), 3-8. Retrieved from http://bmb.oxfordjournals.org

Colak, E., Mutlu, F., Bal, C., Oner, S., Ozdanmar, K., Gok, B., & Cavusoglu, Y. (2012). Comparison of semiparametric, parametric, and nonparametric ROC analysis for continuous diagnostic tests using a simulation study and acute coronary syndrome data. *Computational and Mathematical Methods in Medicine*, online publication. doi: 10.1155/2012/698320

Cole, T.J., Bellizzi, M.C., Flegal, K.M., & Dietz, W.H. (2000). Establishing a standard definition for child overweight and obesity worldwide: International survey. *British Medical Journal, 320* (May 6), 1240. Retrieved from www.bmj.com

Coletta, D.K. & Mandarino, L.J. (2011). Mitochondrial dysfunction and insulin resistance from the outside in: Extracellular matrix, the cytoskeleton, and mitochondria. *American Journal of Physiology – Endocrinology and Metabolism, 301*, E749-E755. doi:10.1152/ajpendo.00363.2011

Conwell, L.S., Brown, W.J., Trost, S.G., & Batch, J.A. (2004). Indexes of insulin resistance and secretion in obese children and adolescents. *Diabetes Care, 27*(2), 314-319. Retrieved from http://care.diabetesjournals.org/

Cook, J.S., Hoffman, R.P, Stene, M.A., & Hansen, J.R. (1993). Effects of maturational stage on insulin sensitivity during puberty. *Journal of Clinical Endocrinology and Metabolism, 77*(3), 725-730. doi:10.1210/jc.77.3.725

Cook, S., Auinger, P., & Huang, T.K. (2009). Growth curves for cardio-metabolic risk factors in children and adolescents. *The Journal of Pediatrics, 155*(Suppl. 6), e15-e26. doi: 10.1016/j.jpeds.2009.04.051

D'Adamo, E. & Caprio, S. (2011). Type 2 diabetes in youth: epidemiology and pathophysiology. *Diabetes Care, 34*(suppl 2), S161-S165. doi: 10.2337/dc11-S212

d'Annunzio, G., Vanelli, M., Pistoria, A., Minuto, N., Bergamino, L., Iafusco, D., Lorini, R., and the Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetes (ISPED) (2009). Insulin resistance and secretion indexes in healthy Italian children and adolescents: A multicenter study. *Acta Biomedica, 80*, 21-28. Retrieved from www.actabiomedica.it

da Conceicao-Machado, M.E., Silva, L.R., Santana, M.L., Pinto, E.J., Silva, R.R., Moraes, L.T., …Assis, A.M. (2013). Hypertriglyceridemic waist phenotype: Association with metabolic abnormalities in adolescents. *Jornal de Pediatria, 89*(1), 56-63 doi: 10.1016.j.jped.2013.02.009

da Luz, P.L., Favarato, D., Faria-Neto, Jr, J.R., Lemos, P, & Chagas, A.C.P. (2008). High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease. *Clinics, 63*, 427-432. doi: 10.1590/S1807-593220080000-400003

Data Resource Center for Child and Adolescent Health, *National Survey of Children's Health, NSCH 2011/12,* Child and Adolescent Health Measurement Initiative, Retrieved from www.childhealthdata.org

de Ferranti, S.D., Gauvreau, K. Ludwig, D.S., Neufeld, E.J., Newburger, J.W. & Rifai, N. (2004). Prevalence of the metabolic syndrome in American adolescents: findings from the Third National Health and Nutrition Examination Survey. *Circulation, 110*(16), 2494-2497. doi:10.1161/01.CIR.0000145117.40114.C7

DeFronzo, R.A., Tobin, J.D., & Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *American Journal of Physiology – Endocrinology and Metabolism, 237*, E214-E223.

Delwiche, L.D., & Slaughter, S.J. (2008). *The Little SAS Book*, 4th ed. Cary, NC: SAS Institute

Despres, J.P. (2006). Is visceral obesity the cause of the metabolic syndrome? *Annals of Medicine, 38*(1), 42-51. doi: 10.1080/07853890500415358

Di Bonito, P., Moio, N., Scilla, C., Cavuto, L., Sibilio, G., Sanguigno, E., …Capaldo, B. (2011). Usefulness of the high triglyceride-to-HDL cholesterol ratio to identify cardiometabolic risk factors and preclinical signs of organ damage in outpatient children. *Diabetes Care, 35*, 158-162. doi: 10.2337/dc11-1456

Dixon, D.B., Steffen, L.M., Kornblum, A., & Steinberger, J. (2013). Implementation of pediatric lipid screening guidelines among primary pediatric providers [Abstract]. *Journal of Clinical Lipidology, 7,* 261-262.

