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Walden University

College of Health Sciences

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David Kershner

has been found to be complete and satisfactory in all respects,
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the review committee have been made.

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Walden University
2018

Abstract

Oral Glucose Insulin Secretion Test for Identifying Patients with Insulin Resistance

by

David Kershner

MSPA, MD

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

Walden University

August 2018

Abstract

Insulin resistance is an increasing public health issue with the current literature, suggesting reduced sensitivity of insulin leads to adult onset diabetes and associated downstream pathologies that reduce life expectancy. The main objectives of this study were to evaluate the ability of the Oral Glucose Insulin Secretion Test (OGIST) to identify insulin resistance and examine differences in the insulin sensitivity based on gender, age, and ethnicity. This study was supported by the insulin resistance theory which focuses on the reduced ability of insulin to bind to the cellular insulin receptor, reducing the sensitivity of insulin. The OGIST lab results of a total of 250 patients, aged 18–65, were included in this study from a major city in the midwestern United States. Binomial logistic regression was used to evaluate the relationship between the dependent variables and the validation independent variables and analyze the possible differences seen in insulin, proinsulin, C-peptide, and HbA1c with age. The OGIST demonstrated the ability to identify elevated levels of insulin, proinsulin, and C-peptide at the end of the first phase insulin secretion to glucose. The results of this study demonstrated patients with insulin resistance exhibited a greater reduction in insulin production with age compared to those without insulin resistance. There were no changes observed between gender or ethnicity. The OGIST was the only test that demonstrated the ability to identify the individual's insulin sensitivity, β-cell function, and progression to diabetes. The ability of the OGIST to identify both insulin resistance and β-cell function can contribute to positive social change by encouraging further research for the early diagnosis and treatment of insulin resistance and the reduction in adult onset diabetes.

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Chapter 1: Introduction to the Study

Background

Insulin resistance (IR) is characterized by the reduced response of target tissue to the polypeptide hormone insulin (DeFronzo 1988). The reduced response of the tissue to insulin is the major pathogenesis of Type 2 diabetes mellitus (T2DM; Cerf, 2013; DeFronzo, 1988; Lorenzo et al., 2010; Groop et al., 1996; Samuel & Shulman, 2012; Warram et al., 1990; Weir & Bonner-Weir; 2004). Insulin is produced by the pancreatic β-cells and is responsible for glucose uptake by tissues. Although the mechanism of IR is complex, the reduced skeletal muscle sensitivity to insulin is the primary defect leading to impaired glucose synthesis and insulin resistance. Skeletal muscle is primarily responsible for 80%–90% of postprandial glucose uptake mediated by insulin during euglycemic hyperinsulinemic conditions (Thiebaud et al., 1982). The glucose transporter isoform 4 (GLUT4) is responsible for the transport of glucose into the cell for phosphorylation in the skeletal muscle and adipose tissue. The translocation of GLUT4 to the cell surface occurs after insulin binds to the PPAR gamma receptor. The binding of insulin to the PPAR gamma receptor triggers the phosphorylation of the insulin receptor substrate (IRS)-1, p85 regulatory substrate, and p110 catalytic subunit which increases phosphatidylinositol-3,4,5 triphosphate. This, in turn, triggers the phosphorylation of the Akt substrate 160 which translocates GLUT4 to the cell surface (Cerf, 2013; DeFronzo, 1988; Groop et al., 2005; Olefsky & Saltiel, 2000; Reaven, 1988; Samuel & Shulman, 2013; Savage, 2005).

As complex as the insulin-stimulated glucose uptake is, any one abnormality of this cascade can lead to IR. Regardless of the etiology of IR, the reduced sensitivity of insulin by target cells results in a normal increased secretion of insulin by the β-cells to maintain glucose homeostasis. As the muscle tissue further becomes insulin resistant, postprandial glucose plasma concentrations rise further. This, in turn, further stimulates insulin response to maintain glucose levels. The stimulated oversecretion of insulin promotes pancreatic β-cell exhaustion/death with the progression of time, which results in chronic hyperglycemia and T2DM.

By the time impaired glucose tolerance or impaired fasting glucose (IFG) is elevated a significant amount of β-cell exhaustion has occurred (Butler et al., 2008; DeFronzo & Abdul-Ghani, 2011; DeFronzo & Muhammad, 2011; Groop et al., 1996; Lorenzo et al., 2010). It would then seem likely that identifying IR early in the individual to preserve β-cell function would be more successful in preventing T2DM than after cell injury has occurred. This problem creates the need for a simple test to identify IR in the individual for both preventative diagnosis and evaluation of treatment in those with the condition.

IR has been simply defined as the reduced biological effectiveness of insulin-requiring larger amounts to maintain normal glucose levels (Wallace & Matthews, 2002). This condition evolves from hyperinsulinemia, after meal consumption, to glucose intolerance and finally full diabetes. Metabolic syndrome is the result of IR and results in lipid and lipoprotein dysregulation, hyperinsulinemia, and hyperglycemia

which act individually or together to worsen the effects of the disease state progressively.

IR is responsible for the proinflammatory state found in metabolic syndrome. This is secondary to up-regulation of inflammatory adipokine tumor necrosis factor α, C-reactive protein, and interleukin 6 with a reduction in adiponectin (Avramoglu et al., 2006). The increased concentrations of these inflammatory mediators are not due to infection, tissue injury, or autoimmunity but secondary to the chronic release of inflammatory cytokines generated by adipocytes during oxidative stress form increased free fatty acids (FFA). The increased release of insulin drives serum glucose into the adipocyte for storage, increasing the production of FFA which increases the overexpression of inflammatory proteins (Hotamisligil, 2006). The overexpression of inflammatory proteins contributes to the reduction of the signaling pathways of insulin and increases lipid peroxidation.

The mechanisms of hypertension seen in patients with metabolic syndrome are thought to be secondary to obesity. Essential hypertension has been documented to be closely associated with abnormal metabolic effects of glucose intolerance, dyslipidemia, and obesity (Landsberg, 2001; Scott, 2003). While obesity played the strongest role in uncontrolled hypertension studies, increased insulin activates the Renin-Angiotensin System , increasing the production and release of angiotensinogen, AII, and overexpression of the AT1 receptor which increases vasoconstriction and blood pressure (Landsberg et al., 2001; Scott, 2003).

The atherogenic problem seen with metabolic syndrome is due to a combination of increased triglycerides, apolipoprotein B, small-dense low-density lipoprotein (LDL), with reduced high-density lipoprotein (HDL; Albertti et al., 2006). These changes are secondary to increased triglyceride production stimulated by hyperinsulinemia (Deedwania et al., 2006). As the triglycerides are increased and not used they are then converted to LDL cholesterol.

IR increases platelet aggregation by increasing serum fibrinogen in addition to increasing plasminogen activator inhibitor 1. Thrombin generation is also increased during hyperinsulinemia, which potentiates clot formation, leading to increased stroke and myocardial infarction found in patients with metabolic syndrome (Grundy et al., 2002). Aso et al. (2005) demonstrated that in the presence of metabolic syndrome and hyperlipidemia-elevated plasma concentrations of thrombin-activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor (PAI)-1 while inhibiting fibrinolysis. This finding was confirmed by Kraja et al. (2007) who also added that CRP, IL6 contributes with TAFI and PAI risk factors for the prothrombotic state in metabolic syndrome.

Problem Statement

Currently, there is no cost-effective, standardized test for the identification of IR in the context of clinical practice or for the use in large-scale screening in population-based studies. The euglycemic insulin clamp test is considered to be the best available benchmark test for the measuring insulin secretion and insulin action in vivo (DeFranzo et al., 1979). This technique requires the patient to be hospitalized overnight using the

administration of intravenous glucose to maintain serum glucose levels at the euglycemic concentration. The advantage is that is test primarily reflects the uptake of skeletal muscle, which is significant for evaluating IR during the first phase insulin response. The disadvantage of this test is the invasiveness requiring a hospital stay and significant cost involved. These disadvantages prohibit the test to be used in the clinical situation or population studies.

Other tests for IR include the insulin suppression test, oral glucose tolerance test, insulin tolerance test, insulin suppression test, and the frequently sampled intravenous glucose tolerance test with minimal model analysis. These tests are also invasive with the tests ranging from 2–5 hours in length and have a considerable cost involved. Although these tests provide data on skeletal muscle sensitivity to insulin, they also provide secondary hepatic insulin sensitivity and glucose production. Because of the importance of identifying IR and the disadvantages of this tests, surrogate tests were developed which include the homeostasis model (HOMA) and quantitative insulin-sensitivity check index (QUICKI). These tests can be performed under clinic conditions for patient diagnosis but are based on mathematical fasting insulin and glucose and are unable to generate data for skeletal muscle insulin sensitivity (Chen et al., 2004; Hanley et al., 2003; Matthews et al., 1985). The ability to predict IR in the normoglycemic patient is important for the success of any diabetes prevention program.

Purpose of the Study

The purpose of this study was to examine the Oral Glucose Insulin Secretion Test (OGIST), a novel algorithm designed and used to quantify IR in normoglycemic

individuals who otherwise meet the criteria for metabolic syndrome. In this study, I compared this surrogate measure for IR to three established surrogate markers. Serum glucose, insulin, proinsulin, and C-peptide levels are obtained and measured at the final stage of the first phase insulin secretion after a small glucose challenge. Results from the OGIST allow the direct evaluation of insulin sensitivity to target tissue and production of insulin by the β-cells. I received patient data from patient charts and EMR collected by the medical records department and laboratory information system for inclusion in this study.

Research Questions

The objective of this study was to answer two research questions. The research questions and their corresponding hypotheses follow:

RQ1: In patients meeting the criteria for metabolic syndrome will an Oral Glucose Insulin Secretion Test (OGIST) predict insulin resistance in the clinical setting?

$H_0 1$: In patients diagnosed with metabolic syndrome, the Oral Glucose Insulin Secretion Test (OGIST) does not predict insulin resistance in a clinical setting.

$H_A 1$: In patients diagnosed with metabolic syndrome, the Oral Glucose Insulin Secretion Test (OGIST) does predict insulin resistance in a clinical setting.

RQ2: Does age, gender, and ethnicity have an influence on the Oral Glucose Insulin Secretion Test (OGIST) for insulin, proinsulin, glucose, and C-peptide levels.

$H_0$2: The Oral Glucose Insulin Secretion Test (OGIST) does not identify changes in insulin, proinsulin, glucose, and C-peptide in different ages, gender, and ethnic groups.

H_A2: The Oral Glucose Insulin Secretion Test (OGIST) does identify changes in insulin, proinsulin, glucose, and C-peptide in different ages, gender, and ethnic groups.

Framework

The theoretical framework is a tested idea or group of ideas that provide the organizational background for the study and guides the interpretation of the results. Etiologic research is the search for the cause of the disease, the relationship to other factors, and the magnitude of effects of the disease. The causative relation of the disease is the unique postulated connection between the X factors which affects the Y result. The determinants resulting in disease affecting the individual result from causal factors and do not occur from random chance. The identification of these factors begins with an observational study to evaluate the relation between the affected individual, the causative agent, and the individual's environment. With a chronic disease, such as diabetes, the focus is on the relationship between the causative problem and the downstream physiological effects (McKenna & Collins, 2010). Ecological studies are descriptive, using observations from individuals to plot risk factors and outcome values to evaluate and assess the possible relationship of the condition studied. Ecological studies use aggregate, environmental, and global characteristics to identify risk-modifying factors and health outcomes.

I based this study on the theory of IR and insulin resistance syndrome (now known as metabolic syndrome), which was first proposed by Reaven (1988). Reaven's theory of IR initially described the relationship between the reduced ability of insulin to bind to the cellular receptor and stimulate the uptake of glucose for energy production. Reaven further reported the metabolic and physiologic differences observed in patients progressing to T2DM and the observed cardiovascular problems. The association between insulin and peripheral tissue during the postprandial first phase insulin response is the basis for the early detection of IR and β-cell dysfunction. In this study, I attempted to demonstrate the effectiveness of evaluating insulin sensitivity during the first phase insulin response to glucose using the OGIST. As an effective test for IR, the OGIST could potentiate the health action process approach by potentiating improved outcomes and reduce the progression of T2DM through both individual and medical understanding and treatment of IR (Scwarzer et al., 2003).

Nature of the Study

The nature of the study was a causal-comparative/quasi-experimental design using patient laboratory data from the OGIST used at an Omaha, NE area medical center. The main purpose of the OGIST is to identify patients with IR by directly measuring their insulin sensitivity after an initial small glucose challenge at the end of the first phase insulin secretion. The variables measured in the OGIST to identify insulin sensitivity include serum insulin, proinsulin, C-peptide, and glucose. Insulin sensitivity is identified by elevated serum levels of insulin, proinsulin, C-peptide, and glucose at the end of the first phase insulin secretion. The higher these levels are above

normal, the less sensitive the target tissue is for insulin. This allows the identification of IR in the individual long before β-cell exhaustion occurs. The variables used to evaluate β-cell function include insulin, proinsulin, C-peptide, glucose and HbA1c levels. β-cell exhaustion can then be identified with an inverse relationship between insulin, proinsulin, C-peptide, and HbA1c.

Definitions

Prediabetes: A medical condition in which not all findings are adequate to diagnose diabetes but are above normal. WHO criteria for fasting blood sugar are 110mg/dl to 125mg/dl. American Diabetes Association (ADA; 2014) criteria are 100mg/dl to 125mg/dl (Bansal, 2015).

Diabetes: All clinical categories of diabetes which include Type 1 diabetes mellitus, T2DM, gestational diabetes mellitus (GDM), and diabetes related to other causes, such as drug or chemical induced and secondary endocrine pathological conditions such as diabetes insipidus and cystic fibrosis (ADA, 2014).

Type 1 diabetes mellitus: Diabetes due to complete autoantibody destruction of the pancreatic β-cells as seen in Type 1A or Type 1B due to complete exhaustion and death of pancreatic β-cells (ADA, 2014).

Type 2 diabetes mellitus (T2DM): Diabetes due to progressive exhaustion and burn out of the pancreatic β-cells secondary to the reduced sensitivity of insulin to peripheral tissue from IR. T2DM is diagnosed based on plasma glucose levels with fasting plasma glucose (FPG) $\geq$126 mg/dl, HgA1c $\geq$6.5%, 2-hour glucose tolerance test of $\geq$200 mg/dl, or random plasma glucose of $\geq$200 mg/dl (ADA, 2014).

Gestational diabetes mellitus (GDM): Diabetes that is diagnosed using the T2DM criteria during pregnancy only with serum glucose levels reverting to normal after the birth of the child (ADA, 2014).

Impaired fasting glucose (IFG): An elevated fasting serum glucose concentration of ≥ 100 mg/dl and < 126 mg/dl (ADA, 2014).

Normal glucose tolerance (NGT): FPG of < 100 mg/dl and 2-hour glucose tolerance of < 140 mg/dl (ADA, 2014).

Insulin resistance (IR): The reduced response of target tissue to insulin, skeletal muscle, adipose, and liver tissue (ADA, 2014).

Metabolic syndrome: A clustering of risk factors for cardiovascular disease (CVD) in association with IR. The characteristics of metabolic syndrome include abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, and glucose intolerance. According to the NCEP-ATP III guidelines, any three of the above characteristics constitute metabolic syndrome (Grundy et al., 2004).

Assumptions

I made four major assumptions in this study and with the use of the OGIST. The first assumption was that the specific cross-section of patients recruited for this study was a true representation of the general U.S. population suffering from IR. Another assumption was that the individuals selected for the OGIST accurately met the criteria for metabolic syndrome. The third assumption was, since the OGIST is a timed test, that the blood samples were obtained at the appropriate time and issues, such as difficult venous access or short clinical staffing, did not skew the data used in the study by

extending the time in which the samples were obtained. My final assumption was that the medical records used to obtain the patient data were correct.

Assumptions of the comparison surrogate measures from fasting basal-steady-state insulin/glucose concepts. The HOMA, QUICKI, and McAuley's index rely on single fasting blood glucose, insulin, and/or triglycerides representing the basal-state-state of the individual. A major assumption of this type of study is that the individual is truly fasting and in a basal-steady-state. In those individuals that are unknowingly impaired glucose tolerant (IGT), with normal fasting glucose, the fasting insulin levels could be inappropriately low and insufficient to maintain normal glucose homeostasis during the first phase insulin response.

Limitations

One limitation of this study was my use of secondary data collected by nonresearch clinical personnel. The OGIST blood samples need to be drawn specifically 15 minutes after the patient has orally taken 36 grams of glucose to evaluate insulin, proinsulin, C-peptide, and glucose levels at the end of the first phase insulin response to glucose. Since the data from the OGIST are used for the specific purpose of identifying and treating IR during normal clinic hours, situations that impeded the staff from collecting the blood sample at the appropriate time could have skewed the test results.

Another possible limitation was that patients should have fasted prior to the test. Fasting dependability relies completely on the patient's truthfulness and perception of fasting prior to the test. The major limitation of the surrogate measures from fasting basal-steady-state insulin/glucose indices is the lack of standardization of the assays and

the inability to define universal cutoff points for IR. Another limitation existed in the inability of these surrogate indices to accurately measure the first phase insulin response which could accurately identify early IR in the normal glucose tolerant individual.