Eckel, R.H., Grundy, S.M. & Zimmet, P.Z. (2005). The metabolic syndrome. *The Lancet, 365*, 1415-1428. doi:10.1016/S0140-6736(05)66378-7

Elliott, S.S., Keim, N.L., Stern, J.S., Teff, K. & Havel, P.J. (2002). Fructose, weight gain, and the insulin resistance syndrome. *American Journal of Clinical Nutrition, 76*(5), 911-922. Retrieved from http://ajcn.nutrition.org

Enos, W.F., Holmes, R.H., & Beyer, J. (1953). Coronary disease among United States soldiers killed in action in Korea. *Journal of the American Medical Association, 152*(12): 1090-1093. Retrieved from http://jama.jamanetwork.com

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. (2001). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). *Journal of the American Medical Association, 285*(19), 2486-2497. Retrieved from http://jama.jamanetwork.com

Eyzaguirre, F., & Mericq, V. (2009). Insulin resistance markers in children. *Hormone Research, 71*, 65-74. doi: 10.1159/000183894

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. *Journal of Pediatrics, 145*, 439-444. doi: 10.1016/j.jpeds.2004.06.044

Florkowski, C.M. (2008). Sensitivity, specificity, receiver-operating characteristic (ROC) curves and likelihood ratios: Communicating the performance of diagnostic tests. *The Clinical Biochemist Reviews, 29* (Supp 1), S83-S87. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/issues/172516/

Franssen, R., Monajemi, H., Stroes, E.S.G., & Kastelein, M.J.P. (2011). Obesity and dyslipidemia. *Medical Clinics of North America, 95*,893-902. doi: 10.1016/j.mcna.2011.06.003

Friis, R.H. & Sellers, T.A. (2009). *Epidemiology for public health practice*. Sudbury, MA: Jones and Bartlett.

Ganong, W.F. (2005). *Review of medical physiology*. New York: McGraw-Hill.

Gasevic, D., Frohlich, J., Mancini, G.B.J., & Lear, S.A. (2012). The association between triglyceride to high-density-lipoprotein cholesterol ratio and insulin resistance in a multiethnic primary prevention cohort. *Metabolism Clinical and Experimental, 61*, 583-589. doi: 10.1016/j.metabol.2011.09.009

Gavin, J.R., Alberti, K.G.M.M., Davidson, M.B., DeFronzo, R.A., and The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997). Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care, 20*(7), 1183-1197.

Geloneze, B, Vasques, A.C.J., Stabe, C.F.C., Pareja, J.C., Paez de Lima Rosado, L.E.F., de Queiroz, E.C., Tambascia, M.A. (2009). HOMA 1-IR and HOMA 2-IR indexes in identifying insulin resistance and metabolic syndrome – Brazilian Metabolic Syndrome Study. *Arquivos Brasileiros de Endocrinologia & Metabologia, 53*(2), 281-287. Retrieved from www.scielo.br

George, L., Bacha, F., Lee, S., Tfayli, H., Andreatta, E., & Arsianian, S. (2011). Surrogate estimates of insulin sensitivity in obese youth along the spectrum of glucose tolerance from normal to prediabetes to diabetes. *Journal of Clinical Endocrinology and Metabolism, 96*(7), 2136-2145. doi: 10.1210/jc.2010-2813

Giannini, C., Santoro, N., Caprio, S., Kim, G., Lartaud, D., Shaw, M., …Weiss, R. (2011). The triglyceride-to-HDL cholesterol ratio: Association with insulin resistance in obese youths of different ethnic backgrounds. *Diabetes Care, 34*, 1869-1874. doi:10.2337/dc10-2334

Go, A.S., Mozaffarian, D., Roger, V.L., Benjamin, E.J., Berry, J.D., Borden, W.B. …Turner, M.B. (2013). Heart disease and stroke statistics – 2013 update: A report from the American Heart Association. *Circulation, 127,*e6-e245. doi: 10.1161/CIR.0b013e31828124ad

Goldenberg, E. & Bathina, J. (2013). The adipocyte: Friend or foe? *Lipid Spin, 12*(1), 17-19. Retrieved from www.lipid.org

Goran, M.I. & Gower, B.A. (2001). Longitudinal study on pubertal insulin resistance. *Diabetes, 50*, 2444-2450. doi:10.2337/diabetes.50.11.2444

Greiner, M., Sohr, D., & Gobel, P. (1995). A modified ROC analysis for the selection of cut-off values and the definition of intermediate results of serodiagnostic tests. *Journal of Immunological Methods, 185*, 123-132. Retrieved from http://www.sciencedirect.com/science/journal/00221759

Grimes, D.A., & Schulz, K.F. (2002). Uses and abuses of screening tests. *The Lancet, 359*; 881-884. Retrieved from www.thelancet.com

Gungor, N., Saad, R., Janosky, J., & Arslanian, S. (2004). Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. *Journal of Pediatrics, 144*(1), 47-55. 10.1016/j.peds.2003.09.045

Gurol, Y., Akan, H., Izbirak, G., Teffanat, Z.T., Gunduz, T.S., Hayran, O., & Yilmaz, G. (2012). The sensitivity and the specificity of rapid antigen test in streptococcal upper respiratory tract infections. *International Journal of Pediatric Otorhinolaryngology, 74*, 591-593. doi: 10.1016/j.ijporl.2010.02.020

Hadaegh, F., Khalili, D., Ghasemi, A., Tohidi, M., Sheikholeslami, F., & Azizi, F. (2009). Triglyceride/HDL-cholesterol ratio is an independent predictor for coronary heart disease in a population of Iranian men. *Nutrition, Metabolism & Cardiovascular Diseases, 19*, 401-408. doi: 10.1016/j.numecd.2008.09.003

Haffner, S.M., Miettinen, H., & Stern, M.P. (1997). The homeostasis model in the San Antonio Heart Study. *Diabetes Care, 20*(7), 1087-1092. Retrieved from http://care.diabetesjournals.org/

Hajian-Tilaki, K. (2013). Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. *Caspian Journal of Internal Medicine, 4*(2), 627-635. Retrieved from www.caspjim.com

Hajian-Tilaki, K.O., Hanley, J.A., Joseph, L., &Collet, J-P. (1997). A comparison of parametric and nonparametric approaches to ROC analysis of quantitative diagnostic tests. *Medical Decision Making, 17*(1), 94-102. doi: 10.117/0272989X9701700111

Hannon, T.S., Bacha, F., Lee, S.J., Janosky, J., & Arslanian, S.A. (2006). Use of markers of dyslipidemia to identify overweight youth with insulin resistance. *Pediatric Diabetes, 7*, 260-266. doi: 10.1111/j.1399-5448.2006.00199.x

Har, D., Carey, M., & Hawkins, M. (2013). Coordinated regulation of adipose tissue macrophages by cellular and nutritional signals. *Journal of Investigative Medicine, 61*(6), 937-941. doi: 10.231/JIM.0b012e31829ceb39

Harris, M.I., & Eastman, R.C. (2000). Early detection of undiagnosed diabetes mellitus: a US perspective. *Diabetes/Metabolism Research and Reviews, 16*(4), 230-236. doi: 10.1002/1520-7560(2000)9999:9999<::AID-DMRR122>3.0.CO;2-W

Hayman, L.L., Meininger, J.C., Daniels, S.R., McCrindle, B.W., Helden, L., Ross, J., … & Williams, C.L.(2007). Primary prevention of cardiovascular disease in nursing practice: Focus on children and youth: A scientific statement from the American Heart Association Committee on Atherosclerosis, Hypertension and Obesity in Youth of the Council on Cardiovascular Disease in the Young, Council on Cardiovascular Nursing, Council on Epidemiology and Prevention, and Council on Nutrition, Physical Activity, and Metabolism. *Circulation, 116*, 344-357. doi: 10.1161/CIRCULATIONAHA.107.184595

Haynes, R.B. (1981). How to read clinical journals: II. To learn about a diagnostic test. *Canadian Medical Association Journal, 124*(6), 703-710. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/issues/138758/

Hermans, M.P., Ahn, S.A., & Rousseau, M.F. (2012). The atherogenic dyslipidemia ratio [log(TG)/HDL-C] is associated with residual vascular risk, beta-cell function loss and microangiopathy in type 2 diabetes females. *Lipids in Health and Disease, 11*, 132. Retrieved from http://www.lipidworld.com/content/11/1/132

Hobkirk, J.P., King, R.F., Gately, P., Pemberton, P., Smith, A., Barth, J.H., …Carroll, S. (2013). The predictive ability of triglycerides and waist (Hypertriglyceridemic Waist) in assessing metabolic triad change in obese children and adolescents. *Metabolic Syndrome and Related Disorders, 11*(5), 336-342. doi: 10.1089/met.2012.0152

Hofvind, S., Geller, B.M., Skelly, J., & Vacek, P.M. (2012). Sensitivity and specificity of mammographic screening as practiced in Vermont and Norway. *The British Journal of Radiology, 85*, 41226-e1232. doi: 10.1259/bjr/15168178

Hosker, J.P., Matthews, D.R., Rudenski, A.S., Burnett, M.A., Darling, P., Bown, E.G., & Turner, R.C. (1985). Continuous infusion of glucose with model assessment: Measurement of insulin resistance and β-cell function in man. *Diabetologia, 28*(7), 401-411. Retrieved from http://link.springer.com/journal/125

Hue, L., & Taegtmeyer, H. (2009). The Randle cycle revisited: A new head for an old hat. *American Journal of Physiology – Endocrinology and Metabolism, 297*, E578-E591. doi: 10.1152/ajpendo.00093.2009

Hurt, L, De Pinto, C., Watson, J., Grant, M., & Gielner, J. (2014). Diagnosis and screening for obesity-related conditions among children and teens receiving Medicaid – Maryland, 2005-2010. *Morbidity and Mortality Weekly Report, 63*(14), 305-308. Retrieved from www.cdc.gov/mmwr

Jaquith, B.C., Harris, M.A., & Penprase, B. (2013). Cardiovascular disease risk in children and adolescents. *Journal of Pediatric Nursing, 28*(3), 58-266. doi:10.1016/j.pedn.2012.11.003

Janssen, I, Katzmarzyk, P.T., Ross, R. (2004). Waist circumference and not body mass index explains obesity-related health risk. *American Journal of Clinical Nutrition, 79*(3), 379-384. Retrieved from ajcn.nutrition.org

Jeffery, A.N., Streeter, A.J., Metcalf, B.S., Voss, L.D., Hosking, J., & Wilkin, T.J. (2012). Age before stage: Insulin resistance rises before the onset of puberty. *Diabetes Care, 35*, 536-541. doi: 10.2337/dc11-1281

Johnson, C.L., Dohrmann, S.M., Burt, V.L., Mohadjer, L.K. (2014*). National Health and Nutrition Examination Survey: Sample design, 2011-2014.* National Center for Health Statistics. Vital Health Stat2, (162).Washington, DC: Government Printing Office. Retrieved from www.cdc.gov/nchs/nhanes.htm

Johnson, C.L., Paulose-Ram, R., Ogden, C.L., Carroll, M.D., Kruszan-Moran, D., Dohrmann, S.M., & Curtin, L.R. (2013). *National Health and Nutrition Examination Survey: Analytic guidelines, 1999-2010.* National Center for Health Statistics. Vital Health Stat 2(161) (DHHS Publication No. 2013-1361). Retrieved from http://www.cdc.gov/nchs/nhanes/analytic_guidelines.htm