Significance

The American Heart Association Statistics Committee reported that in 2010 more than 8.3% of adults (over the age of 20 years of age) in the United States had been diagnosed with diabetes. An additional 3.5% of the adults had undiagnosed diabetes and more than 38.2% additional adults having prediabetes (Go et al., 2013). The 2003–2006 National Health and Nutrition Examination Survey estimated that nearly one third of adults in the United States have metabolic syndrome. IR has been cited as the initial problem responsible for T2DM secondary to the impaired tissue responsiveness found in skeletal muscle, adipose tissue, and the liver to the insulin molecule (Groop et al., 2005). IR not only leads to T2DM but significantly increases an individual's risk to CVD and death (Mottillo et al., 2010). The ADA estimated that the average cost to manage healthcare for one patient is $6,649 per year with a 15% increase in expenditure for those patients with the associated CVD. The ADA also determined that patients with metabolic syndrome alone incur $2,000 dollars more per year in health care costs than those patients without the condition.

The OGIST is the first true clinical test for identifying and evaluating the treatment of IR. By evaluating the efficacy of this test, patients with IR can be identified sooner within the clinical setting, provided with early tailored treatment with an evaluation of outcomes ensuring the best outcome and reduced progression to T2DM.

The early detection and treatment of patients with IR in the clinical setting allows for improved insulin sensitivity and β-cell function, reducing the progression of this disease to T2DM and the associated cardiovascular complications. According to the National Center for Chronic Disease Prevention and Health Promotion and the Centers for Disease Control and Prevention (CDC; 2011), there are nearly 131,776 Nebraskans with diagnosed T2DM and 100,224 additional Nebraskan with prediabetes. This equates to nearly $1,438,400,000 annual medical expense to treat T2DM and the associated CVD in these patients. By identifying IR and successfully reducing or preventing the progression to T2DM, a 20% reduction would equal a reduced annual T2DM patient volume of 46,400 with a cost savings of $287,680,000 for the state of Nebraska alone.

The OGIST provides several positive social change implications to include the early identification of patients with IR, the early and accurate treatment of IR, the reduced progression of IR to diabetes, and the reduced comorbid complications seen with diabetes such as heart disease. One of the most important changes providers will see when using the OGIST is the patient's actual insulin response to glucose at the end of the first phase insulin response to glucose. The results from OGIST will demonstrate whether the patient is overproducing insulin due to poor binding of insulin to the cells insulin receptor and the actual insulin production by the pancreatic β-cells. The actual insulin levels at the end of the first phase insulin response to glucose will lead the appropriate treatment for the patient and evaluation of treatment protocols. The OGIST will provide documentation of the individuals treatment and response for IR to

healthcare insurance companies improving the knowledge of IR and opening the doors for further research in this area.

Summary

The first step in preventing T2DM is to identify those individuals that are most susceptible to acquire the disease and are in need of aggressive lifestyle modification and medical treatment. Traditional models to reduce the environmental contribution in patients with T2DM are well understood and include age, blood pressure, body mass, and family history and have been validated using fasting glucose or glycated hemoglobin (A1C; Buijsse et al., 2011). The unfortunate fact is that these measures are designed to detect patients who have developed T2DM and will not identify IR early in the patient's life. By the time a patient is diagnosed with having hyperglycemia more than 80% of the pancreatic β-cell function is lost (DeFronzo & Muhammad, 2011). A significant advancement in this area has been made recently with the ADA establishing specific criteria for diagnosing prediabetes, which includes fasting glucose of 100 to 125 mg/dl, HgA1C of 5.7% to 6.4%, and 2-hour OGTT of 140 to 200 mg/dl. Although these tests are inexpensive, they can only identify prediabetic conditions after IR has progressed to the point of pancreatic β-cell strain and blood glucose levels are inadequately controlled. Consequently, there is a tremendous need for a simple diagnostic test for IR that can identify this large at-risk population to reduce the progression to T2DM and the associated morbidity and mortality.

I designed this study to evaluate the OGIST, which is currently being used for the early identification and treatment of IR to reduce the progression to T2DM in these

patients. This test was designed at the Joshua Medical Center and to my knowledge is the only clinic test used currently for IR. Chapter 2 will contain a review of the literature supporting the theory of IR, mechanisms, and the relationship between IR, metabolic syndrome, and T2DM.

Chapter 2: Literature Review

Introduction

The International Diabetes Federation estimated that as many as 175 million adults worldwide may have diabetes that is currently undiagnosed. In addition to the undiagnosed individuals with diabetes, an additional 316 million individuals are estimated to be IGT or have IFG. These conditions are recognized as having elevated serum glucose levels above normal and below that considered to be diabetic (International Diabetes Federation, 2015). This condition is considered prediabetic in that more than 70% of these patients advance to T2DM (Knowler et al., 2002; Santaguida et al., 2006; Vendrame & Gottlieb, 2014).

The progression of IR to IGT and early T2DM is insidious in that symptoms of the disease may not be noted by the individual for several years. Early symptoms of IR include hypoglycemia, weight gain, and those symptoms associated with polycystic ovarian syndrome in women; while those symptoms associated with T2DM include those of overt hyperglycemia.

The overproduction and release of insulin with time results in pancreatic β-cells becoming exhausted with reduced insulin production causing serum glucose levels to elevate slowly over time. This increase in serum glucose may be experienced as simple fatigue by the individual and later accepted as a normal feeling until other symptoms arise resulting in the need for medical attention. By the time an individual has been diagnosed with full-blown diabetes, they have lost nearly 80% of the β-cell function. Researchers have also suggested that at the early onset of β-cell exhaustion, the

individual likely has an associated CVD, which is the leading cause of death and a majority of health care costs for patients with diabetes (Butler et al., 2008). The field of public health is faced with this global epidemic-level increase in individuals diagnosed with diabetes and the large numbers of those with prediabetes unaware that they have the condition. The epidemiology of IR may be faced with several confounders in that the problems associated with diabetes, β-cell dysfunction, hyperlipidemia, hypertension, obesity, neuropathy, renal failure, and CVD, may in fact, be associated problems with an underlying condition responsible for diabetes.

IR, or the reduced sensitivity of insulin to target tissue, has recently been identified as the primary defect responsible for T2DM. The reduced tissue sensitivity to insulin causes the pancreatic β-cells to release larger amounts of insulin to maintain normal serum glucose levels. The overproduction of insulin over several years results in β-cell exhaustion and burn out, resulting in T2DM. Considering the epidemic increase in diabetic individuals globally and the magnitude of those individuals with prediabetes or IGT, the identification of insulin resistance early in life must take precedence in the public health sector. This chapter will contain pertinent literature on IR, T2DM, the progression of IR to diabetes, and current testing for IR.

Literature Search Strategy

I used a comprehensive approach during the literature search phase of this review to obtain all current studies, reports, and data pertaining to IR, IFG, IGT, and metabolic syndrome. The search was not limited to diagnosis, treatment, prognosis, age, gender, or ethnicity. By using this technique, I reduced the amount of missed data

important to the study. To locate literature for the review, I accessed the PubMed, MEDLINE, Medscape databases and used advanced Google Search. I also reviewed current textbooks on diabetes, endocrinology, internal medicine, IR, and metabolic syndrome. The following key research terms were used (as well as combinations of them) during my data search/inquiry: *insulin resistance, metabolic syndrome, insulin sensitivity, diabetes, T2DM, insulin resistance syndrome, reduced insulin sensitivity, type 2 diabetes primary defect, insulin action, insulin secretion, insulin resistance and obesity, childhood obesity, insulin testing, insulin resistance testing, oral glucose tolerance test, insulin clamp test, skeletal muscle resistance to insulin, biphasic insulin release, biphasic insulin response, pathogenesis of insulin resistance, maturity-onset diabetes of the young, impaired glucose tolerance,* and *impaired fasting glucose*. Initially, the data search was conducted for sources published from January 2000 to the present, but this limitation did not generate the needed data for the aforementioned subjects. I increased the limits of the search to sources published from 1998 to the present to obtain complete data on both IR and metabolic syndrome.

Epidemiology of Insulin Resistance

The epidemiology of a disease or condition is concerned with the distribution and determinants of a disease or health-related condition in specific populations and the application to control the related disease or condition (Remington et al., 2010). The determinants of a disease or health condition are the factors that decisively affect or influence the nature of the outcome of the condition. The epidemiology of IR is difficult to research due to the lack of a clinical test to diagnose and evaluate the condition on

large populations. The second issue with the epidemiology of IR is the lack of understanding or misunderstanding of the condition.

With the global increase in diabetes, the research focus has primarily been on diabetes as the primary chronic condition with the prevention efforts based on diet, exercise, obesity, and maintaining blood pressure and cholesterol. Studies designed to reduce cholesterol and hypertension and for an intense reduction in HbA1c levels have failed to demonstrate any significant improvement in cardiovascular outcomes in patients with T2DM (The ACCORD Study Group, 2010; The Action to Control Cardiovascular Risk in Diabetes Study Group, 2008; The Looking AHEAD Research Group, 2013). Considering the mechanism of IR and the IDF estimation of 316 million individuals with IGT, it would seem reasonable to concentrate epidemiological research on IR to identify and treat these individuals early in life before they progress to the prediabetes stage. By the time an individual progresses to prediabetes, they have a 60%–80% reduction in β-cell function and many demonstrate complications associated with full T2DM (Abdul-Ghani et al., 2006; Ferrannini et al., 2005). When lifestyle modifications and the use of metformin was studied in individuals who were diagnosed with prediabetes (Diabetes Prevention Program, 2014; Diabetes Prevention Program Research Group, 2002). The combination of lifestyle modification, increasing exercise, and improving diet with the use oral metformin daily improved prediabetic glucose and reduced the onset of T2DM by 58% (Diabetes Prevention Program Research Group, 2002; National Diabetes Information Clearinghouse, 2014). However close examination of DPP results on intense lifestyle codifications, motivation, and individual counseling

was found to be the most effective in ages 60 and older. The National Health and Nutrition Examination Survey's 2003–2006 report of one third of men and women having metabolic syndrome and the CDC's estimation of nearly 38% of those 20 years of age and older that have progressed to prediabetes strengthens the CDC's prediction of 1 in 3 Americans expected to be diagnosed with diabetes by 2050. This increase in diabetes would also suggest a significant increase in chronic vascular and inflammatory disease, which begins years prior the identification of prediabetes due to the tissues impaired responsiveness to insulin (Go et al., 2013).

More than 87 million adults in the United States over the age of 20 are affected with prediabetes with more than 50% having a lifetime risk of progressing to T2DM (DeFronzo & Abdul-Ghani, 2011; Go et al., 2013). Prediabetes patient numbers were obtained from the National Health and Nutrition Examination Survey, 2009–2012 and the National Diabetes Statistics Report (2014) from those people with blood glucose levels equal to that of IGT concentrations. Around 3% of the U.S. population is estimated to have IR based on small sample studies that used the HOMA-IR criteria for diagnosis (DeFronzo & Abdul-Ghani, 2011; Go et al., 2013; Lee et al., 2006; Viner et al., 2005). The differences noted between individuals estimated with prediabetes and IR would suggest a problem with diagnosing IR. The possible confusion may either be due to the lack of diagnostic criteria or diagnostic testing. These issues make the study of the distribution and determinants of IR frequency in human populations and any possible application to treat or control the problem difficult.

Worldwide determinates for IR are based on metabolic syndrome (Grundy, 2008; Moadab et al., 2010 Sarti & Gallager, 2006), and it is estimated that nearly a quarter of the world's population has IR. However, there was a large variance between populations, such as the percentage of individuals estimated with IR in Japan is 2.3%–7.8%, while in the Netherlands the percentage is 16%–46%. There were similar variations throughout Europe and South America that were thought to be due to marked differences in methodologies and diagnostic criteria (Moadab et al., 2010, Sarti & Gallager, 2006).

The age distribution is dependent more on associated symptoms of the disease. The growing obesity epidemic is seen to be increasing in all ages over the last 50 years, secondary to IR and the associated progression to T2DM (Cerf, 2013; DeFronzo, 1988; DeFronzo & Abdul-Ghani, 2011; DeFronzo & Muhammad, 2011; Groop et al., 1996; Keane et al., 2015; Lorenzo et al., 2010; Samuel & Shulman, 2012; Warram et al., 1990; Weir & Bonner-Weir, 2004). The largest age group to suffer from obesity is children with an increase of more than 50% in the last 10 years (Chiarelli & Marcovecchio, 2008; Levy-Marchal et al., 2006). There is a lack of a recommendation to screen children for IR (Levy-Marchal et al., 2006). Increased polycystic ovarian syndrome (PCOS) presents primarily in women during there early 20s in patients with IR and may be the only presenting problem at the time to their doctor (Rojas et al., 2014). The number of patients presenting to their physician for PCOS may be due to insidious onset of the disease and secondary complications or the pain and problems associated with PCOS condition. IR and increased age have demonstrated both an

increase in CVD and progression to T2DM (Cerf, 2013; DeFronzo, 1988; DeFronzo & Abdul-Ghani, 2011; DeFronzo & Muhammad, 2011; Groop et al., 1996; Keane et al., 2015; Lorenzo et al., 2010; Samuel & Shulman, 2012; Warram et al., 1990; Weir & Bonner-Weir, 2004).

IR has been observed throughout all races with Blacks and East Asians having the highest prevalence of CVD (Beck-Nielsen, 1999; Kodama et at., 2013). Other studies using HOMA-IR to identify insulin resistance among Whites, Blacks and Hispanics demonstrated only slight variations in insulin sensitivity among the groups and only a slight increase in CVD among Blacks and Hispanics (Qu et al., 2011). Most insulin resistance population-based studies use metabolic syndrome criteria for diagnosis with the newer studies using HOMA-IR. Both types of studies lack standard guidelines and largely rely on interpretation.

Insulin Resistance Theory

Reaven first tested and established the theory of IR and developed the first qualitative test to measure insulin-mediated glucose uptake (Reaven et al., 1967). This technique allowed Reaven to measure insulin levels and establish the importance of IR in T2DM and human disease (Ginsberg et al., 1975; Olefsky et al., 1973). In nondiabetic patients, Reaven further demonstrated the role of the IR compensation to maintain normal glucose levels and hyperinsulinemia and the association to hypertriglyceridemia (Reaven et al., 1967); low or reduced HDL levels (Golay et al., 1987); reduced urinary uric acid clearance (Facchini et al., 1991); reduced LDL particle size and increased postprandial lipemia (Reaven et al., 1993); increased serum levels of

PAI-1 (Abbasi et al., 1999); essential hypertension (Fuh et al., 1987); and hyperactivity of the sympathetic nervous system with hyperinsulinemia (Reaven et al., 1996). Reaven (1988) later described the combined or clustering of symptoms secondary to IR in a medical community lecture called Syndrome X. Syndrome X is now referred to as metabolic syndrome to describe the associated symptoms of IR.

Insulin Resistance

IR is a condition defined as the tissues fail to respond to serum insulin causing the reduced cellular uptake and utilization of glucose and glucose homeostasis. Clinically the term *insulin resistance* suggests that an elevated level of insulin above the normal level is required to maintain normal glucose levels. On a cellular level IR consists of abnormal or reduced binding of insulin to the cellular insulin receptor and abnormal downstream signaling of the insulin molecule reducing the uptake and metabolism of glucose (Wallace & Matthews, 2002). This condition propagates compensatory overproduction and release of insulin by the pancreas resulting in hyperinsulinemia due to the reduced uptake and elevated serum levels of glucose (Groop et al., 2005). The overproduction and release of insulin by the pancreatic β-cells are to maintain euglycemia state postprandially by triggering the uptake of glucose of skeletal muscle and adipose tissue. Insulin concomitantly suppresses hepatic glycogen release thus suppressing gluconeogenesis during periods of post-prandial insulin release (DeFronzo & Muhammad, 2011). The overproduction and release of insulin secondary to insulin resistance then progress to the eventual exhaustion and failure of the

pancreatic β-cells to maintain glucose homeostasis and eventually Type 2 diabetes (Knowler et al., 2002; Santaguida et al., 2006; Vendrame & Gottlieb. 2014).

Insulin Resistance Syndrome

Reaven (1988) first proposed a cluster of related metabolic conditions that were secondary to hyperinsulinemia found in patients with IR. Reaven initially referred to this condition as Syndrome X from the cluster of metabolic abnormalities associated with IR. These conditions include glucose intolerance, increased serum triglycerides and very low-density lipoproteins (VLDL), reduced serum HDL, and hypertension. Reaven further summarized that these conditions determined to a large extent which patients would develop cardiovascular disease. Since Reaven first proposed the concept of Syndrome X further studies confirm these conditions associated with insulin resistance to including the later development of diabetes Type 2 as the patient ages (Expert Panel {NCEP}, 2001; Hill, 2003; Reaven, 2003; Srinivasan et al., 2003).

Since the initial description of Syndrome X by Reaven other studies have identified this condition using various names to include: diabesity, deadly quartet, deadly pentad disease, dysmetabolic syndrome, polymetabolic syndrome, coronary risk syndrome, insulin-resistant syndrome, and hyperinsulinemia/insulin-resistant syndrome (Reaven, 2003). Since the NCEP-ATP III Panel the term *metabolic syndrome* has been deemed appropriate to use for this condition.