Johnson, R.J. & Murray, R. (2010). Fructose, exercise and health. *Current Sports Medicine Reports, 9*(4): 253-258. doi: 10.1249/JSR.0b013e3181e7def4

Johnson, R.J., Segal, M.S., Sautin, Y., Nakagawa, T., Feig, D.I., Hang, D., …Sanchez-Lozada, L.G. (2007). Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *The American Journal of Clinical Nutrition, 86*(4): 899-906. Retrieved from http://acjn.nutrition.org

Kahn, S.E., Hull, R.L., & Utzschneider, K.M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature, 444*, 840-846. doi: 10.1038/nature05482

Kannel, W.B., Vasan, R.S., Keyes, M.J., Sullivan, L.M., & Robins, S.J. (2008). Usefulness of the triglyceride-high-density lipoprotein versus the cholesterol-high density lipoprotein ratio for predicting insulin resistance and cardiometabolic risk

(from the Framingham Offspring Cohort), *American Journal of Cardiology, 101*, 497-501. doi: 10.1016/j.am.card.2007.09.109

Kashyap, S.R., & DeFronzo, R.A. (2007). The insulin resistance syndrome: Physiological considerations. *Diabetes and Vascular Disease Research, 4*, 13-19. doi:10.3132/dvdr.2007.001

Katzmarzyk, P., 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-e205. Retrieved from http://www.pediatrics.org/cgi/content/full/114/2/e198

Keim, V., Teich, N., Fiedler, F., Hartig, W., Thiele, G., & Mossner, J. (1998). A comparison of lipase and amylase in the diagnosis of acute pancreatitis in patients with abdominal pain. *Pancreas, 16*(1), 45-49. Retrieved from http://journals.lww.com/pancreasjournal/toc/1998/01000

Khan, H., & Valdez, R. (2003). Metabolic risks identified by the combination of enlarged waist and elevated triacylglycerol concentration. *American Journal of Clinical Nutrition, 78*(5), 928-934. Retrieved from http://ajcn.nutrition.org

Khoury, M., Manlhiot, C., Dobbin, S., Gibson, D., Chahal, N., Wong, H., …McCrindle, B. (2012). Role of waist measures in characterizing the lipid and blood pressure assessment of adolescents classified by body mass index. *Archives of Pediatric and Adolescent Medicine, 166*(8), 719-724. doi: 10.1001/archpediatrics.2012.126

Khoury, P., Morrison, J.A., Kelly, K., Mellies, M., Horvitz, R., & Glueck, C.J (1980). Clustering and interrelationships of coronary heart disease risk factors in schoolchildren, ages 6-19. *American Journal of Epidemiology, 112*(4): 524-538. Retrieved from http://aje.oxfordjournals.org/content/112/4/524.

Kim, J.Y., van de Wall, E., Laplante, M., Azzara, A., Trujillo, M.E., Hofmann, S.M., … Scherer, P.E. (2007). Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *Journal of Clinical Investigation, 117*, 2621-2637. doi: 10.1172/JCI31021

Kim-Dorner, S-J., Deuster, P.A., Zeno, S.A., Remaley, A.T., & Poth, M. (2010). Should triglycerides and the triglycerides to high-density lipoprotein cholesterol ratio be used as surrogates for insulin resistance? *Metabolism Clinical and Experimental, 59*, 299-304. doi: 10.1016/j.metabol.2009.07.027

Klop, B., Elte, J.W.F., & Cabezas, M.C. (2013). Dyslipidemia in obesity: Mechanisms and potential targets. *Nutrients, 5*(4), 1218-1240. doi: 10.3390/nu5041218

Kulasingam, V., & Diamandis, E. (2008). Strategies for discovering novel cancer biomarkers through utilization of emerging technologies. *Nature Clinical Practice Oncology, 5*(10), 588-599. doi:10.1038/ncponc1187

Labarthe, D.R., Dai, S., Day, R.S., Fulton, J.E., & Grunbaum, J.A. (2009). Findings from Project HeartBeat!: Their importance for CVD prevention. *American Journal of Preventive Medicine, 37*(1S), S105-S115. doi: 10.1016/j.amepre.2009.04.013

LaMonte, M.J., Ainsworth, B.E., DuBose, K.D., Grandjean, P.W., Davis, P.G., Yanowitz, F.G., & Durstine, J.L. (2003). The hypertriglyceridemic waist phenotype among women. *Atherosclerosis, 171*(1), 123-130. doi: 10.1016/j.atherosclerosis.203.07.008

Lang, T.A. & Secic, M. (2006). *How to report statistics in medicine: Annotated guidelines for authors, editors, and reviewers* (2nd ed.). Philadelphia, PA: American College of Physicians

Lee, J.M., Davis, M.M., Woolford, S.J., & Gurney, J.G. (2009). Waist circumference percentile thresholds for identifying adolescents with insulin resistance in clinical

practice. *Pediatric Diabetes, 10*, 336-342. doi: 10.1111/j.1399-5448.2008.00474.x

Lee, J.M., Herman, W.H., Okumura, M.J., Gurney, J.G., & Davis, M.M. (2006). Prevalence and determinants of insulin resistance among U.S. adolescents. *Diabetes Care, 29*(11), 2427-2432. doi: 10.2337/dc06-0709

Leeflang, M.M.G., Bossuyt, P.M.M., & Irwig, L. (2009). Diagnostic test accuracy may vary with prevalence: Implications for evidence-based diagnosis. *Journal of Clinical Epidemiology, 62*(1), 5-12. doi: 10.1016/j.clinepi.2008.04.007

Leeflang, M.M.G., Rutjes, A.W.S., Reitsma, J.B., Hooft, L., & Bossuyt, P.M.M. (2013). Variation of a test's sensitivity and specificity with disease prevalence. *Canadian Medical Association Journal, 185*(11); e537-e544. doi: 10.1503/cmaj.121286

Lemieux, I., Pascot, A., Couillard, C., Lamarche, B., Tchernof, A, Almeras, N., …Despres, J. (2000). Hypertriglyceridemic waist: A marker of the atherogenic metabolic triad (hyperinsulinemia, hyperapolipoprotein B, small, dense LDL) in me? *Circulation, 102*, 179-184. doi: 10.1161/01.CIR.102.2.179

Lemieux, I., Poirier, P., Bergeron, J., Almeras, N., Lamarche, B., Cantin, B., …Despres, J. (2007). Hypertriglyceridemic waist: A useful screening phenotype in preventive cardiology? *Canadian Journal of Cardiology, 23*(Suppl. B), 23B-31B. Retrieved from www.onlinecjc.ca

Lieberman, M., Marks, A., & Peet, A. (2013). *Marks' basic medical biochemistry: A clinical approach.* Philadelphia, PA: Lippincott, Williams & Wilkins

Lusted, L.B. (1960). Logical analysis in roentgen diagnosis*. Radiology, 74*(2), 178-193. doi:10.1148/74.2.178

Malcom, G.T., Oalmann, M.C., & Strong, J.P. (1997). Risk factors for atherosclerosis in young subjects: The PDAY study. *Annals of the New York Academy of Sciences, 817*, 179-188. doi: 10.1111/j/1749-6632.1997.tb48205.x

Marotta, T., Russo, B., & Ferrara, L. (2010). Triglyceride-to-HDL-cholesterol ratio and metabolic syndrome as contributors to cardiovascular risk in overweight patients. *Obesity, 18*(8), 1608-1613. doi: 10.1038/oby.2009.446

Marshall, W.A. & Tanner, J.M. (1969). Variations in the pattern of pubertal changes in girls. *Archives of Disease in Children, 44*(235), 291-303. doi: 10.1136/adc.44.235.291

Marshall, W.A. & Tanner, J.M. (1970). Variations in the pattern of pubertal changes in boys. *Archives of Disease in Children, 45*(239), 13-23. doi: 10.1126/adc.45.239, 13-23

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., & Turner, R.C. (1985). Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412-419. Retrieved from http://link.springer.com/journal/125

McLaughlin, T., Abbasi, F., Cheal, K., Chu, J., Lamendola, C., & Reaven, G. (2003). Use of metabolic markers to identify overweight individuals who are insulin resistant. *Annals of Internal Medicine, 139*(10), 802-809. doi: 10.7326/0003-4819-139-10-200311180-00007

Mercurio, V., Carlomagno, G., Fazio, V., & Fazio, S. (2012). Insulin resistance: Is it time for primary prevention? *World Journal of Cardiology, 4*(1), 1-7. doi:10.4330/wjc.v4.i1.1

Metz, C.E. (1978). Basic principles of a ROC analysis. *Seminars in Nuclear Medicine, 8*(4), 283-298.

Mirel, L.B., Mohadjer, L.K., Dohrmann, S.M., Clark, J., Burt, V.L., Johnson, C.L., & Curtin, L.R. (2013*). National Health and Nutrition Examination Survey: Estimation procedures, 2007-2010.* National Center for Health Statistics. Vital Health Stat 2(159). (DHHS publication No. 2013-1359). Retrieved from www.cdc.gov/nchs/data/series/sr_02/sr02_159.pdf

Moran, A., Jacobs Jr., D.R., Steinberger, J, Cohen, P., Hong, C.P., Prineas, R. & Sinaiko, A.R. (2002). Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. *The Journal of Clinical Endocrinology & Metabolism, 87*(10), 4817-4820. doi: 10.1210/jc.2002-020517

Moran, A., Jacobs Jr., D.R., Steinberger, J, Hong, C.P., Prineas, R., Luepker, R., & Sinaiko, A.R. (1999). Insulin resistance during puberty: Results from clamp studies in 357 children. *Diabetes, 48*, 2039-2044. doi:10.2337/diabetes.48.10.2039

Morrison, J.A., Glueck, C.J., Horn, P.S., Schreiber, G.B., & Wang, P. (2009). Homeostasis model assessment of insulin resistance * body mass index interactions at ages 9 to 10 years predict metabolic syndrome risk factor aggregate score at ages 18 to 19 years: a 10-year prospective study of black and white girls. *Metabolism Clinical and Experimental, 58*, 290-295. doi: 10.1016/j.metabol.2008.09.027

Muniyappa, R, Lee, S. Chen, H. & Quon, M. (2007). Current approaches for assessing insulin sensitivity and resistance in vivo: Advantages, limitations, and appropriate usage. *American Journal of Physiology – Endocrinology and Metabolism, 294*, E15-E26. doi: 10.1152/ajpendo.oo645.2007

Murdock, D.K., Olson, K.J., Juza, R.M., & Hendricks, B.L. (2006). *The Journal of Cardiometabolic Syndrome, 1*, 242-247. Retrieved from www.lejacq.com