Metabolic Syndrome

Metabolic syndrome is a group of conditions that increase the chance of developing cardiovascular disease. These conditions include abdominal obesity,

atherogenic dyslipidemia, elevated blood pressure, glucose intolerance, proinflammatory state, and prothrombotic state. The Paris Prospective Study conducted in 1991 by Fontbonne and Eschwège demonstrated that patients with hyperinsulinemia and hypertriglyceridemia were similar to those with IR and central obesity. They concluded that these metabolic conditions even at mild elevations could increase in death from cardiovascular disease. Diagnostic guidelines established by the NCEP-ATP III for metabolic syndrome include three confirmed factors from the five established by the NCEP-ATP III. The NCEP-ATP III diagnostic guidelines are easy to apply to patient intake vital signs, but lack specificity for insulin sensitivity and β -cell function.

Current Direct Tests for Insulin Resistance

These are the most common tests used currently to test IR in studies. These tests are expensive, invasive, and very time-consuming. The only test that may be considered for clinical use would be the oral glucose tolerance test. During this test, serum sampling begins at 30 minutes post glucose load and would miss the use of insulin by skeletal muscle tissue during the first phase insulin secretion.

Oral Glucose Tolerance Test (OGTT)

This test is one of the earliest tests for in vivo insulin sensitivity. The standard oral glucose load of 75g (calculated 1.75 g/kg body mass) is given to fasting patients over 5 minutes. Blood is then sampled every 15-30 minutes for 2-5 hours for serum glucose and insulin concentrations. The initial change in insulin/glucose ratio (Δinsulin/Δglucose) during an OGTT had been used as an index of insulin sensitivity. This insulin sensitivity calculation inspired the calculation of fasting and postload

glucose and insulin values. The concept of this type of test is that the higher the increase in plasma glucose per unit of insulin then the lower of insulin sensitivity is observed. Pacini and Mari (2003) suggested the use of a modified OGTT for insulin sensitivity considering the area under the OGTT curve after 15 minutes. The standard clinical protocol for utilizing OGTT for diagnosing and treating diabetes has evolved to 2 hours with serum blood testing at fasting, 30 minutes, 60 minutes, and at 120 minutes.

Insulin Tolerance Test (ITT)

This test is one of the earlier tests to evaluate in vivo insulin action. Fasting patient's baseline serum glucose is calculated and is given a bolus of fast acting insulin (0.1 unit/kg/day) intravenously. Serum glucose levels are obtained every 5 minutes for a 70-minute duration. The calculated insulin-induced glucose metabolism index is expressed as the glucose disappearance rate (KITT=[0.693/t1/2] X 100%/min). In this equation, t 1/2 is equal to the half-life of the plasma glucose decay. Normal values of KITT are greater than 2%/min with abnormal values being less than 1.5. Values between 1.5 and 2 are considered equivocal. The advantages of this test are that it is relatively simple as an intravenous test. There is a modified Short Insulin Tolerance Test (SITT) version that can be completed over 15 minutes and reduces the incidence of hypoglycemia. The test is reproducible and accurate both the standard version and the short version as Hirst et al. (1993) demonstrated. The disadvantages of the test are hypoglycemia in patients during the test. Hypoglycemia stimulates hepatic glucose release and impairs peripheral glucose uptake. The Inability of the test to allow insulin-

induced glucose metabolism secondary to the hypoglycemia/hyperinsulin response. The test is not indicated in pediatrics due to the problems associated with hypoglycemia.

Insulin Suppression Test (IST)

This test is used to evaluate the disposal of an intravenous glucose load by a constant/fixed level of hyperinsulinemia. This test is completed on patients that have been fasting overnight and given 5mg of propranolol intravenously. Propranolol inhibits hepatic glucose release during the test. Intravenous epinephrine is given continuously at 6 mcg/min to suppress insulin secretion. Propranolol is given IV at a continuous rate of 0.08 mg/min with regular insulin 80mU/min, and glucose 6 mg/kg/min, all over 180 minutes. Steady-state plasma glucose (SSPG) and steady-state plasma insulin (SSPI) are observed after 90 minutes. Blood samples are obtained every 15 minutes for the first 90 minutes and then every 10 minutes for the last 90 minutes. The concentration of SSPG is proportional to the insulin-mediated glucose metabolism with an increase in SSPG being proportional to an increase in IR. This test demonstrates a high correlation between IR and insulin suppression and the euglycemic clamp test $r = 0.092$, $p<0.001$ Greenfield et al. (1981). The disadvantages of this test include: high amount of complexity rendering the test inappropriate for clinic use, propranolol may not be adequate to suppress hepatic glucose release particularly in the presence of IR or diabetes, SSPG levels can be different between individuals and the effects of hyperglycemia and hyperinsulinemia can cause additional metabolic changes that could alter the results, diabetic patients and those with IR could respond differently to epinephrine with the increased possibility of arrhythmias in the elderly.

The Frequently Sampled Intravenous Glucose Tolerance Test With Minimal Model Analysis (FSIGTT)

In this model fasting patients receive a bolus of 50% glucose solution which is calculated at 0.3g/kg body weight over 1 minute. Basal blood samples are collected at -15, -10, -5 and -1 minutes with post bolusing samples being obtained at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes. This model allows frequent sampling of glucose and insulin which are analyzed using a minimal computer model (MINMOD). There have been two modifications to the FSIGTT in an attempt to improve endogenous insulin response to the glucose bolus and enhance the computer model's ability to analyze the data. The tolbutamide enhanced FSIGTT utilizes intravenous tolbutamide after 20 minutes with additional blood testing at 23, 24, and 27 minutes. Tolbutamide is typically given at 300 mg for normal individuals and 500 mg for overweight or obese patients. The second modified FSIGTT is the insulin-modified FSIGTT. This test utilizes insulin at 0.03 units/kg by intravenous bolus after the start of a standard FSIGTT protocol. This modification was established for the use of FSIGTT in diabetic patients to reduce the possibility of hyperglycemia.

Glucose Clamp Test (GCT)

This type of test is considered the current benchmark test to evaluate pancreatic insulin release, insulin-induced glucose disposal, and insulin sensitivity. The glucose clamp test is designed based on the glucose/insulin feedback loop that regulates the β -cell release of insulin and glucose uptake by the peripheral tissue. There are two

variations of the GCT; euglycemic-hyperinsulinemic clamp test and hyperglycemic clamp test.

Euglycemic-Hyperinsulinemic Clamp Test

The goal of this test is to raise and maintain the serum insulin levels while maintaining glucose levels at the basal level. The euglycemic-hyperinsulinemic clamp test allows measurements of insulin sensitivity; insulin-stimulated glucose uptake/metabolism (M), and the metabolic clearance rate of insulin (MCR). This test is complicated, invasive, and time consuming in that the test requires an antecubital venous catheter for infusions of both glucose and insulin, arterial heated-catheter for frequently sampled blood testing, hand-held glucometer for rapid testing of blood glucose levels, intravenous dextrose 20% for infusion, and regular crystalline insulin for intravenous infusion. After an overnight fast, patients are given regular insulin per IV at 5-120 mu/m2 /min to maintain a steady state insulin level. The average rate of insulin infusion is 40 mu/m2 /min; this will increase blood glucose levels above baseline by ~ 100 µu/ml. intravenous glucose is infused (milligrams/minute) and clamped to maintain basal level during the test while suppressing hepatic glucose production. Blood glucose readings are normally obtained every 5 minutes over the 3-hour test and samples are obtained every 15 minutes for insulin. The amount of glucose infused to maintain euglycemia then reflects the amount of glucose metabolized in the peripheral tissue and measured in milligrams/kilograms/minute (M value) reflecting an indirect index of peripheral tissue sensitivity (capability to bind) to the insulin molecule. The higher the M value the higher the sensitivity to insulin which is seen in individuals with normal

metabolism. This is much different than patients with insulin resistance that require less glucose to maintain basal glucose levels. This is likely due to inability of insulin to bind and stimulate peripheral tissue cells to uptake and metabolize insulin.

Peripheral Insulin Sensitivity Index (ISI)

ISI is the ratio of M/I where (M) equals the rate of stabilized glucose levels during infusion and (I) equal the mean insulin level during the test. This measurement is useful when comparing different groups that have different steady-state concentrations of insulin from a euglycemic-hyperinsulinemic clamp study. The metabolic clearance rate of insulin (MCR) is calculated by (ml/kg/min) dividing the fusion rate of insulin by the change (or increase) in circulating insulin concentrations from the euglycemic-hyperinsulinemic clamp study.

Hyperglycemic Clamp

The hyperglycemic clamp test is designed to evaluate the pancreatic β -cell response to a controlled hyperglycemic condition. This test requires the patient to fast overnight and receive an intravenous bolus of glucose of 20% dextrose over 2 minutes increase basal serum glucose to 225 mg/dl. The amount of glucose give is calculated by (225 – the mean of three fasting serum glucose readings) then multiplied by the patient's weight (kg) with the results being glucose distribution-glucose distribution factor which is 1 for normal adults, 1.1 for overweight children, and 1.5 for normal weight children. This calculation allows for the initial bolus calculation and is adjusted as needed during the test. Glucose measurements are obtained every 2.5 minutes with insulin and C-peptide during the first 15 minutes of the test and every 5 minutes during

the remainder of the 120-minute test. Data from the hyperglycemic clamp test are used to calculate serum insulin response to glucose and whole body glucose metabolism.

Surrogate Indexes for Insulin Sensitivity/Resistance

The HOMA is an interaction model between glucose and insulin used to predict fasting steady-state glucose and insulin concentrations (Matthews et al., 1985). The formula to calculate HOMA is (fasting insulin x fasting glucose)/22.5. The denominator 22.5 is normalization factor derived from testing healthy normal individuals fasting plasma insulin and fasting plasma glucose.

The QUICKI is a derived mathematical conversion of fasting plasma insulin and glucose levels producing a predictive index of insulin sensitivity. This index is a variation of the HOMA equation converting the data by using the logarithm and reciprocal of both glucose and insulin product. The formula for the QUICKI index is 1/[log (Insulin μU/mL) + log (Glucose mg/dL; Duncan, et al., 1995).

The McA is a novel surrogate index that uses both fasting plasma insulin and triglyceride levels to predict insulin sensitivity. The formula for McA is exp[2.63 - 0.28 ln(insulin mU/L) – 0.31 ln(triglycerides mmol/L)] This index uses triglycerides as an index in the equation since triglyceride dyslipidemia is a primary finding in IR (McAuley et al., 2001).

Challenges With the Current Tests for Insulin Resistance

The current studies available to identify IR include the frequently sampled intravenous glucose tolerance test which is conducted over 2 hours after a bolus of 70 grams of glucose is ingested (Pacini et al. 1986), hyperinsulinemic-euglycemic clamp

technique conducted utilizing intravenous insulin and glucose concentrations in a hospital setting (DeFronzo et al., 1979), and the insulin suppression test which is similar to the hyperinsulinemic-euglycemic test (Harano et al., 1977; Shen et al., 1970). These tests are all very expensive, demonstrate some risk for the patient, and are difficult or impossible to complete in the clinical setting. The HOMA-IR study has also been suggested for identifying individuals with IR and is based on calculations of the patient's height, weight, age, and cholesterol levels. This test is relatively inexpensive but is based on general numbers from groups of patient's results from insulin suppression and hyperinsulinemic-euglycemic clamp tests (Cheatham et al., 1995). These tests are very time consuming and impractical for daily patient diagnosis and/or treatment of IR. These tests also lack the ability to provide accurate clinical identification and treatment evaluation of the disease (Matthews et al., 1985).

Cost-Effectiveness of Treating Insulin Resistance

Researchers have not identified cost-effective methods for treating IR; but, there are two studies on treating impaired glucose tolerance (Diabetes Prevention Program Control Group, 2003, Herman et al., 2005). These studies concluded that a combination of both lifestyle modifications of improved diet and exercise with generic metformin effectively reduced improved IGT over 3 years compared to the control groups. The combination of lifestyle modifications and metformin increased insulin sensitivity and reduced serum glucose in the treated subjects (Diabetes Prevention Program Control Group, 2003; Herman et al., 2005). The use of pioglitazone in patients to improve IGT demonstrated a 72% reduced conversion to T2DM (DeFronzo et al., 2011). This is the

only study found that used a thiazolidinedione class of insulin sensitizer, pioglitazone, to improve IGT. Pioglitazone was also found to reduce triglyceride levels in these patients which improved cardiovascular outcomes. There is no data on GLP-1 receptor agonists for the treatment of IGT or IR although these medications have been demonstrated to increase insulin sensitivity they have only been studied in T2DM patients to demonstrate the capability to improve serum glucose and reduce HbA1c.

Multiple researchers demonstrated the effectiveness of treating IGT prediabetes with lifestyle interventions, metformin, and now with pioglitazone to reduce the progression to T2DM in most cases patients would progress to T2DM later in life than their control counterparts (DeFronzo et al., 2011). This continued progression is primarily due to the 70%-80% loss in β-cell function, had the treatment been started prior the loss β-cell function could have been preserved (DeFronzo & Muhammad, 2011). Each treatment has demonstrated improvement a combination utilizing all three lifestyle interventions (diet and exercise) with pioglitazone and metformin may provide the best treatment option. Both pioglitazone and metformin are generic and inexpensive and have proven to be safe (Dormandy et al., 2005). When considering that IGT represents a midphase progression from insulin resistance to T2DM the results from the above treatment arms could be used to treat early insulin resistance. Considering the 176 billion dollar cost of T2DM health care cost in 2012 and an additional 69 billion dollar reduction in productivity in the US, the early identification and treatment of insulin resistance is extremely cost-effective.

Summary and Conclusions

The medical community is focused and committed to treating diabetes effectively to reduce the morbidity and mortality of the disease. The current treatment emphasis is education on diet and excise and treating serum glucose, cholesterol while monitoring blood pressure to reduce complications. However, Butler et al. (2003) demonstrated that by the time an individual is diagnosed with T2DM they have lost significant β-cell mass and function. Other studies suggested that early T2DM patients have cardiovascular disease due to the increased insulin during the reduced insulin sensitivity before becoming diabetic (Defronzo & Abdul-Ghani, 2011; Duckworth et al., 2009; Ferrannini et al., 2005, Ginsberg, 2000). Because IGT and T2DM patients continue to progressively express increased morbidity, it would seem reasonable to institute early identification and treatment of IR to reduce the growing diabetic epidemic. A valid clinical test is needed for the identification and evaluation of treatment regimens for insulin resistance. Chapter 3 is an explanation of this study's research methods, variables, and statistical analysis.

Chapter 3: Research Method

Study Methods

The main objective of this study was to evaluate the ability of the OGIST to diagnose IR in clinical evaluation for those patients that meet the criteria for metabolic syndrome. My second objective with this study was to evaluate the ability of the OGIST to evaluate possible differences that gender, age, and ethnicity may have on IR. The OGIST was originally designed to directly measure serum insulin, proinsulin, C-peptide, and glucose levels after a small glucose challenge at the end of the first phase insulin release to accurately evaluate insulin resistance (sensitivity). In this study, I used clinical laboratory data obtained from patient results that were tested using the OGIST conducted at the Joshua Medical Center. The primary purpose of the OGIST is to accurately identify IR in those patients meeting the criteria for metabolic syndrome and evaluate treatment in those patients diagnosed and treated for IR. The correct and accurate measurement of insulin sensitivity allows for the correct diagnosis and evaluation of a therapeutic intervention for both epidemiological studies and clinical practice. In this chapter, I will describe the research hypothesis, design, study population, study variables and data analysis in this study.

Research Questions and Hypotheses

RQ1: In patients meeting the criteria for metabolic syndrome will an Oral Glucose Insulin Secretion Test (OGIST) predict insulin resistance in the clinical setting?

$H_0$1: In patients diagnosed with metabolic syndrome, the Oral Glucose Insulin Secretion Test (OGIST) does not predict insulin resistance in a clinical setting.

H_A1: In patients diagnosed with metabolic syndrome, the Oral Glucose Insulin Secretion Test (OGIST) does predict insulin resistance in a clinical setting.

RQ2: Does age, gender, and ethnicity have an influence on the Oral Glucose Insulin Secretion Test (OGIST) for insulin, proinsulin, glucose, and C-peptide levels.

$H_0$2: The Oral Glucose Insulin Secretion Test (OGIST) does not identify changes in insulin, proinsulin, glucose, and C-peptide in different ages, gender, and ethnic groups.

H_A2: The Oral Glucose Insulin Secretion Test (OGIST) does identify changes in insulin, proinsulin, glucose, and C-peptide in different ages, gender, and ethnic groups.

Study Population

The medical center providing patient results is located in Omaha, Nebraska with a total population of 443,885 in 2016. The Omaha population is made up of approximately 73.1% Caucasian, 12.7% African American, 13.7% Hispanic, 6% other, 3% Asian, and 0.4% Native American. Study patient demographics included both male and female Asian, African American, Caucasian, Hispanic, and Native American

patients' ages 15 to 70 years of age. The participant demographics for this study were somewhat different from the normal distribution in the Omaha area (due to the location of the medical center) and consisted of 52% Caucasian, 32% Hispanic, 12% African American, 2% Native American, and 2% Asian.

The participant dataset I used in this study consisted of patients meeting the criteria for metabolic syndrome established by NCEP-ATP III and those patients that have been diagnosed with IR to evaluate their treatment. Inclusion criteria for patients and their data in this study included: being 18–65 years of age, must not have been previously diagnosed with diabetes or be on any diabetic medication to include insulin, and must not have any uncontrolled endocrine disorders such as hypo-hyperthyroidism or adrenal cortex. Exclusion criteria for subjects in this study included patients that had uncontrolled hypertension with blood pressure exceeding 170/109 mmHg, uncontrolled or abnormal thyroid function at the time of study, had been hospitalized within the prior 3 months, elevated liver enzymes greater than twice the normal levels, or had abnormal renal function of serum creatinine > 1.5 mg/dl. Patients that were pregnant at the time of the study were also excluded.

Measurements and Instrumentation

In this study, I used various laboratory techniques to measure serum levels of insulin (normal range 3-19 uIU/ml), proinsulin (normal range < 12.8 pmol/L), C-peptide (normal range 1.1–4.4 ng/ml), glucose (70–100 mg/dl), and HgA1C (normal range 4.5%–5.7%). To accurately measure the data required in this study, I first needed to define the structure of the type of measurement used. First, I conducted isomorphic

measurements of each different study aspect. This equals to the similarity in numerical properties utilized for each aspect. When serum glucose levels were measured in each individual , the established unit of measure and the established normal level of serum glucose at 70–100 mg/dl were used. This established unit of measure for both normal levels and measured units helped establish the abnormal levels of hyperglycemia and hypoglycemia in this study. These units of measure are also represented as a nominal level of measure since each unit of measure is represented as a number (Frankfort-Nachmias et al., 2008).

When considering the different hormones and substances evaluated in this study, to accurately evaluate each one I placed them in similar isomorphic groups together. For example, glucose in mg/dl units were calculated and measured together, but separate from insulin uIU/ml, C-peptide ng/ml, proinsulin pmol/L, and HgA1C which were measured as a percentage of serum concentration. Each hormone and substance was evaluated in its own isomorphic group.

I obtained interval measurements for each value since the measurements were placed in fixed and equal units of measure for each isomorphic group. This was found true since the comparison of insulin at the normal range is 3-19 uIU/ml and then compared to the individual and each group for the evaluation of hyper/hypo insulin secretion of the β-cells. This was true for each isomorphic group studied which allowed for the evaluation of the binding of insulin to the cellular insulin receptor. I also conducted ordinal measurement evaluations between the groups of individuals, glucose,

insulin, proinsulin, C-peptide, HbA1c, and BMI. The ordinal evaluations took place between all groups for each isomorphic group tested.

I measured the key variables in this study using the OGIST. This test measures insulin, proinsulin, C-peptide, glucose, and HbA1c at the end of the first phase insulin response to glucose and was designed to evaluate IR (insulin sensitivity) by measuring serum insulin, C-peptide, proinsulin, glucose, and HbA1c at the end of the first phase insulin response to glucose. The OGIST is carried out by giving the patient 30 grams of oral glucose, and 15 minutes later, postprandial serum levels of insulin, C-peptide, proinsulin, glucose, and HbA1c are obtained. This test was based on the IR theory and the results from the glucose clamp technique to quantify insulin secretion and the results from the euglycemic-hyperinsulinemic clamp test (DeFronzo & Abdul-Ghani, 2011; DeFronzo et al., 1979; Reaven, 1988, 2003; Reaven et al., 1993). An accredited laboratory, through the College of American Pathologists or Commission on Office Laboratory Accreditation, that demonstrated performance specifications for accuracy, precision, and reportable range of the patient test results performed all lab testing.

Proinsulin

Proinsulin is produced, within the islets of Langerhans located in the pancreatic β-cells, as a large polypeptide and has minimal biological activity in this state. Proinsulin is cleaved into insulin, as the active hormone, and C-peptide by convertase in both the β-cells and circulating blood supply. Proinsulin levels are found to be only 20% of the total insulin systemic circulation in normal metabolic individuals having normal insulin sensitivity and β-cell function. Proinsulin levels are measured during the

OGIST and a key variable at the end of the first phase insulin response to glucose. Normal proinsulin levels should be 1.7–12 pmol/L and will be found elevated within individuals with advanced IR due to β-cell exhaustion. As IR advances with age, pancreatic β-cells respond slower to glucose stimulation resulting in reduced insulin and increased proinsulin release at the end of the first phase insulin response.

Insulin

Insulin is produced initially by the pancreatic β-cells as proinsulin and cleaved in the bloodstream into insulin and C-peptide. Insulin is a polypeptide composed of two amino acid chains linked by disulfide bonds and is the active hormone responsible for several important actions. In this study, I focused on the ability of insulin to bind to muscle cells and upregulate glucose entry into the cell, glucose homeostasis, increased triglyceride synthesis, and increased fatty acid synthesis (weight gain). The OGIST measures serum insulin as one of the key variables at the end of the first phase insulin response to glucose challenge allowing the evaluation of insulin sensitivity. At the end of 15 minutes, serum insulin will be at normal levels 3.0–25.0 mU/L in those individuals with normal insulin sensitivity. Serum insulin levels will be elevated in individuals with IR and normal β-cell production of insulin.

C-Peptide

C-peptide is a peptide cleaved from proinsulin in the production of insulin and released in concentrations equimolar to insulin into circulation. C-peptide has no biological activity and is considered a waste product during insulin production. Unlike insulin, C-peptide is excreted by the kidneys from the circulating blood supply over 30

minutes. Normal fasting serum C-peptide levels are found to be 0.51–2.70 ng/ml. Reference laboratory levels have been established 0.81–3.85 ng/ml for this population. C-peptide levels have been found to be as high as 5.6 ng/ml in normal patients after glucose load. C-peptide levels are equal to insulin produced by the β-cells and have been suggested as a test for true diabetes prior exogenous insulin use (Jones & Hattersley., 2013).

Glycated Hemoglobin (HbA1c)

HbA1c is glycated hemoglobin formed by the reaction between serum glucose and hemoglobin, and the rate at which glycated hemoglobin is formed is proportional to the concentration of blood glucose. Red blood cells can survive up to an average of 120 days while in circulation, this then allows an average index of the individual's blood glucose to be made over 120 days. This allows for a more accurate blood glucose average than spot glucose testing using a glucometer which can vary over 30 minutes. Any condition that can affect the life of the red blood cell can falsely lower HbA1c levels (Sherwani et al., 2016). These conditions include hemolytic anemia, hemodialysis, and chronic kidney disease. HbA1c is one of the variables used in OGIST and this study, allowing the evaluation of the average steady-state glucose levels of the individual. Normal HbA1c levels are 4.3%–5.6% and 5.7%–5.9% is considered prediabetes with early β-cell exhaustion and over 6.0% is classified as being diabetic with β-cell exhaustion and poor glucose control (Abdul et al., 2006; ADA, 2014; Cerf, 2013; Gerich, 2002; Lorenzo et al., 2010). For proinsulin insulin ratio measurements will be obtained to evaluate β-cell function further. Tura et al. (2003) developed this

proinsulin: insulin ratio as a test to evaluate β-cell function. This study included this test to try and understand the β-cell function in regard to insulin release as the patient with IR ages. This allowed the identification of possible exhaustion of the β-cell and reduced secretion of insulin in response to glucose stimulation during the first phase insulin release. This test was used to evaluate the progression of IR to true diabetes Type 2 so that clinical correlation can be made for aging patients with IR.

Ratio levels of measurement were conducted on proinsulin: insulin to estimate β-cell function in patients with advanced IR. This will allow correlation of proinsulin: insulin ratio to predict Type 2 diabetes in advanced patients.

Study Validity

Laboratory instrumentation validity was maintained by each laboratory utilized for analysis of the individual serum insulin, proinsulin, glucose, and C-peptide as required by the American Association of Bioanalysts. Study design validity was evaluated at the completion of the study for the use of the oral glucose insulin secretion test as a valid clinical test for the evaluation of patients with IR.

Internal validity can be affected by design problems of the study or interpretation of the study data is flawed (Frankfort-Nachmias et al., 2008). Protocols were established to improve internal validity, patient, and control selection process, study participant accountability, central study coordinator, nursing and laboratory personnel classes before starting the study, correct dosing of glucose and timing of serum draws, proper storing and shipping of laboratory samples.

External validity can also be affected by the study through centralized patient population, environment, and patient drop-out rate, researcher effect, external laboratory problems (Frankfort-Nachmias et al., 2008). Although any one of these issues can affect the validity of the study and data, a close assessment of all possible external factors was evaluated closely in an attempt to eliminate or reduce the possibility of any external issues reducing the studies validity.

Content validity would cover the aspect that this study is truly evaluating the functional binding of insulin receptor to the insulin during the first phase insulin release and to some degree the functional ability of the β-cells ability to release insulin. Content validity includes face validity and sampling validity (Frankfort-Nachmias et al., 2008). Face validity is based on subjective evaluation of the study's true ability to measure the receptors binding ability as opposed to the concept theorized. Sampling validity focuses on whether the population sampled is adequate for the intended response and further, can the data be generalized. Because there is not a true clinical test for IR face validity was considered true until the final data either proves or disproves the hypothesis that this test adequately evaluates the insulin binding function to the insulin during the first phase insulin release. Sampling validity was maintained by including multiple ethnic groups in all arms of the study.

Empirical validity reflects the measured outcome relationship to the measuring tool (Frankfort-Nachmias & Nachmias, 2008). This study has two aspects to empirical validity. First, the laboratory instrumentation that was used to study serum levels of insulin, proinsulin, C-peptide, glucose, and HbA1c. Because this laboratory's testing,

the serum values are accredited and undergo quarterly proficiency testing I relied on the laboratory's accredited as valid. The second is that I accurately evaluated the IR's binding function to insulin during the first phase insulin release and that this test can accurately identify a patient with IR and can later be used to monitor treatment efficacy. The completed data allowed the predictive validity of the test to be established at the conclusion.

Construct validity relates to the weather I was able to empirically and logically link to the concepts and theoretical assumptions utilized in the study (Frankfort-Nachmias & Nachmias, 2008). To demonstrate construct validity, I needed to demonstrate that I accurately identified a patient with IR. This was accomplished by the use and comparison of all aspects of the study. The results of this study will be compared to the findings of DeFronzo's results of the euglycemic insulin clamp studies on normal glycemic subjects (DeFronzo, Tobin, & Andres, 1979) and Kabadi's results on normal glycemic subjects during the first phase insulin response for insulin sensitivity (Kabadi & Kabadi, 2011). First phase insulin and glucose response results from OGIST were compared to first phase insulin and glucose results from DeFronzo et al., 1979; Kabadi & Kabadi, 2011).

Sample Size Calculation

For the purpose of this study, 251 patient data sets were used that had met the above criteria and were placed in groups of 5 years of age (i.e., 18-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, and 60-65) with the youngest and oldest group collapsed into 18-24 and 60-65 for adequate sample size. Patients were placed in these

age groups to rule out possible variance in insulin/glucose response due to age and were compared to the HbA1c differences found with age from the Framingham Offspring Study and the National Health and Nutrition Examination survey 2001-2004 (Pani et al., 2008). This allowed a comparative analysis of the progression of prediabetic expression to full T2DM. Insulin, proinsulin, C-peptide, glucose, and HbA1c were analyzed using binomial logistic regression to evaluate changes in predictor variables age, gender, and ethnicity.

Sample size determination is an important aspect of study design and must be specified in the protocol before beginning the study. Calculation of the study's sampling is also important for credibility and control of the study's probability in translating to real effect as being statistically significant (Bland & Altman, 1986). Determining the specification of the statistical power and the level of significance is needed in calculating the sample size to ensure the correct amount of subjects are included in the study and that the investigator has not over or underestimated the sample size. The sample size is then based on the power of the study, an acceptable level of significance, and the effect size. The statistical power is the probability of detecting a real relationship between the variables being studied. The statistical power is the false negative rate or the proportion of positive findings reported as being negative or having no relationship when a true relationship existed and called Type II error. The statistical power is referred to as β and equal to $(1-\beta)$ and is set at 0.80 in this study. Level of significance is referred to as α and is predetermined prior the study and is set at 0.05. Meaning that 5% of the results are due to chance and did not have a true relationship

between the variables studied. Accepting a false positive in this case is referred to as Type I error (Myles & Cui, 2007). Effect size is the magnitude or size of an effect between two groups. Because the data sets between the groups have not been completed, there was a small effect size between gender and the comparison within each age group. A larger effect size should be noted between the younger and older patients in the study due to β-cell exhaustion seen in insulin resistance with advanced age.

Statistical analysis of the study results was conducted by the use of student's *t* test, and binomial logistic regression analysis was used for comparison of the changes found in insulin, glucose, HbA1c, proinsulin, and C-peptide with the dependent variables. SPSS software computer analysis was used for the above calculations. OGIST results were compared with the initial normal glucose tolerant group. Patient data were also compared to the calculated HOMA IR results using the formula (fasting glucose mmol/l; fasting insulin mU/l)/22.5 (Bonora et al., 2002). Insulin sensitivity was calculated using the formula ([Δ%I 0-15/ΔG 0-15]/fasting insulin/fasting glucose; Kabadi & Kabadi, 2011).

I indirectly tested the binding ability of the insulin receptor to the insulin during the first phase insulin release which allowed the diagnosis of IR in patients. The clinical test consisted of testing insulin, proinsulin, glucose, and C-peptide after the patient consumes 36 gram of oral glucose stimulating the release of insulin by the pancreatic β-cells. Bland-Altman method of agreement (Bland & Altman, 1986) was used to plot the findings of each group for insulin, proinsulin, glucose, and C-peptide for correlation

analysis. Each group was analyzed individually and comparatively to test both increased insulin secretion found in IR and the reduced insulin response found in advanced IR due to β-cell exhaustion.

These groups were compared for statistical significance between the means of the findings utilizing a one-tailed unpaired *t* test. Total patient numbers are initially planned at 200 and assumed that the standard deviations are the same for simplicity ($\alpha_1 = \alpha_2 = \alpha$). The mean difference between the groups was considered as (μ_1 - μ_2). The calculation used for determining the sample size was:

$$n \approx [(2(Z_{power}+Z_{1-\alpha}))/(2(\mu_1 - \mu_2)/\delta)]^2$$ Smithson (2000)

Z_{1-a} signified 1 – α quantile of the normal standard distribution which can then be taken from the standard statistical table. Sample size determination for the unpaired *t* test had α conversion to α/2 as the only change. For this study, a significance level of 2.5% was used and power set at 0.80. Utilizing the statistical tables $Z_{.08}$ = 0.8416 and $Z_{.0975}$ = 1.96. Considering the above equation the sample size would be considered:

$$45.2 \approx [(2(0.8416+1.96))/(5/6)]^2$$

This equation would then suggest that a sample size of 23 patients could be used for each group in place of the previously considered 200 patients. This allowed for a reduced expense of the study. This, however, would not take into account for any patient, lab error, or other unforeseen problems that could arise which may reduce patient numbers in each group.

Research Design and Methods

This study design was quantitative, quasi-experimental in nature to analyze the diagnostic effectiveness of the OGIST for insulin resistance. The data utilized in this study were provided from patient laboratory and chart data that have been tested for IR at the Joshua Medical Center utilizing OGIST. To meet the objective in this study data from all patients tested for IR ranging in age from 18 to 65 years old obtained from the medical center laboratory records and patient charts all of which met the criteria for metabolic syndrome and were tested using the OGIST. The OGIST evaluates the first phase insulin secretion after the ingestion of 36 grams of concentrated glucose. In a normal individual first phase, insulin release by the pancreatic β-cells occurs 2-3 minutes postprandial and returns to normal levels after 15 minutes along with glucose. Proinsulin levels are found to be normal after 15 minutes with slightly elevated C-peptide levels.

Early detection of reduced β-cell function can be made by testing first phase insulin secretion (Gerich, 2002). Testing first phase insulin secretion can also be used to evaluate the sensitivity of insulin thus evaluate IR in the patient. The OGIST allows the indirect testing of insulin binding to the cellular insulin receptor and cellular uptake of glucose during the first phase insulin release.

Study Variables and Data Analysis

The independent variables in this study consisted of the initial oral glucose load of 36 grams given during the OGIS, age, gender, and ethnicity groups (Table 1 and 2). The dependent variables, or response variables, are insulin sensitivity. In those patients

included that are being treated for IR the dependent or response variable would also include HbA1c, weight/BMI, and possibly liver enzymes and triglycerides based on any improvement of insulin sensitivity/resistance due to treatment (Table 1). Statistics used in this study are based on changes found in the variable outcomes which include mean/median, standard deviation, standard error and effect size. Confidence intervals will be calculated at 95% for all population means within the study (Table 3).

Table 1

Dependent Variables: Descriptions and Measurements

Dependent variable	Description of variable	Measurement
First phase serum insulin	Serum insulin obtain at 15min after oral challenge during OGIST	Insulin measured in mU/L
First phase proinsulin	Serum proinsulin obtain at 15min after oral challenge during OGIST	Proinsulin measured in pmol/L
First phase C-peptide	Serum C-peptide obtain at 15min after oral challenge during OGIST	C-peptide measured in ng/mL
Hemoglobin A1c	Glycated Hemoglobin (Hb A1c), provides an index of the average blood sugar for a 2-4 month period	Measured as % of glycated hemoglobin
Serum triglycerides	Complex lipids of esterified glycerol influenced by insulin	Triglycerides measured in mg/dl

Table 2

Independent Variables: Descriptions and Measurements

Independent variables	Description of variable	Level of measurement
Glucose (Oral)	Oral glucose is given during OGIST	Measured in grams of actual glucose
Age	Age of patients	Continuous
Gender	Gender of patients	Categorical; Male or Female
Ethnicity	Ethnic group stated by patient	Categorical; Caucasian, Black, Hispanic, Asian, American Indian, Other
BMI	Body Mass Index. Body mass divided by the square of the body height expressed as units of kg/m^{2}.	Continuous

β -cell function is important is evaluating the effects of IR. Because β-cell function decreases due to exhaustion with advanced IR, both insulin, and C-peptide levels may be near normal levels while proinsulin is elevated at 15 minutes during the OGIST. HbA1c levels may be found at or near those for impaired glucose tolerant, but not high enough to document full onset diabetes. Because of this transitional phase, this I evaluated the OGIST for each patient individually and by group to identify this phenomenon.