Musso, C., Graffigna, M., Soutelo, J., Honfi, M., Ledesma, L., Miksztowicz, V., …Berg, G.A. (2011). Cardiometabolic risk factors as apolipoprotein B, triglyceride/HDL-cholesterol ratio and C-reactive protein, in adolescents with and without obesity: Cross-sectional study in middle class suburban children. *Pediatric Diabetes, 12*, 229-234. doi: 10.111/j.1399-5448.2010.00710.x

Narasimhan, S. & Weinstock, R.S. (2014). Youth-onset type 2 diabetes mellitus: Lessons learned from the TODAY study. *Mayo Clinic Proceedings, 89*(6), 806-816. doi:10.1016/j.mayocp.2014.01.009

Nguyen, Q.M., Srinivasan, S.R., Xu, J-H., Chen, W., Kieltyka, L., & Berenson, G.S. (2010). Utility of childhood glucose homeostasis variables in predicting adult diabetes and related cardiometabolic risk factors. *Diabetes Care, 33*(3), 670-675. doi: 10.2337/dc09-1635.

Nichols, G.A., Arondekar, B, & Herman, W.H. (2008). Complications of dysglycemia and medical costs associated with nondiabetic hyperglycemia. *The American Journal of Managed Care, 14*(12), 791-798. Retrieved from www.ajmc.com

Ogden, C. & Carroll, M. (2010*). Prevalence of obesity among children and adolescents: United States, trends 1963-1965 through 2007-2008.* NCHS Health E-Stat Retrieved from National Center for Health Statistics website: www.cdc.gov/nchs/data/hestat/obesity_child_07_08/obesity_07_08.htm

Olson, K., Hendricks, B., & Murdock, D.K. (2012). The triglyceride to HDL ratio and its relationship to insulin resistance in pre- and post-pubertal children: Observation from the Wausau SCHOOL Project. *Cholesterol,* Article ID 794252, pp.4 doi: 10.1155/2012/794252

Pan, D.A., Lillioja, S., Kriketos, A.D., Milner, M.R., Baur, L.A., Bogardus, C., …Storlien, L.H. (1997). Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes, 46*, 983-988. doi:10.2337/diab.46.6.983

Quijada, Z., Paoli, M., Zerpa, Y., Camacho, N., Cichetti, R., Villarroel, V., …Lanes, R. (2008). The triglyceride/HDL-cholesterol ratio as a marker of cardiovascular risk in obese children; association with traditional and emergent risk factors. *Pediatric Diabetes, 9*, 464-471. doi: 10.1111/j.1399-5448.2008.00406.x

Randle, P.J., Garland, P.B., Hales, C.N., & Newsholme, E.A. (1963). The glucose fatty-acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *The Lancet, 281*(7285), 785-789. doi: 10.1016/S0140-6736(63)91500-9

Roden, M., Price, T.B., Perseghin G., Petersen, K.F., Rothman, D.L., …Shulman, G.I. (1996). Mechanism of free fatty acid-induced insulin resistance in humans. *Journal of Clinical Investigation, 97*(12), 2859-2865. doi:10.1172/JCI118742

Roehrig, C.S. & Rousseau, D.M. (2010). The growth in cost per case explains far more of US health spending increases than rising disease prevalence. *Health Affairs, 30*(9): 1657-1663. doi: 10.1377/hltaff.2010.0644

Saltiel, A.R. & Kahn, C.R. (2001). Insulin signaling and the regulation of glucose and lipid metabolism. *Nature, 414*, 799- 806. doi:10.1038/414799a

Samuel, V., Petersen, K.G., & Shulman, G.I. (2010). Lipid-induced insulin resistance: Unraveling the mechanism, *The Lancet, 375*, 2267-2277. doi:10.1016/S0140-6736(10)60408-4

Schipper, H. Prakken, B. Kalkhoven, E, & Boes, M. (2012). Adipose tissue – resident immune cells: key players in immunometabolism. *Trends in Endocrinology and Metabolism, 23*(8), 407-415. doi: 10.1016/j.tem.2012.05.011

Schwartz, B., Jacobs, D.R., Moran, A., Steinberger, J.S., Hong, C.P., & Sinaiko, A.R. (2008). Measurement of insulin sensitivity in children. *Diabetes Care, 31*(4), 783-788. doi: 10.2337/dc07-1376

Sesti, G. (2006). Pathophysiology of insulin resistance. *Best Practice & Research: Clinical Endocrinology & Metabolism, 20*(4):665-679. doi: 10.1016/j.beem.2006.09.007

Shah, A.S., Dolan, L.M., Gao, Z., Kimball, T.R., & Urbina, E.M. (2011). Clustering of risk factors: A simple method of detecting cardiovascular disease in youth. *Pediatrics, 127*, e312-e318. doi: 10.1542/peds.2010-1125

Shulman, G.I. (2000). Cellular mechanisms of insulin resistance. *The Journal of Clinical Investigation, 106*(2): 171-176. doi:10.1172/JCI10583

Singh, B. & Saxena, A. (2010). Surrogate markers of insulin resistance: A review. *World Journal of Diabetes, 1*(2), 36-47. doi: 10.4239/wjd.v1.i2.36

Smoak, C.G., Burke, G.L., Webber, L.S., Harsha, D.W., Srinivasan, S.R. & Berenson, G.S. (1987). Relationship of obesity to clustering of cardiovascular disease risk factors in children and young adults: The Bogalusa Heart Study. *American Journal of Epidemiology, 125*(3): 364-372.