Demographics and OGIST data in this study are presented as the mean ± SE. The dependent variables were analyzed using a student *t* test for variance comparison. The relationship between the variables was evaluated using one samples *t* test. The relationship between insulin and HbA1c was evaluated utilizing binomial logistic regression with graphing of insulin and HbA1c with age to observe possible changes.

Protection of Human Subjects and Patient Information

The OGIST results in this study were obtained from patient charts, laboratory records and EMR that were seen at the medical center providing study data. The data used in this study were collected maintaining Health Insurance Portability and Accountability Act of 1996 (HIPPA) standards for patient information protection and safety. Walden IRB approval granted 12/05/2016, IRB 12-05-16-007795. This study was conducted with the highest professional and ethics standards and over seen by both the medical director and compliance officer. No personal patient identification or information was used and complete anonymity of the individuals was maintained.

Table 3

Summary of Data Analyses for Research Question 1 and Comparative Surrogate Test

Variable	Analysis	Research Question/ Surrogate test
Insulin	Binomial logistic regression	RQ1/HOMA/McA/QUICKI
Proinsulin	Binomial logistic regression	RQ1
Glucose	Binomial logistic regression	RQ1/HOMA/QUICKI
HbA1c	Binomial logistic regression	RQ1
C-peptide	Binomial logistic regression	RQ1
Triglycerides	Binomial logistic regression	Comparative study with surrogate test McAuley's index (McA)

Summary

I evaluated the ability of the OGIST to accurately measure IR (sensitivity) in patients that initially meet the criteria for metabolic syndrome. The OGIST primarily measures the individual's proinsulin, insulin, C-peptide, glucose, and HbA1c at the end of the first phase insulin response to glucose. In those individuals with normal insulin sensitivity, or in other words normal binding of insulin to the cell and insulin production, these levels will be normal at the end of the first 15 minutes. In those individuals with IR, these levels will be abnormal depending on the severity of the individual's resistance or lack of binding of insulin to the insulin receptors on the cell surface. By measuring proinsulin, insulin, C-peptide, glucose, and HbA1c at the end of the first phase insulin response to glucose, the β-cell function can also be evaluated. In

early insulin resistance, insulin and C-peptide levels will be elevated with normal proinsulin, glucose, and HbA1c. As IR continues with overproduction of insulin by the pancreatic β-cells, proinsulin levels will increase due to a strained response of the β-cells with a gradual increase in both glucose and HbA1c levels. With continued overproduction of insulin by the β-cells with age insulin levels will drop to near normal while proinsulin levels remain slightly elevated and glucose levels increase. HbA1c levels now will increase to prediabetic or early diabetic stage. C-peptide levels will be found to be elevated as long as the patient continues to produce insulin. When the β-cells fail and no longer produce insulin C-peptide levels will be normal or below normal. This then provides a solid indicator for exogenous insulin to maintain normal serum glucose homeostasis.

The patients test results are similar to the general population of the Omaha, Nebraska region and should give a good representation of that of the U.S. population as a whole. The main hypothesis of this study was whether the OGIST can predict IR in those patients meeting metabolic syndrome and if age, gender, and ethnicity will influence the results of OGSIT. The study design used was causual-comparitive/quasi-experimental in nature to analyze patient results from the OGIST. The key dependent variables analyzed were insulin, proinsulin, C-peptide, glucose, and HbA1c. Chapter 4 includes the analysis and findings from this study.

Chapter 4: Results

Overview

IR and the reduced sensitivity of insulin to target tissues has been identified as the major pathology for T2DM and the associated cardiovascular complications seen in later life (Butler et al., 2008; DeFranzo & Abdul-Ghani, 2011: DeFronzo & Muhammad, 2011; Groop et al., 1996; Lorenzo et al., 2010). Considering the epidemic type increase in individuals diagnosed and those undiagnosed with T2DM worldwide increases the need for an accurate clinical test for IR.

The main objective of this study was to evaluate the ability of the OGIST to diagnose IR in clinical evaluation for those patients that met the criteria for metabolic syndrome. My second objective with this study was to evaluate the ability of the OGIST to evaluate possible differences that gender, age, and ethnicity may have on IR. The variables I measured to identify insulin sensitivity in this study included serum insulin, proinsulin, C-peptide, and glucose. Insulin sensitivity is identified by elevated serum levels of insulin, proinsulin, C-peptide, and glucose at the end of the first phase insulin secretion. These levels are at normal levels in those individuals with normal metabolism and are elevated in those individuals with IR. The higher insulin, proinsulin, and C-peptide levels are above normal, the less sensitive the target tissue is for insulin, allowing for the identification of IR in the individual long before β-cell exhaustion occurs. The variables I used to evaluate β-cell function included insulin, proinsulin, C-peptide, glucose, and HbA1c levels. Patients with prediabetes (early β-cell exhaustion) should demonstrate an inverse relationship between insulin and HbA1c (average serum

glucose levels). β -cell exhaustion can then be identified with an inverse relationship between insulin, proinsulin, C-peptide, and HbA1c.

The OGIST is a significantly different way to evaluate IR than current surrogate models such as HOMA, QUICKI, and the McA calculations. The OGIST is a dynamic test to measure the amount of insulin, proinsulin, glucose, and C-peptide at the end of the first phase insulin response to glucose. The first phase insulin response has been shown to be the most accurate time to measure insulin sensitivity (Abdul-Ghani et al., 2006; Gerich, 2002; Nessher & Cerasi, 2002; Salinari et al., 2009). The primary purpose of the OGIST is to identify IR in those patients meeting the criteria for metabolic syndrome and evaluate treatment in those patients diagnosed and treated for IR. The correct and accurate measurement on insulin sensitivity allows for the correct diagnosis and evaluation of therapeutic intervention for both epidemiological studies and clinical practice.

In this chapter, I will describe the methods for data management and procedures used for data analysis along with interpretation of the study results of the analysis design that was described in Chapter 3. The collection of the data files, handling and input of the data, complete descriptive statistics, and the results of *t*test and binomial logistic regression models will be included with answers to the research questions. The following research questions and hypotheses guided this study:

> RQ1: In patients meeting the criteria for metabolic syndrome, will an Oral Glucose Insulin Secretion Test (OGIST) predict insulin resistance in the clinical setting?

$H_0$1: In patients diagnosed with metabolic syndrome, the Oral Glucose Insulin Secretion Test (OGIST) does not predict insulin resistance in a clinical setting.

H_A1: In patients diagnosed with metabolic syndrome, the Oral Glucose Insulin Secretion Test (OGIST) does predict insulin resistance in a clinical setting.

RQ2: Does age, gender, and ethnicity have an influence on the Oral Glucose Insulin Secretion Test (OGIST) for insulin, proinsulin, glucose, and C-peptide levels.

$H_0$2: The Oral Glucose Insulin Secretion Test (OGIST) does not identify changes in insulin, proinsulin, glucose, and C-peptide in different ages, gender, and ethnic groups.

H_A2: The Oral Glucose Insulin Secretion Test (OGIST) does identify changes in insulin, proinsulin, glucose, and C-peptide in different ages, gender, and ethnic groups.

Data Collection and Handling

I obtained Walden University IRB approval prior to collecting any data for this study (IRB Approval Number: 12-05-16-0077958). A study coordinator nurse was trained on the study protocol and was responsible for identifying patients that met the criteria for metabolic syndrome who were then tested using OGIST. Patient data were obtained from patient charts, an EMR system newer to medical center study site, and the Laboratory Information System (LIS) 18 months prior to the study and collected 2017. I obtained the OGIST results 3 weeks after approval of the study by the IRB. The International Classification of Disease Version 10 codes used in the identification of patients meeting the criteria for this study included E16.3 (increased secretion of

glucagon), E16.8 (other specific disorders of pancreatic secretion), E74.8 (other specific disorders of carbohydrate metabolism), and E88.81 (metabolic syndrome). The EMR numeric patient identifier was maintained to allow access of the files by the granting medical center, and all patient information, such as patient's name, was disallowed. I imported all variables into an Excel 2010 spreadsheet and then imported them from there into the SPSS Statistics 22 program for analysis. Variables included in the SPSS program for analysis consisted of age, gender, ethnicity, BMI, insulin, proinsulin, C-peptide, HbA1c, and triglycerides. My descriptive analysis of the patients' data found that the study population comprised of White 50.4%, Hispanic 43.7%, Black 2.0%, and Asian 2.8% (see Table 3). Although these demographics are somewhat different from the normal Omaha, NE population they are similar to that of the medical center demographics.

Table 4

Descriptive Statistics for Ethnicity and Percentages

		Frequency	Percent	Valid Percent
Valid	Asian	7	2.8	2.8
	Black	5	2.0	2.0
	White	128	50.4	51.0
	Hispanic	111	43.7	44.2
	Total	251	98.8	100.0
Missing	System	3	1.2	
Total		254	100.0	

Table 5

Descriptive Statistics for Gender and Percentages

		Frequency	Percent	Valid Percent
Valid	Male	120	47.2	47.8
	Female	131	51.6	52.2
	Total	251	98.8	100.0
Missing		3	1.2	
Total		254	100.0	

The gender demographics for this study equaled 131 women (51.6%) and 120 men (47.2%; see Table 5). These findings are similar to that seen in the Omaha area, with women making up 51.3% of the population and men 48.7%. The patient lab results that I used in this study included glucose, HbA1c, insulin, proinsulin, C-peptide, and triglyceride cholesterol levels. These results were obtained at the end of the first phase insulin response to glucose after a 36-gram glucose oral bolus was given to the patient. Normal levels of these results (Deska-Pagana & Pagana, 2017) are included in Table 6 below.

Oral Glucose Insulin Secretion Test Procedure

The OGIST is given to those patients that initially meet the criteria for metabolic syndrome or have been previously diagnosed with IR and are under treatment. Patients were initially given 36 grams of oral concentrated glucose, (EASYDEX 100 Bvo free, by Aero-Med LTD.), and at 15 minutes (the end of the first phase insulin response to glucose) insulin, glucose, C-peptide, proinsulin, HgA1c levels are obtained. Those patients responding normally to glucose will demonstrate normal levels of insulin, glucose, proinsulin, and HgA1c. C-peptide will remain elevated since this peptide is secreted in 30–40 minutes by the kidneys. In those patients with IR, cellular sensitivity

to insulin is reduced due to poor binding of insulin to the cellular insulin receptor. This results in higher levels of insulin, glucose, and proinsulin at the end of the first phase insulin response to glucose. Patients with advanced IR, insulin, proinsulin, and C-peptide will start to drop due to β-cell exhaustion, while glucose and HbA1c levels will increase based on both the sensitivity of insulin to the cell and the ability of the β-cell to produce insulin to maintain glucose homeostasis. Glucose and HbA1c levels were analyzed using a Beckman Coulter UniCel DxC800 chemistry analyzer. Insulin, proinsulin, and C-peptide were analyzed by chemiluminescent immunoassay on a Centaur/Centaur XP immunoassay platform. I used SPSS version 18.0 analytics software to analyze the data in this study.

I calculated the surrogate indexes of HOMA, QUICKI, and McA from the patient results and used them for validation comparison with the results from the OGIST. The equations for surrogate indexes calculations for insulin sensitivity and IR included the following HOMA equation: $HOMA = \frac{\text{fasting insulin x fasting glucose}}{22.5}$ Where the denominator 22.5 is the normalization factor initially calculated by testing normal healthy individuals (Matthews et al., 1985). This model calculates the steady-state insulin and glucose concentrations. The following QUICKI equation: $\text{QUICKI} = \frac{1}{[\log(Insulin\ \mu U/mL) + (\log(Glucose\ mg/dL)]}$ mathematically converts fasting insulin and glucose levels using the logarithm and reciprocal of both (Duncan et al., 1995). The McA equation is McA = exp[2.63-0.28 log(Insulin µU/mL) – 0.31 log(Triglycerides mmol/L)] and uses both insulin and triglycerides levels to predict

insulin sensitivity since insulin is the feedback hormone for triglyceride production (McAuley et al., 2001).

Results

In this study, I included a total of 251 patients with IR, either newly diagnosed or in treatment, with a mean age of 49.2 and a mean BMI of 34. Because these patients had confirmed IR, it was reasonable to expect a higher BMI than normal since insulin levels higher than 25mU/L stimulate adipogenesis during periods of hyperinsulinemia at the end of the first phase insulin response to glucose. The descriptive statistics for the participants can be found in Table 6.

Table 6

Descriptive Statistics

	N	Minimum	Maximum	*M*	*SD*	Kurtosis	
							Std. Error
Age	250	12.0	79.0	50.13	14.55	-.301	.307
BMI	251	3.37	468.29	240.47	162.69	-1.69	.306
Insulin (1U/L)	251	6.8	940.9	95.28	116.51	24.40	.306
Glucose (1g/dL)	251	71.0	452.0	147.85	52.08	6.15	.306
HgA1c (%)	251	3.5	138.0	6.42	8.46	236.29	.306
Triglycerides (1g/dL)	251	4.8	961.0	163.58	128.68	11.22	.306
HOMA	251	3.56	1520.24	330.0	261.49	.842	.306
QUICKI	251	.159	.274	.188	.022	2.425	.30
McA	251	.587	9.05	1.35	1.38	15.66	.30
Valid N (listwise)	250						

The mean insulin level at the end of the first phase insulin response to glucose was 99.84 mU/L instead of the normal limits of 3–25 mU/L. The maximum level of insulin was 940.9 mU/L, which was observed in newly diagnosed patients, and a

minimum of 6.8 mU/L, seen in patients with β-cell exhaustion. The insulin standard deviation was 118.87 mU/L.

The mean level of proinsulin was 71.75 pmol/L at the end of the first phase insulin response to glucose. The maximum was 840 pmol/L, seen in newly diagnosed patients and those with early β-cell exhaustion, and a minimum of 1 pmol/L was seen in those with severe β-cell exhaustion/burn out and early diabetes. The proinsulin standard deviation was found to be pmol/L.

The mean C-peptide levels at the end of the first phase insulin response to glucose was 5.26 ng/ml with the maximum levels at 26 and minimum C-peptide level 0 pmol/L. The C-peptide standard deviation was 3.24 pmol/L. C-peptide levels at the end of the first phase insulin response to glucose should always be slightly higher than normal due to its secretion through the kidneys 30–40 minutes after release.

Normal glucose levels at the end of the first phase insulin response to glucose are 80-120mg/dl. The mean glucose levels in this study were 147.46 mg/dl with the maximum 452 mg/dl and minimum 71 mg/dl with *SD* of 53.13 mg/dl. The higher levels of glucose were seen in those patients with higher HbA1c levels and lower C-peptide levels suggesting early onset diabetes and severe β-cell exhaustion.

Normal HbA1c levels are below 5.6%, with 5.7-5.9% being considered prediabetes with early β-cell exhaustion and 6.0% and above are classified as diabetic. In this study the mean HbA1c was 5.9% (prediabetes) with the maximum 13.8% (uncontrolled diabetes), and minimum of 3.5% with *SD* 1.53%. The higher levels of HbA1c were seen with β-cell exhaustion.

Triglycerides cholesterol is produced by the liver and used for cell wall production and energy for certain cells of the body. Insulin is the feedback hormone for triglyceride production. In this study the mean triglyceride level was 186.9mg/dl with the maximum of 696 mg/dl and minimum level at 32 mg/dl with *SD* 1.86 mg/dl. Newly diagnosed patients with IR demonstrated higher levels of triglycerides with the lower levels found in those patients that were well treated.

One sample *t*-test was conducted to evaluate the dependent variables of the OGIST results mean against the null hypothesis. The mean results were then compared to the normal values for each dependent variables glucose, insulin, proinsulin, C-peptide, and HbA1c. The null hypothesis $H_0 1$ was rejected because $p <.000$ was observer for all the dependent variables.

Table 7

One Same t Test

	t	df	*p* (2-tailed)	Mean Difference	Lower	Upper
Insulin (mU/L)	12.57	223	.000	99.84	84.18	115.49
Proinsulin (pmol/L)	12.34	222	.000	71.75	60.30	83.21
C-Peptide (ng/mL)	24.08	222	.000	5.23	4.81	5.66
Glucose (mg/dL)	41.54	223	.000	147.46	140.46	154.46
HbA1c (%)	57.78	223	.000	5.91	5.71	6.11
Triglycerides (mg/dL)	25.66	223	.000	186.92	172.57	201.27
HOMA	9.82	223	.000	11.30	9.037	13.57
QUICKI	162.26	223	.000	.25	.25	.25
McA	46.30	223	.000	4.07	3.90	4.24

Regression Analysis

Binomial logistic regression analysis was conducted to evaluate the relationship of insulin, proinsulin, and C-peptide with the independent variables. Because the dependent variables demonstrated a dichotomous tendency and did not meet the criteria assumptions for linear regression, binomial logistic regression was used in this study. The first step was to create a binary categorical dependent variable for each continuous variable. Each dependent variable was changed from continuous to categorical binary variables by using the median value of each group as a cutoff point and separating into two groups above and below the median value. This allowed the dependent variables to meet assumption testing for binomial logistic regression. This information was then used to answer Research Question 1 to determine if the OGIST is capable of predicting insulin resistance in the patient meeting the criteria for metabolic syndrome. Tables 8-20 demonstrate the results of this analysis. Bonferroni multiple-comparison correction was used since multiple tests were performed simultaneously resulting in statistical significance being accepted with $p < 0.05$ (Tabachnick & Fidell, 2014).

Binomial logistic regression was used to evaluate the relationship between the predictors and the dependent variable insulin Table 8.

Table 8

Binomial Regression Analysis Summary: Dependent Variable-Insulin

		B	S.E.	Wald	*df*	*p*	*OR*	95% C.I.for OR Lower	Upper
Step 1[a]	In_age	-2.60	1.14	5.15	1	.023	.074	.008	.700
	In_BMI	1.07	1.58	.45	1	.500	2.915	.130	65.18
	Ln_Ethnicity	-1.97	1.16	2.85	1	.091	.139	.014	1.37
	Ln_HOMA	-47.23	14.98	9.93	1	.002	.000	.000	.000
	Ln_McA	-5.00	1.84	7.36	1	.007	.007	.000	.249
	Ln_QUICKI	-513.38	152.44	11.34	1	.001	.000	.000	.000
	Constant	-600.89	179.50	11.20	1	.001	.000		

a. Variable(s) entered on Step 1: In_age, In_BMI, Ln_Ethnicity, Ln_HOMA, Ln_McA, Ln_QUICKI.

The results in Table 8 demonstrate the model is statistically significant, $x^2(6) = 231.548$, $p < .005$. Nagelkerke R^2 was 87.1% and the model correctly identified 95.2% of the cases. Sensitivity of the test was 91.4% and specificity was 96.6%. Positive predictive value was 91.4% with a negative predictive value of 96.6%. The predictor variables used in this model included age, BMI, ethnicity, HOMA, McA and QUICKI. Out of these variables age, HOMA and QUIKI, and McA were found to be significant. This analysis demonstrates that the odds or reduced insulin sensitivity in older adults is 7.4% higher than younger adults. The significance of the surrogate tests for insulin resistance compared to the OGIST were HOMA: 0.002, McA: 0.007, and QUICKI: 0.001

Binomial logistic regression was used to evaluate the relationship between the predictors and the dependent variable proinsulin Table 9.

Table 9

Binomial Regression Analysis Model Summary: Dependent Variable-Proinsulin

		B	S.E.	Wald	*df*	*p*	*OR*	95% C.I.for OR	
								Lower	Upper
Step 1[a]	In_age	.247	.43	.32	1	.568	1.280	.548	2.991
	In_BMI	1.089	.75	2.06	1	.151	2.971	.672	13.140
	Ln_Ethnicity	-.109	.52	.04	1	.834	.897	.323	2.491
	Ln_HOMA	-7.961	3.13	6.44	1	.011	.000	.000	.163
	Ln_McA	-.650	.76	.71	1	.397	.522	.116	2.347
	Ln_QUICKI	-84.349	29.67	8.07	1	.004	.000	.000	.000
	Constant	-103.842	34.87	8.86	1	.003	.000		

Variable(s) entered on step 1: In_age, In_BMI, Ln_Ethnicity, Ln_HOMA, Ln_McA, Ln_QUICKI.

The results in Table 9 demonstrates the model is statistically significant, $x^2(6) = 67.665$, $p < .005$. Nagelkerke R^2 was 31.8% and the model correctly identified 70.7% of the cases. Sensitivity of the test was 71.8% and specificity was 69.7%. Positive predictive value was 67.7% with a negative predictive value of 73.6%. The predictor variables used in this model included age, BMI, Ethicity, HOMA, McA and QUICKI. Age, BMI, and ethnicity did not demonstrate any significance in proinsulin production. The significance of the surrogate tests for proinsulin compared to the OGIST were HOMA: 0.011 and QUICKI: 0.004. Binomial logistic regression was used to evaluate the relationship between the predictors and the dependent variable C-peptide Table 10.

Table 10

Binomial Regression Analysis Model Summary: Dependent Variable-C-peptide

		B	S.E.	Wald	*df*	*p*	*OR*	95% C.I.for OR	
								Lower	Upper
Step 1[a]	In_age	-.62	.51	1.47	1	.225	.536	.196	1.467
	In_BMI	-.39	.87	.197	1	.657	.677	.121	3.792
	Ln_Ethnicity	-.58	.62	.851	1	.356	.560	.163	1.921
	Ln_HOMA	-12.63	3.93	10.309	1	.001	.000	.000	.007
	Ln_McA	-2.45	.89	7.585	1	.006	.086	.015	.493
	Ln_QUICKI	-129.71	37.50	11.96	1	.001	.000	.000	.000
	Constant	-145.12	43.91	10.92	1	.001	.000		

a. Variable(s) entered on step 1: In_age, In_BMI, Ln_Ethnicity, Ln_HOMA, Ln_McA, Ln_QUICKI.

The results in Table 10 demonstrate the model is statistically significant, $x^2(6) = 116.545$, $p < .005$. Nagelkerke R^2 was 49.8% and the model correctly identified 81.1% of the cases. Sensitivity of the test was 83.2% and specificity was 79.0%. Positive predictive value was 80% with a negative predictive value of 82.3%. Age, BMI, and ethnicity did not demonstrate any significance in C-peptide. The significance of the surrogate tests for C-peptide compared to the OGIST were HOMA: 0.001, McA: 0.006, and QUICKI: 0.001. Binomial logistic regression was used to evaluate the relationship between the predictors and the dependent variable HbA1c Table 11.

Table 11

Binomial Regression Analysis Model Summary: Dependent Variable-HbA1c

		B	S.E.	Wald	*df*	*p*	*OR*	95% C.I.for OR Lower	Upper
Step 1[a]	In_age	1.86	.44	17.44	1	.000	6.46	2.692	15.509
	In_BMI	1.18	.73	2.57	1	.109	3.25	.770	13.797
	Ln_Ethnicity	1.83	.60	9.25	1	.002	6.27	1.922	20.500
	Ln_HOMA	5.43	2.53	4.59	1	.032	229.71	1.594	33105.192
	Ln_McA	.71	.734	.950	1	.330	2.04	.485	8.609
	Ln_QUICKI	43.00	22.62	3.61	1	.057	47.5	.263	8.59
	Constant	34.004	25.86	1.72	1	.189	58.60		

a. Variable(s) entered on step 1: In_age, In_BMI, Ln_Ethnicity, Ln_HOMA, Ln_McA, Ln_QUICKI.

The results in Table 11 demonstrate the model is statistically significant, $x^2(6) = 36.34$, $p <.0005$. Nagelkerke R^2 was 18.2% and the model correctly identified 66.7% of the cases. Sensitivity of the test was 77.5% and specificity was 53.2%. Positive predictive value was 67.29% with a negative predictive value of 65.5%. The predictor variables used in this model included age, BMI, and ethnicity. Both age and ethnicity demonstrated significance in HbA1c. The significance of the surrogate tests for HbA1c compared to the OGIST was HOMA: 0.032.

Binomial logistic regression was used to evaluate the relationship between the predictors and the dependent variable triglycerides. Table 12 to allow the comparison of the surrogate test McA with OGIST.

Table 12

Binomial Regression Analysis Validation Model Summary: Dependent Variable Triglycerides

		B	S.E.	Wald	df	*p*	OR	95% C.I.for OR	
								Lower	Upper
Step 1[a]	In_age	-2.704	.756	12.804	1	**.000**	.067	.015	.294
	In_BMI	1.119	1.357	.680	1	.409	3.062	.214	43.720
	Ln_Ethnicity	-2.627	1.182	4.938	1	**.026**	.072	.007	.734
	Ln_HOMA	-7.514	5.176	2.107	1	.147	.001	.000	13.902
	Ln_McA	22.945	3.380	46.082	1	**.000**	92271826	12243677.52	6953866483000.00
	Ln_QUICKI	-111.22	46.514	5.718	1	**.017**	.000	.000	.000
	Constant	-158.93	54.879	8.387	1	.004	.000		

Variable(s) entered on step 1: In_age, In_BMI, Ln_Ethnicity, Ln_HOMA, Ln_McA, Ln_QUICKI.

The results in Table 12 demonstrate the model is statistically significant, $x^2(6) = 36.34$, $p < .0005$. Nagelkerke R^2 was 78.4% and the model correctly identified 92.4% of the cases. Sensitivity of the test was 94.6% and specificity was 89.9%. Positive predictive value was 91.1% with a negative predictive value of 93.8%. The predictor variables used in this model included age, BMI, and ethicity. HOMA, McA and QUICKI were surrogate predictors. Out of these variables only age and ethnicity demonstrated a correlation with elevated triglycerides. The significance of the surrogate tests for triglycerides compared to the OGIST were McA: 0.001 and QUICKI: 0.017.

Scatter plot graph analysis was completed on insulin, proinsulin, C-peptide, and HbA1c. The dependent variables insulin, proinsulin, C-peptide, and HgA1c were placed on the X-axis with age placed on the Y-axis for possible differences seen during advanced age.

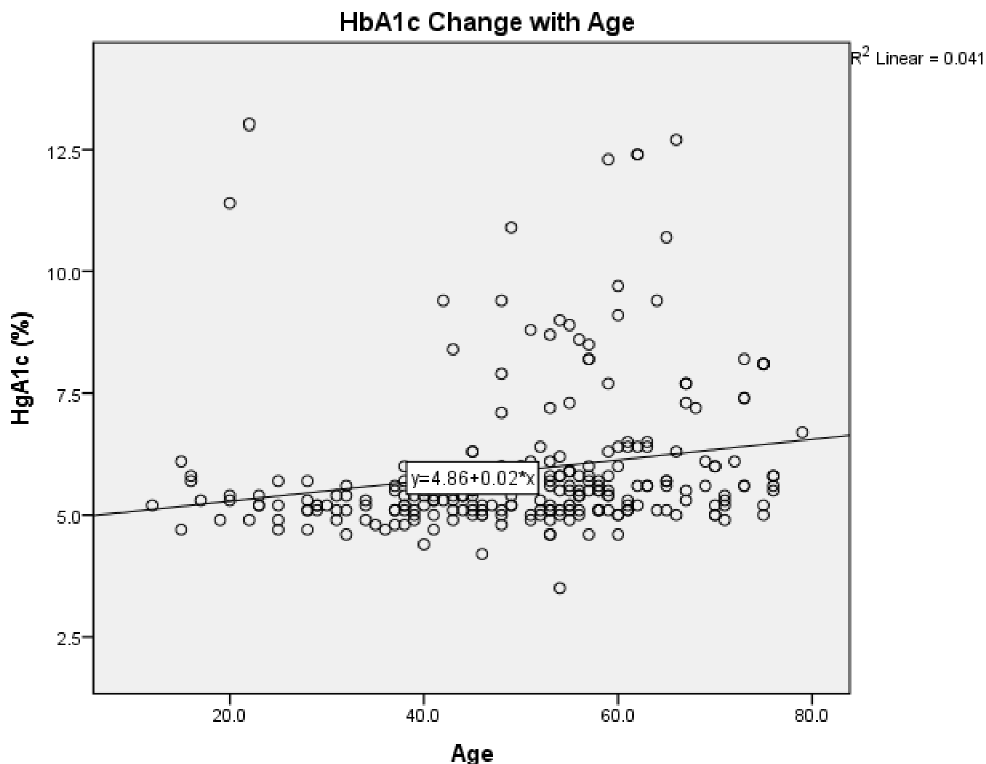

Figure 1. Analysis of HbA1c with age.

Figure 1 demonstrates the increase in HbA1c levels with increased age in those patients diagnosed with IR. The higher HbA1c levels found in patients over 40 years of age is due to advanced β-cell exhaustion due to IR. The higher HbA1c levels observed in younger patients was observed with severe IRThose patients having elevated insulin levels above 500 mU/L at the end of the first phase insulin response to glucose.

Table 13

Binomial Regression Analysis Validation Model Summary: Dependent Variable Age

		B	S.E.	Wald	df	*p*	OR	95% C.I.for OR	
								Lower	Upper
Step 1[a]	InsulinCat	1.130	.364	9.625	1	.002	3.095	1.516	6.319
	ProinsulinCat	-.220	.309	.510	1	.475	.802	.438	1.469
	CPeptideCat	-.154	.329	.220	1	.639	.857	.450	1.633
	HbA1cCat	-.898	.274	10.721	1	.001	.407	.238	.697

a. Variable(s) entered on step 1: InsulinCat, ProinsulinCat, CPeptideCat, HbA1cCat.

The results in Table 13 demonstrate the model is statistically significant, $x2(4) = 23.14$, $p < .0005$. Nagelkerke R2 was 11.9% and the model correctly identified 64.3% of the cases. Sensitivity of the test was 71.2% and specificity was 58.0%. Positive predictive value was 60.4% with a negative predictive value of 69%. The predictor variables used in this model were InsulinCat, ProinsulinCat, CPeptideCat, and HbA1cCat. Only InsulinCat and HgA1cCat were found to be significant with InsulinCat: 0.002 and HbA1cCat: 0.001.

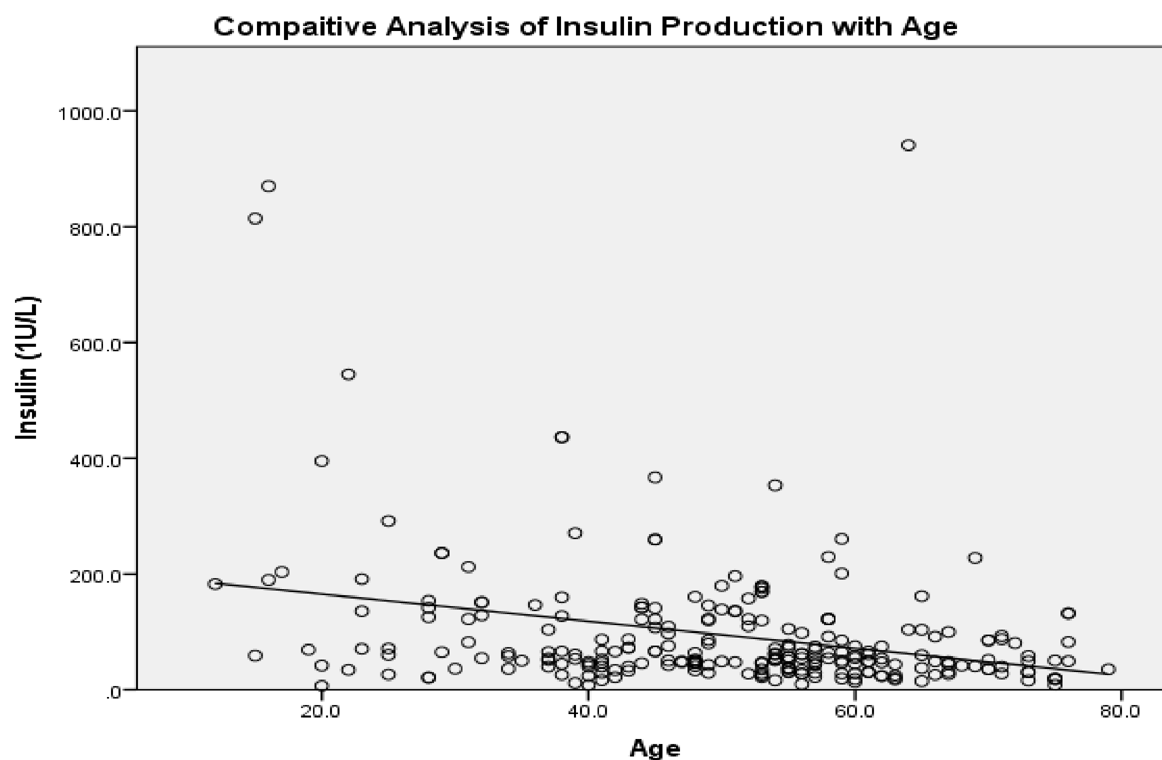

Figure 2. Analysis of insulin secretion with age.

Binomial logistic regression analysis was completed to confirm the changes seen in insulin production with age. To accommodate the requirements for binomial regression patient's ages were divided into those under 50 years of age and those 50 years of age and older. Figure 2 demonstrates the results of this test.

Summary

This study demonstrates the effectiveness of the OGIST and the ability of the test to predict insulin resistance in a clinical setting without invasive hospital testing as seen in the glucose clamp technique (DeFronzo et al., 1979). The OGIST allowed the direct measurement of all insulin indices to include insulin, proinsulin, C-peptide, and

HbA1c at the end of the first phase insulin response to glucose. The OGIST allowed evaluation of both the insulin binding to target tissue and insulin production of the pancreatic β-cells.

The objective of this study was to evaluate the ability of the OGIST to diagnose IR in the clinical setting that has met the criteria for metabolic syndrome. The OGIST is designed to evaluate insulin sensitivity by directly measuring the patients' insulin, proinsulin, C-peptide, and glucose levels at the end of the first phase insulin response to glucose. An oral glucose challenge of 36 grams is given is initially given to the patient with labs being obtained 15 minutes later. HbA1c is also obtained to evaluate the patient's glucose control. This allows evaluation of insulin production by direct measure of C-peptide at the end of the first phase insulin response to glucose and glucose homeostasis by HbA1c. Insulin sensitivity is then evaluated by direct measure of insulin and proinsulin at the end of the first phase insulin response to glucose. This test also allows the evaluation of the pancreatic β-cells function/exhaustion by the measurement of insulin, proinsulin, C-peptide, and comparing with HgA1c levels.