Somerville, M., Kumaran, K., & Anderson, R. (2012). *Public health and epidemiology at a glance.* Hoboken, NJ: John Wiley & Sons, Ltd.

Spolidoro, J.V., Pitrez Filho, M.L., Vargas, L.T., Santana, J.C., Pitrez, E., Hauschild, J.A., …Piva, J.P. (2013). Waist circumference in children and adolescents correlate with metabolic syndrome and fat deposits in young adults. *Clinical Nutrition, 32*(1), 93-97. doi: 10.1016/j.clnu.2012.05.020

Srikanth, S., & Deedwania, P. (2011). Primary and secondary prevention strategy for cardiovascular disease in diabetes mellitus. *Cardiology Clinics, 29*, 47-70. doi: 10.1016/j.ccl.2010.11.004

Stanhope, K.L., Griffen, S.C., Bair, B.R., Swarbrick, M.M., Keim, N.L., & Havel, P.J. (2008). Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. *American Journal of Clinical Nutrition, 87*, 1194-1203. Retrieved from http://ajcn.nutrition.org

Stanhope, K.L., Schwarz, J.M., Keim, N.L., Griffen, S.C., Bremer, A.A., Graham, J.L., …Havel, P.J. (2009). Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *The Journal of Clinical Investigation, 119*, 1322-1334. doi: 10.1172/JCI37385

Strausser, T. (1980). Prevention in childhood of major cardiovascular diseases of adults. In: Falkner, F. (Ed.), *Prevention in childhood of health problems in adult life*. Geneva: WHO

Strong, J.P., Malcom, G.T., Oalmann, M.C., & Wissler, R.W. (1997). The PDAY study: Natural history, risk factors, and pathobiology. *Annals of the New York Academy of Sciences, 811*,226-235. doi: 10.1111/j.1749-6632.1997.tb2004.x

Strong, J.P. & McGill, Jr., H.C (1962). The natural history of coronary atherosclerosis. *American Journal of Pathology, 40*(1): 37-49. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1949572/pdf/amjpathol00326-0044.pdf

Strong, J.P. & McGill, Jr., H.C. (1969). Pediatric aspects of atherosclerosis. *Journal of Atherosclerosis Research, 9*(3): 251-265. Retrieved from

http://www.sciencedirect.com.ezproxyhost.library.tmc.edu/science/journal/03681319/9/3

Sumner, A.E., Finley, K.B., Genovese, D.J., Criqui, M.H., Boston, R.C. (2005). Fasting triglyceride and the triglyceride-HDL cholesterol ratio are not markers of insulin resistance in African Americans. *Archives of Internal Medicine, 165*, 1395-1400. Retrieved from www.archinternmed.com

Sumner, A.E., Harman, J.L., Buxbaum, S.G., Miller III, B.V., Tambay, A.V., Wyatt, S.B., …Sarpong, D.F. (2010). The triglyceride/high-density lipoprotein cholesterol ratio fails to predict insulin resistance in African-American women: An analysis of Jackson Heart Study. *Metabolic Syndrome and Related Disorders, 8*(6), 511-514. doi: 10.1089/met.2010.0028

Tateya, S., Kim, F., & Tamori, Y. (2013). Recent advances in obesity-induced inflammation and insulin resistance. *Frontiers in Endocrinology, 4*(article 93), 1-14. doi: 10.3389/fendo.2013.00093

Tanko, L., Bagger, Y., Qin, G., Alexandersen, P., Larsen, P., & Christiansen, C. (2005). Enlarged waist combined with elevated triglycerides is a strong predictor of accelerated atherogenesis and related cardiovascular mortality in postmenopausal women. *Circulation, 111*(15), 1883-1890. doi: 10.1161/01.CIR.0000161801.65408.8D

Tobisch, B., Blatniczky, L., & Barkai, L. (2013). Cardiometabolic risk factors and insulin resistance in obese children and adolescents: Relation to puberty. *Pediatric Obesity*. Advance online publication. doi: 10.1111/j.2047-6310.00202.x

Travers, S.H., Jeffers, B.W., Bloch, C.A., Hill, J.O., & Eckel, R.H. (1995). Gender and Tanner stage differences in body composition and insulin sensitivity in early

pubertal children. *Journal of Clinical Endocrinology and Metabolism, 80*(1), 172-178.

Tresaco, B., Bueno G., Pineda, I., Moreno, L.A., Garagorri, J.M., & Bueno, M. (2005). Homeostatic model assessment (HOMA) index cut-off values to identify the metabolic syndrome in children*. Journal of Physiology and Biochemistry, 61*(2), 381-388. Retrieved from http://link.springer.com/journal/13105

U.S. Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute. (2012). *Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents* (NIH Publication 12-7486A). Retrieved from www.nhlbi.nih.gov/guidelines/cvd_ped/index.htm#guidelines

Urbina, E., Khoury, P., McCoy, C., Dolan, L., Daniels, S., Kimball, T. (2013). Triglyceride to HDL-C ratio and increased arterial stiffness in children, adolescents and young adults. *Pediatrics, 131*, e1082-e1090. doi: 10.1542/peds.2012-1726