A total 251 patients were included into the study. A total of 26 patient's data were not considered for the study due to the lack of critical data. The mean study patient BMI was found to be higher than that of the normal population (34 vs 28) which would be expected with the increased insulin production. Mean insulin levels in this study at the end of the first phase insulin response was 99.84 mU/L compared to normal levels of 3-25 mU/L. The higher level of insulin was found to be in a newly diagnosed patient with 940.9 mU/L and a low of 6.8 mU/L which was observed in a patient with

prediabetes and β-cell exhaustion. C-peptide levels should normally be elevated at the end of the first phase insulin response due to the clearance of this peptide by the kidneys between 30 and 40 minutes. The C-peptide levels in this study ranged between 0 pmol/L and 26 pmol/L with mean levels at 5.26 pmol/L. Lower than normal levels were seen in those patients with β-cell exhaustion and diabetes with no or very poor insulin production. The higher C-peptide levels were found in patients with uncontrolled IR producing very high levels of insulin. Glucose levels were found to parallel HgA1c levels and based primarily on the patients' glucose and HgA1c homeostasis. Triglycerides were found to be higher in patients diagnosed with IR and higher insulin levels. The mean triglyceride level in the study was 189.6mg/dl with the minimum of 32mg/dl in patients being treated for IR and a high of 696mg/dl found in patients newly diagnosed with higher insulin levels.

Research Question H1

In patients meeting the criteria for metabolic syndrome will an OGIST predict IR in the clinical setting? This study demonstrates that patients that are insulin resistant demonstrate an increase in insulin, proinsulin, C-peptide, and glucose at the first phase insulin response to glucose when tested using the OGIST. The degree of insulin production appears to be associated directly with the patient's insulin sensitivity and β-cell function. The less sensitive the patient is to insulin the higher the production to maintain glucose homeostasis. Those older patients with IR demonstrate β-cell exhaustion which is seen with the increase in glucose and HbA1c and reduced levels of insulin, proinsulin, and C-peptide at the end of the first phase insulin response to

glucose. Binomial logistic regression analysis demonstrated a statistical significance thus allowing the rejection of the null hypothesis.

Research Question H2

Does age, gender, and ethnicity, have an influence on OGIST. Serum insulin, proinsulin, and C-peptide were elevated at the end of the first phase insulin secretion phase in patients with IR tested using OGIST. When comparing the patients in this study with IR, there was a significant reduction in insulin production with age with an increase in glucose and HbA1c inversely equal to the reduced insulin production. This change was significant in insulin and C-peptide levels tested using OGIST. Changes in proinsulin levels were not significant with increasing age but were elevated in younger patient with insulin resistance. The reduction in insulin production with increasing HgA1c then demonstrates β-cell exhaustion. These results suggest the overproduction of insulin with age leading to β-cell exhaustion and diabetes. There were no comparative changes observed in insulin, proinsulin, or C-peptide, levels between gender and ethnic groups. There was a statistically significant change noted in HbA1c and triglyceride levels with ethnicity. The above findings allowed the rejection of the null hypothesis for Research Question 2.

When comparing the OGIST to HOMA, QUICKI, and McA, HOMA had similar predictive quality to OGIST for insulin and C-peptide alone. The QUICKI test demonstrated similar predictive qualities to OGIST for insulin, proinsulin, and C-peptide. McA demonstrated similar predictive qualities to OGIST for triglycerides only. OGIST demonstrated significant predictive qualities for insulin, proinsulin, C-peptide, HbA1c,

and triglycerides. When evaluating the ability of the tests to evaluate the individuals' insulin sensitivity, production and β-cell function OGIST was superior to the other tests. The OGIST superiority could be due to the ability of the test to evaluate the combination of insulin, proinsulin, glucose, C-peptide, and HgA1c together at the end of the first phase insulin response to glucose.

Chapter 5: Interpretation, Study Limitations, Recommendations, and Conclusion

Introduction

IR is characterized as the reduced response of target tissue to the polypeptide hormone insulin (DeFronzo, 1988) and is theorized to be the leading pathogenesis of T2DM in adult life (Cerf, 2013; DeFronzo, 1988; Groop et al., 1996; Lorenzo et al., 2010; Samuel & Shulman 2012; Warram et al., 1990; Weir & Bonner-Weir, 2004). Skeletal muscle has been cited as the primarily tissue responsible for 80%–90% of postprandial glucose up-take during the first phase insulin phase insulin response (Kahn, 2001). Within the first 15 minutes after a glucose load, this phase could allow testing for insulin sensitivity and β-cell function. The OGIST measures levels of insulin, proinsulin, C-peptide, and HbA1c at the end of the first phase insulin response to a primary glucose challenge. This allows direct evaluation of both the individual's insulin sensitivity and β-cell function. The OGIST then allows for early detection of IR, evaluation of treatment, and beta cell function by identifying changes in insulin and insulin indices at the end of the first phase insulin response to glucose (Cerf, 2013; Kahn, 2001).

The main objective of the study was to evaluate the ability of the OGIST to diagnose IR in the clinical outpatient setting in those individuals meeting the criteria for metabolic syndrome. The second objective of the study was to evaluate the ability of the OGIST to evaluate possible differences that gender, age, and ethnicity may have on IR.

The results of this study demonstrated that the OGIST identified an increase in mean levels of insulin during the first phase insulin response to glucose in those patients

meeting the criteria for metabolic syndrome compared to normal levels. I found proinsulin levels to be higher in those patients in earlier stages of IR compared to advanced stages of IR, demonstrating a lower proinsulin production due to beta cell exhaustion. C-peptide levels were found to be subtly elevated throughout the study except in those patients with advanced IR and increased HbA1c, demonstrating β-cell exhaustion and lower than normal levels of insulin production. Glucose levels were found follow HbA1c levels in patients with IR, reflecting β-cell function. The higher the glucose and HbA1c, the lower of the β-cell function, demonstrating early diabetes. I found triglyceride levels to be higher in all patients with elevated levels of insulin at the end of the first phase insulin response due to the over stimulation of triglyceride production by the elevated insulin (see Avramoglu et al., 2006; Bayes et al., 2004; Cruz-Garcia et al., 2014).

Patients meeting the criteria for metabolic syndrome and tested using the OGIST with elevated insulin levels also demonstrated increased obesity and BMI. This finding was reasonable since elevated levels of insulin stimulate adipose genesis (see Angeloni & Hanson, 2002; Aso et al., 2005; Avramoglu et al., 2006; Bayes et al., 2004). Younger patients demonstrated normal glucose and HbA1c levels with increased insulin proinsulin levels compared to older patients demonstrating reduced levels of insulin and proinsulin with elevated glucose and HbA1c levels, suggesting pancreatic β-cell exhaustion from overproduction of insulin during their lifetime. There was no significant differences in insulin, proinsulin, C-peptide, and triglycerides among gender or ethnic groups.

When comparing the results of the OGIST with the mathematical surrogate tests including HOMA, QUICKI, and McA, each had similar predictive characteristics for IR in the fasting patient. These tests were unable to identify dynamic changes in insulin sensitivity or β-cell function and were inferior to the OGIST in this regard. This could be due to the use of fasting insulin and glucose levels used in the surrogate tests compared to the OGIST using results at the end of the first phase insulin response to glucose (Bonora et al., 2002; Gayoso-Diz et al., 2013; Katz et al., 2000; Mattes et al., 1985; McAuley et al., 2001; Qu et al., 2011).

When comparing the OGIST with surrogate tests including HOMA, QUICKI, and McA, I found that both HOMA and QUICKI demonstrated comparative predictive ability for IR to that of the OGIST. The surrogate studies were unable to evaluate individual's insulin sensitivity, insulin production, and beta cell function compared to the OGIST.

Interpretation of the Findings

When considering the first research question: In patients meeting the criteria for metabolic syndrome, does the OGIST predict IR in the clinical outpatient setting? The results of this study demonstrated that the OGIST could predict IR in the individual at the end of the first phase insulin response to glucose by measuring serum insulin, proinsulin, C-peptide, glucose, and HbA1c levels. The OGIST was effective in determining insulin sensitivity within the individual and β-cell function.

The findings of this study revealed that patients that are insulin resistant demonstrated an increase in insulin, proinsulin, C-peptide, and glucose at the first phase

insulin response to glucose when tested using the OGIST. The results from this study suggest that the amount of insulin production is directly associated with the patient's insulin sensitivity and β-cell function (see Abdul-Ghani et al., 2006; Cerf, 2013; DeFronzo & Tripathy, 2009; Gerich, 2002; Lorenzo et al., 2010; Reaven, 2004; Weir & Bonner-Weir, 2004). The less binding of insulin to the cell's insulin receptor, the less sensitive the patient is to insulin and the higher the production is needed to maintain glucose homeostasis (Abdul-Ghani et al., 2006; Cerf, 2013; DeFronzo & Tripathy, 2009; Gerich, 2002; Reaven, 2004). The OGIST results in this study suggest that those older patients with IR demonstrate β-cell exhaustion, which is seen with the increase in glucose and HbA1c and reduced levels of insulin, proinsulin, and C-peptide at the end of the first phase insulin response to glucose (see Cerf, 2013; DeFronzo & Tripathy, 2009; Gerich, 2002). Binomial logistic regression analysis demonstrated a statistical significance thus allowing the rejection of the null hypothesis with $p < 0.005$.

The change in insulin I observed in the 249 participants during the OGIST was an increase in the mean serum level at the end of the first phase insulin response of 99.84 mU/L compared to the normal level of 25 mU/L. The standard deviation was 118.87 mU/L, which was due to those patients with very early IR producing very high amounts of insulin to maintain normal glycemic conditions and those patients in β-cell exhaustion and entering prediabetes or early onset diabetes. The OGIST mean serum insulin level of 99.84 mU/L was similar in part to that of the Hyperinsulinemic Euglycemic Glucose Clamp studies of DeFronzo et al. (1979) and Muhammad et al. (2006). The results of these studies both suggested a higher insulin level at the end of

the first phase insulin response to glucose. The mean proinsulin levels during this study was found to be 71.75 pmol/L with a maximum serum level of 840 pmol/L found in younger patients with IR as opposed to older prediabetic patients which demonstrated a low of 1 pmol/L. Because proinsulin is released initially by the β-cells as a nonactive hormone containing C-peptide, elevated levels of serum proinsulin would suggest a significant demand on the pancreatic β-cells to release increased amounts of insulin secondary to hyperglycemia at the end of the first phase insulin response to glucose. Hyperglycemia was evident in this study with the mean serum glucose level of 147.46mg/dl in the first phase of this response glucose instead of the normal serum glucose level of 80–120mg/dl. Hyperglycemia observed at the end of the first phase insulin responsive glucose was also observed by Muhammad et al. in patients diagnosed with IR and IFG.

I found the mean C-peptide level in the study to be 5.26 ng/ml, with a standard deviation of 3.245 ng/ml, which was slightly higher than normal levels. Because C-peptide is a waste product cleaved from proinsulin in the bloodstream, leaving the active hormone insulin available for use, it would be reasonable for C-peptide levels to be slightly elevated since this peptide is excreted by the kidneys within 30–40 minutes. C-peptide levels were found to be significantly higher in those patients demonstrating elevated levels of HbA1c, which indicated β-cell exhaustion and prediabetes.

The OGIST was validated using HOMA, QUICKI, and McA and I found it to be the most similar in ability to diagnose this resistance with the QUICKI test. Both the HOMA and QUICKI tests are calculated studies based on fasting serum glucose levels

with QUICKI being differentiated in that it uses the log of glucose and insulin in the calculation where HOMA does not (Gerich, 2002; Hettihewa & Weerarathna, 2011; Katz et al., 2000; McAuley, 2001). The McA test uses the exponential of fasting insulin and triglycerides to calculate cut-off levels for IR. All three surrogate tests for IR use fasting serum levels of glucose, insulin, and/or triglycerides and do not give a true evaluation of the patient's insulin, proinsulin, C-peptide, and glucose levels at the most critical time, the end of the first phase insulin response to glucose (Gerich, 2002). The OGIST demonstrated superior ability to evaluate insulin sensitivity, beta cell function, and progression to diabetes. Because the OGIST analyses serum levels of insulin, proinsulin, C-peptide, glucose, and HbA1c at the end of the first phase of responsive glucose, the test was far superior evaluating the individual for IR, beta cell function, and evaluation of treatment.

Research Question 2 was: Does age, gender, and ethnicity have an influence on OGIST? Serum insulin, proinsulin, and C-peptide were elevated at the end of the first phase insulin secretion phase in participants with IR that were tested using the OGIST. These levels were found to be inversely related to glucose and HbA1c in patients with advanced IR experiencing β-cell exhaustion (see Cerf, 2013; DeFronzo & Tripathy, 2009; Gerich. 2002). When comparing the results of the OGIST insulin resistant patients in this study with normal levels, there was a significant reduction in insulin production with age with an increase in glucose and HbA1c inversely equal to the reduced insulin production (see Cerf, 2013; Gerich, 2002; Pani et al., 2008). Changes in proinsulin levels were not increased with age that were diagnosed with IR but were

elevated in younger patients with early IR. β -cell exhaustion was demonstrated in those patients with increased HbA1c and glucose levels and insulin, proinsulin, and C-peptide levels below normal. These results support the overproduction of insulin with age leads to β-cell exhaustion and diabetes (see Abdul-Ghani et al., 2006; Cerf, 2013; DeFronzo & Tripathy, 2009; Gerich, 2002; Reaven, 2004). There were no comparative changes observed in insulin, proinsulin, or C-peptide levels between gender and ethnic groups. There were significant changes noted in HbA1c and triglyceride levels with ethnicity with HbA1c, $p = 0.002$ and triglycerides, $p = 0.026$. These findings allowed for the rejection of the null hypothesis for RQ2, demonstrating that the OGIST can distinguish changes in triglycerides and HbA1c with ethnicity.

The results of this study did not demonstrate any significant change in insulin, proinsulin, glucose, or C-peptide levels when comparing gender and ethnicity. The OGIST did demonstrate changes in insulin, proinsulin, C-peptide, and glucose at the end of the first phase insulin secretion glucose on the individual level. This finding allows for evaluation of an individual's sensitivity to insulin and beta cell function.

When considering age, there was a noted reduction in insulin production in patients diagnosed with IR with age. There was also a noted increase in HbA1c levels with age comparable with that indicated in the literature (see Imamura et al., 2012; Srinivasan et al., 2003). These age-related changes in patients with IR then suggest that as the patient ages with uncontrolled IR, the beta cells become exhausted resulting in T2DM (Abdul-Ghani, et al., 2006; Cerf, 2013; DeFronzo & Abdul-Ghani, 2011). The current literature suggests a 1.5% reduction in insulin production every 10 years in

patients with normal glucose levels. Table 20 demonstrates the results found in this study.

This study included all patients that had been diagnosed with IR using the OGIST. I made no distinction between newly diagnosed patients with IR and those patients currently undergoing treatment. Considering the patients' data included in this study, there remained a significant drop in insulin production between the ages of 47 and 56 based on the severity of the individuals' insulin sensitivity. The overall reduction in insulin production with age was approximately three times greater than that currently described in the literature, $p < 0.001$. This can be appreciated by reviewing the graph below comparing insulin production with age.

Currently, the hyperinsulinemic euglycemic glucose clamp test remains the reference test for IR (DeFronzo et al., 2009). This test was accomplished by creating a hyperinsulinemic state by infusing a constant IV rate of insulin above the normal serum levels of insulin. This rate of infusion is calculated for the individual prior the test to maintain a hyperinsulinemic state. A 20% dextrose solution is given intravenously at the same time of the insulin infusion maintaining a euglycemic state. This allows increase glucose disposal by the skeletal muscle and adipose tissue while suppressing hepatic glucose production. Insulin sensitivity than calculated from the change of insulin above that of steady-state divided by the amount of glucose needed to maintain normal serum glucose levels. This test depends on the ability to achieve and maintain steady-state conditions of hyperinsulinemia and normal serum glucose levels at 30 min (Abdul-Ghani et al., 2006; Cerf, 2013; DeFronzo & Abdul-Ghani, 2011). Because the

hyperinsulinemic euglycemic glucose clamp test was designed and tested on euglycemic patients, the test then assumes that all patients will reach steady-state insulin/glucose levels at 30 minutes and hepatic glucose production being suppressed. This test is not taken to consider the insulin resistant patient that may be experiencing early β -cell exhaustion or delayed response to glucose (Cerf, 2013; Gerich, 2002). Since all current surrogate tests are based on the findings from the hyperinsulinemic euglycemic glucose clamp test results, (Bonora et al., 2002; Gayoso-Diz et al., 2013; Hettihewa & Weerarathna, 2011; Katz et al., 2000; McAuley, 2001), they would also lack the ability to identify changes during the first phase of responsive glucose. This would limit their ability identify those patients having its resistance had either very early stages or later stages, but beta cell exhaustion. This could also reduce the surrogate tests ability to evaluate treatment of IR. The OGIST would then be far superior in not only diagnosing insulin resistance but the evaluation of the patient through all stages of IR to include early beta cell exhaustion, prediabetes, and treatment evaluation.