Uwaifo, G.I., Fallon, E.M., Chin, J., Elberg, J., Parikh, S.J., & Yanovski, J.A. (2002). Indices of insulin action, disposal, and secretion derived from fasting samples and clamps in normal glucose-tolerant Black and White children. *Diabetes Care, 25*(11), 2081-2087. doi:10.2337/diacare25.11.2081

Virtue, S., & Vidal-Puig, A. (2008). It's not how fat you are, it's what you do with it that counts. *PLOS Biology, 6*(9), e237. doi: 10.1371/journal.pbio.0060237

Wang, M. & Fotsch, C. (2006). Small-molecule compounds that modulate lipolysis in adipose tissue: Targeting strategies and molecular classes. *Chemistry & Biology, 13*, 1019-1027. doi: 10.1016/j.chembiol.2006.09.010

Warnick, G.R., Kimberly, M.M., Waymack, P.P., Leary, E.T., & Myers, G.L. (2008). Standardization of measurements for cholesterol, triglycerides, and major lipoproteins. *Labmedicine, 39*(8), 481-490. Retrieved from www.labmedicine.com

Webber, L.S., Voors, A.W., Srinivasan, S.R., Frerichs, R.R., & Berenson, G.S. (1979). Occurrence in children of multiple risk factors for coronary artery disease: The Bogalusa Heart Study. *Preventive Medicine, 8*: 407-418.

Weintraub, W.S., Daniels, S.R., Burke, L.E., Franklin, B.A., Goff, D.C. Jr, Hayman, L.L., …Whitsel, L.P. (2011). Value of primordial and primary prevention for cardiovascular disease: A policy statement from the American Heart Association, *Circulation, 124*, 947-990. doi: 10.1161/CIR.0b013e3182285a81

Weiss, R., Dufour, S., Taksali, S.E., Tamborlane, W.V., Petersen, K.F., Bonadonna, R.C., …Caprio, S. (2003). *The Lancet, 362*(9388), 951-957. Retrieved from http://www.journals.elsevier.com/the-lancet

Weiss, R., Otvos, J.D., Sinnreich, R., Miserez, A.R., Kark, J.D. (2011). The triglyceride to high-density lipoprotein-cholesterol ratio in adolescence and subsequent weight gain predict nuclear magnetic resonance-measured lipoprotein subclasses in adulthood. *The Journal of Pediatrics, 158*, 44-50. doi: 10.1016/j.jpeds.2010.07.002

WHO Expert Committee. (1990). *Prevention in childhood and youth of adult cardiovascular diseases: Time for action.* Technical Report Series 792. Geneva: WHO Retrieved from http://whqlibdoc.who.int/trs/WHO_TRS_792.pdf

Willey, J., Rodriguez, C., Carlino, R., Moon, Y., Paik, M., Boden-Albala, B., …Elkind, M. (2011). Race-ethnic differences in the association between lipid profile components and risk of myocardial infarction: The Northern Manhattan Study. *American Heart Journal, 161*, 886-892. doi: 10.1016/j.ahj2011.01.018

Wilson, J.M.G. & Jungner, G. (1968). *Principles and practice of screening for disease*. (World Health Organization Public Health Papers, No. 34). Retrieved from http://whqlibdoc.who.int/php/WHO_PHP_34.pdf

Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., … Chen, H. (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. doi: 10.1172/JCI200319451

Yao, W. (2013). *Estimation of ROC curve with complex survey data*. (Doctoral dissertation). Available from ProQuest Dissertations and Theses database. (UMI No. 3550591)

Yeckel, C.W., Weiss, R., Dziura, J., Taksali, S.E., Dufour, S., Burgert, T.S., …Caprio, S. (2004). Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. *The Journal of Clinical Endocrinology & Metabolism, 89*(3), 1096-1101. Retrieved from http://www.endo-society.org

Yip, J., Facchini, G., & Reaven, G. (1998). Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. *Journal of Clinical Endocrinology & Metabolism, 83*(8), 2773-2776. Retrieved from http://press.endocrine.org

Yoshida, M., McKeown, N.M., Rogers, G., Meigs, J.B., Saltzman, E., D'Agostino, R., & Jacques, P.F. (2007). Surrogate markers of insulin resistance are associated with consumption of sugar-sweetened drinks and fruit juice in middle and older-aged adults. *The Journal of Nutrition, 137*, 2121-2127. Retrieved from http://jn.nutrition.org

Zweig, M.H., & Campbell, G. (1993). Receiver-operating characteristic (ROC) plots: A fundamental evaluation tool in clinical medicine. *Clinical Chemistry, 39*(4), 561-577.

Biographical Information

Constance Cephus, nurse practitioner, joined Texas Children's Hospital in 2002 where she divides her time between caring for critically ill children with heart disease and "healthy" children with elevated cholesterol. She is a clinical instructor in the Department of Pediatrics at Baylor College of Medicine. Ms. Cephus graduated from Alfred University in New York with a Bachelor of Science in Nursing and earned a Master of Science in Nursing from Loma Linda University in California. She returned to studies after moving to Houston and earned Post-Master's Certificates in primary and acute care pediatrics. She is board certified as a nurse practitioner in neonatology, pediatric primary and pediatric acute care. Ms. Cephus was a fellow at the NIH NINR's Summer Genetic Institute in 2009. She is a member of Sigma Theta Tau and Phi Kappa Phi Honor Societies. She received a Love of Learning Award from Phi Kappa Phi in 2012 and a Bond Fellowship award in 2014. Her current research interests include prevention, early identification and management of elevated cholesterol in children.