When comparing the OGIST to HOMA, QUICKI, and McA, HOMA had similar predictive quality to OGIST for insulin and C-peptide alone. The QUICKI test demonstrated similar predictive qualities to OGIST for insulin, proinsulin, and C-peptide. McA demonstrated similar predictive qualities to OGIST for triglycerides only. OGIST demonstrated significant predictive qualities for insulin, proinsulin, C-peptide, HbA1c, and triglycerides. When evaluating the ability of the tests to evaluate the individuals' insulin sensitivity, production and β-cell function OGIST was superior to

the other tests. The OGIST superiority could be due to the ability of the test to evaluate the combination of insulin, proinsulin, glucose, C-peptide, and HgA1c together at the end of the first phase insulin response to glucose.

Limitations of the Study

The oral glucose, insulin secretion test is reliant upon accurate serum levels of insulin, proinsulin, C-peptide, glucose, HbA1c obtained at the of the first phase insulin secretion test. The main limitation the OGIST would be the patient's blood being obtained after the first phase insulin response to the glucose bolus being given. Increased time past the first phase insulin response would result in skewed insulin, proinsulin, C-peptide, and glucose levels, making interpretation of the data less accurate and more difficult. This sometimes can happen within busy medical practice in which nursing staff initially give the 36g of glucose to the patient orally and in either become busy and other duties or called away. Nursing staff then collects the blood sample after the appropriate 15 minute time being unaware of the problem. The second limitation the study was that the patient needs be fasting 2 hours overnight to ensure the patient is not entered into the second phase insulin response to glucose which would cause increased hepatic glucose production. One limitation that was found to reduce the impact of the study was that the patients included the study included all patients tested with the OGIST. This study included new patients diagnosed with metabolic syndrome untreated for insulin resistance and those patients treated for insulin resistance demonstrating improvement in both insulin sensitivity, glucose utilization, and improves weight/BMI.

A binomial regression analysis was used in this study due to the lack of linear relationship between the independent and dependent variables and that the dependent variables were not all normally distributed. The data used in this study initially failed linear regression assumptions testing, but met the assumptions testing for binomial logistic regression testing.

The OGIST is a dynamic test identifying insulin sensitivity at the end of the first phase insulin response to glucose measuring each individual's specific insulin levels. This allows not only the ability to identify insulin resistance but also beta cell function, the progression to diabetes, and evaluation of a treatment for the individual. The hyperinsulinemic euglycemic glucose clamp test is the current reference for insulin resistance. This test falsely forces the individual into a hyperinsulinemic state requiring additional glucose, via intravenous administration, to maintain normal serum glucose to obtain the needed data to calculate insulin sensitivity. This test is invasive, requires hospital inpatient stay, technically challenging, and expensive surrogate tests have been developed for patients in the clinical outpatient setting (Abdul-Ghani et al., 2006; DeFronzo et al., 2009). Current surrogate tests based on the hyperinsulinemic euglycemic glucose clamp test are based on fasting serum glucose, insulin, and/or levels of triglycerides to mathematically calculate possible insulin resistance (Bonora et al., 2002; Gayoso-Diz, et al., 2013; Hettihewa & Weerarathna, 2011; Katz et al., 2000; McAuley, 2001). The limitation of these studies is that they do not measure the individuals' insulin, proinsulin, C-peptide, glucose at the end of the first phase insulin response which is been identified as a critical time to assess its resistance and beta cell

function (Cerf, 2013; Gerich, 2002). Surrogate tests for IR group everyone into a "one size fit all" calculation which lacs the ability to directly measure insulin sensitivity or β-cell function.

Recommendations

This study demonstrates the ability of the OGIST to identify IR in patients meeting the criteria for metabolic syndrome. Insulin sensitivity, beta cell function, and the progression of the individual to diabetes can be identified with the use of the OGIST making this a valuable test in identifying and treating IR and reducing Type 2 diabetes. Because the study included all patients tested using the OGIST further studies should be conducted to understand the full impact of this test. This test should be replicated to study each phase of IR from early onset and young age to complete β-cell exhaustion and diabetes. One arm of the studies should utilize only newly diagnosed patients with IR, second arm to evaluate treatment of IR with the various methods and medications, and the third arm studying those patients that have entered prediabetes to evaluate the potential improvement β-cell function and cardiovascular disease. Further studies should be developed to evaluate additional pathologies suggested by the literature to include male and female reproductive problems associated with IR, the effects of its resistance on cancer and treatment, cardiovascular disease associated with IR and proinflammatory pathologies secondary to its resistance.

A future study design using the OGIST should include true experimental design using double blinded controls on treatment designs and medications for treating and improving IR and sensitivity. Control group experimental studies should also be

considered using diet and exercise treating IR to confirm a true benefit with insulin sensitivity in order to reduce the development of adult diabetes and cardiovascular disease Boudreau et al., 2009; Gallagher et al., 2010; Kim et al., 2010; Knowler et al., 2006; Vendrame et al., 2004).

Further studies should be completed on those patients diagnosed with IR at an early age to evaluate specific treatment modalities, to include medications, and the ability to increase insulin sensitivity and the preservation of β-cell function.

Implications

Currently the medical community assumes and teaches that IR is a true form of diabetes and educates patients in this matter to include treatment with diet and exercise. Although diet and exercise is important and beneficial according to recent literature only provides small improvement IR (Diabetes Prevention Program Research Group, 2003; Herman et al., 2005; Knowler et al., 2006; National Diabetes Clearing House, 2014; Wong & Wang, 2006). The OGIST provides the ability to diagnose IR by accurately testing the individual's insulin sensitivity, beta cell production, and progression to diabetes in the outpatient clinical setting. This test allows true individual testing which will increase the ability of individual treatment evaluation to occur. This will also increase medical community's knowledge and treatment of IR by actually seeing the individual's insulin sensitivity and beta cell function at the end of the first phase insulin response glucose. But having all indices available to the provider, insulin, proinsulin, C-peptide, glucose, and HbA1c understanding of the pathological change of IR will increase.

Currently, health insurance companies place IR as a secondary condition with a low importance and priority to the individual's health and does not provide testing, education, or treatment for the condition. Placing resistance in a low priority continues to allow patients with IR progress to diabetes and the secondary pathological changes that follow to include cardiovascular disease. By utilizing the OGIST healthcare providers will help educate both the public and health insurance on the importance of diagnosing and treating its resistance reducing the progression to diabetes. The first step in reducing healthcare costs on diabetes is identifying the true pathological problem causing the disorder. IR has been suggested by many in the literature to be the initial problem resulting in Type 2 diabetes (Butler et al., 2008; DeFronzo & Abdul-Ghani, 2011; DeFronzo &Muhammad, 2011; Groop et al., 1996; Lorenzo et al., 2010). By having the capability to identify the initial problem responsible for adult onset diabetes the medical community, public, and health insurance regulators may understand the importance of diagnosing and treating this condition.

The OGIST provides positive social change in that the test provides early and accurate identification of IR in patients. This test can be used in both the clinical setting to identify and treat patients and in large epidemiological studies. The test is relatively easy and cost effective. The OGIST provides the evaluation of insulin sensitivity and β-cell function which ultimately improves β-cell function through proper identification and treatment of the patient. The OGIST increases further research in IR and the reduction of the progression of diabetes.

Conclusion

More than 175 million adults worldwide have undiagnosed diabetes with an additional 316 million adults with impaired glucose tolerance (International Diabetes Federation, 2015). These individuals may have lost 60 to 80% of their pancreatic β-cell function and would have early cardiovascular disease secondary to the elevated triglyceride production stimulated by IR (Butler et al., 2008; DeFronzo & Abdul-Ghani, 2011; DeFronzo &Muhammad, 2011; Groop et al., 1996; Lorenzo et al., 2010). The worldwide cost of this problem in both loss of man-hours and medical costs are staggering. Current surrogate mathematical tests for IR are based on the current reference standard hyperinsulinemic euglycemic glucose clamp test which possesses significant limitations. Although surrogate tests for IR can help identify the problem they are poor that measuring individual's sensitivity to insulin, beta cell function, ability to evaluate treatment of insulin resistance. The OGIST demonstrates significant possibilities in the diagnosis and treatment of IR the reduction of both diabetes and related pathologies and β-cell preservation.

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Appendix A: Normal Glucose Homeostasis

Following the normal ingestion of a typical mixed meal, plasma glucose levels increase, stimulating the release of insulin by the pancreatic β-cells. The combination of both increased serum glucose and an elevation of serum insulin stimulate the uptake of glucose by the skeletal muscles, gastrointestinal tract, and liver, while suppressing primary production of glucose by the liver. Fifty percent of the entire glucose utilization occurs by the brain tissue while the liver and gastrointestinal tissue account for 25% of the post-absorptive insulin-independent glucose uptake. The primary skeletal muscle tissue of the body accounts for the majority of the remaining 25% insulin-dependent glucose uptake with adipose tissue accounting for a small amount. Peripheral uptake of glucose is primarily found in skeletal muscle tissue (80-85%) while adipose (fat tissue) account for only a small amount (4-5%). Adipose tissue plays an important role in the maintenance of total body glucose by regulating free fatty acid (FFA) release. As serum glucose rise following a meal insulin levels rise in the serum which in turn act as a strong antilipolytic hormone inhibiting the breakdown of FFA from adipose cells. The reduced serum FFA then stimulates the muscle glucose uptake, in combination with insulin, and inhibits hepatic glucose production and release (Bays et al., 2004).

Glucagon, a polypeptide hormone secreted by the pancreatic α-cells, acts as a counter-regulatory hormone to suppress hepatic glucose production and release. In a fasting state glucagon maintains normal serum glucose levels by stimulating hepatic glucogenolysis and gluconeogenesis both are hepatic functions to increase basal glucose

levels during periods of low blood sugar (Radziuk & Pye, 2001). Both increased serum levels of insulin and glucose suppresses glucagon and hepatic glucose production.

- Gluconeogenesis: The formation of glycogen from noncarbohydrates, such as proteins and fats, by converting pyruvate to D-glucose.
- Glucogenolysis: The hydololysis of glycogen to glucose.

As serum glucose levels increase after oral ingestion of a meal, splanchnic nerves are stimulated thus activating a neural reflex enhancing vagal nerve activity while inhibiting sympathetic nerves within the liver (Radziuk & Pye, 2001). This neural change augments glucose uptake by the liver and stimulates glycogen synthase by the liver. This increase in both glucose uptake and glycogen synthase by the liver during oral intake of a meal simultaneously inhibits glycogen phosphorylase. Thus serum insulin response after oral consumption of glucose is nearly twice as much as that seen after intravenous glucose is administered due to the above. This increase is also related to a release of glucose dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1 (GLP1) and from the intestinal epithelium. Both GIP and GLP1 both augment the regulation of serum glucose levels (Defronzo & Abdul-Ghani, 2011). GIP expresses inhibitory changes on gastric acid secretion and gastrointestinal motility while augmenting the stimulation of insulin secretion with increased serum levels of glucose. GLP-1 inhibits gastric emptying and glucagon secretion while aiding in the stimulation of insulin secretion. Due to these actions the serum response after oral glucose consumption is nearly twice as great as that found after an intravenous infusion of glucose (Defronzo & Abdul-Ghani, 2011).

Normal Cellular Function of Insulin

Insulin action at the cellular level begins with insulin binding to the insulin receptor. The insulin receptor is a large transmembrane glycoprotein belonging to the receptor tyrosine kinase family. Additional members of the tyrosine kinase family include insulin-like growth factor receptor-1 and epidermal growth factor receptor. The insulin receptor is a heterotetrameric protein composed of two identical subunits (α-subunits and β-subunits) which are linked by two disulfide bonds. The extracellular α-subunits contain the ligand-binding domain which binds to the insulin molecule and triggers the one internal β-subunit to undergo intramolecular autophosphorilation of tyrosine residues on the adjacent β-subunit (Le Marchand-Brustel et al., 2003). During basal conditions the α-subunits exert tonic inhibition of the intrinsic kinase activity of the receptor. When insulin binds to the α-subunits they move closer together which allows ATP to bind activating the intracellular kinase. Tyrosine residues found in the receptor’s juxta membrane and C-terminal domains phosphorylated by activated kinase which then creates a high affinity docking site for insulin receptor substrate to become bound and phosphorylated. Three main signaling transduction pathways operate in the primary insulin response of skeletal muscle, adipose tissue, and liver. These pathways include IRS1/2/PI3K, CAP-cbl/crkIII-C3G/TC10, and Shc/Grb2/ras/MAPK pathway. The IRS group is composed of four related proteins IRS1/2/3/4 and is triggered by the binding of activated IR. IRS proteins have multiple functioning domains Pleckstin homology (PH) located at the terminus of IRS-1 mediates the binding to charged groups of phosphatidylinositides that target IRS-1 to membrane structures (Hemmings &

Restuccia, 2012). Phosphotyrosine binding (PTB) domain is found next to the PH domain and is required in recognizing phosphotyrosine of the insulin receptors amino acid sequence NPXpY (asparagine-proline-any amino acid-phosphotyrosine). IRS-1 that has undergone tyrosinephosphorilation contains multiple SH2 docking sites, one of which is the p85 regulatory subunit of type 1A phosphatidylinositides-3 kinase (PI3-kinase), also p50, Grb-2, Nck smaller adapter proteins. Because of this the IRS functions as a pivotal point of the insulin signaling pathway which allows insulin to regulate gene expression, cell growth and differentiation, and carbohydrate, lipid, and protein metabolism (Moxhan & Jacobs, 1992). IRS-1 stimulation increases PI3-kinase activity to translocate to plasma membranes and the cytosol to generate phosphatidylinositol-3-phosphate (PI3P), phosphatidylinositol-3, 4-bisphosphate (PI3, 4P2) and phosphatidylinositol 3, 4, 5, triphosphate (PIP3). In insulin stimulated IRS activated PI3-kinase regulation has been shown to be essential in the regulation and activation of a family of glucose transporters (GLUT) which facilitate glucose movement into the cell (Backer, et al., 1992; Clark, et al., 1998; & Angeloni & Hansen, 2002). There have been four glucose transporter proteins identified; GLUT1 found in fetal cells and in adult erythrocytes and endothelial cells within the blood-brain barrier, GLUT2 found in the liver and pancreatic β-cells functioning primarily as a glucose sensor, GLUT3 found primarily in neuronal axons and dendrites, GLUT4 is found primarily in striated muscle tissue and adipose tissue. GLUT4 is the insulin regulated glucose transporter allowing the uptake of glucose within the cell for energy production. Patients with insulin resistance have been found to have a normal amount of GLUT4

transporters within the cell but the ability of insulin to effect the translocation for glucose transport is disrupted (Sakamoto & Holman, 2008).

Akt is an important substrate for PDK-1 with phosphorylation/activation of the substrate by PDK-1 resulting in two important signaling events. The first pathway occurs when Akt is activated, phosphorylation of glycogen synthase kinase (GSK) begins the primary pathway for insulin-mediated glycogen synthesis. The second signaling event is that Akt activation enhances Target of Rapamycin (mTOR) activity responsible for the mediated protein synthesis increase by insulin.

Akt kinase is activated by specific serine/threonine residues secondary to insulin stimulation. After Akt has been activated by insulin it has have the ability to regulate protein synthesis, lipid synthesis, and glycogen synthesis within the cell. Three different forms of Akt have been identified; these forms include Akt1, Akt2, and Akt3. Akt phosphorylation occurs at the threonine 308 location and the serine 473 location. Phosphorylation must occur at both sites for full activation of Akt to occur. Akt is phosphorylated at the threonine location by PDK-1 and by PDK-2 at the serine site (Whiteman, et al., 2002). Scheid and Woodgett's, (2001) genetic studies utilizing PDK-1-null embryonic stem cells demonstrated that serine 473 phosphorylation can occur when stimulated by insulin even when phosphorylation did not occur at the threonine 308 site suggesting a possible autonomous PDK-2 involvement. Studies that used RNA blocking the expression of Akt in3T3-L1 adipocytes demonstrated that both Akt1 and Akt2 are important mediators in insulin stimulated GLUT4 translocation and glucose transportation (Herr et al, 2005). Further research based on Herr and colleges work

(2005) demonstrated that Akt2-null mice became insulin resistant and displayed both fed and fasting hyperglycemia results with additional hyperinsulinemia, glucose intolerance, with impaired muscle glucose muscle uptake (Garofalo, et al., 2003). Murine studies in which Akt was blocked resulted in insulin resistance progressing to diabetes (Cho, et al., 2001). These studies demonstrate the involvement of Akt in the regulation of glucose homeostasis in several tissues.

PDK-1 has also been demonstrated to directly activate and phosphorylate atypical PKCs (aPKC) which includes PKCϚ and PKCλ. These aPKCs are found as auto-inhibitory configuration in that the NH2-terminal regulatory pseudosubtrate sequence is folded into the substrate binding site of the carboxy-terminal catalytic region which blocks activation and phosphorylation. Enzyme activity is increased when PIP3 binds to aPKC regulatory domains which stimulates unfolding of protein. Farese, (2002) demonstrated that insulin activation of aPKCs in skeletal muscle is reduced in those patients with diabetes type 2 and insulin resistant animal models and concluded that this problem contributed to the reduction in insulin stimulated glucose up-take seen in those patients with type 2 diabetes.

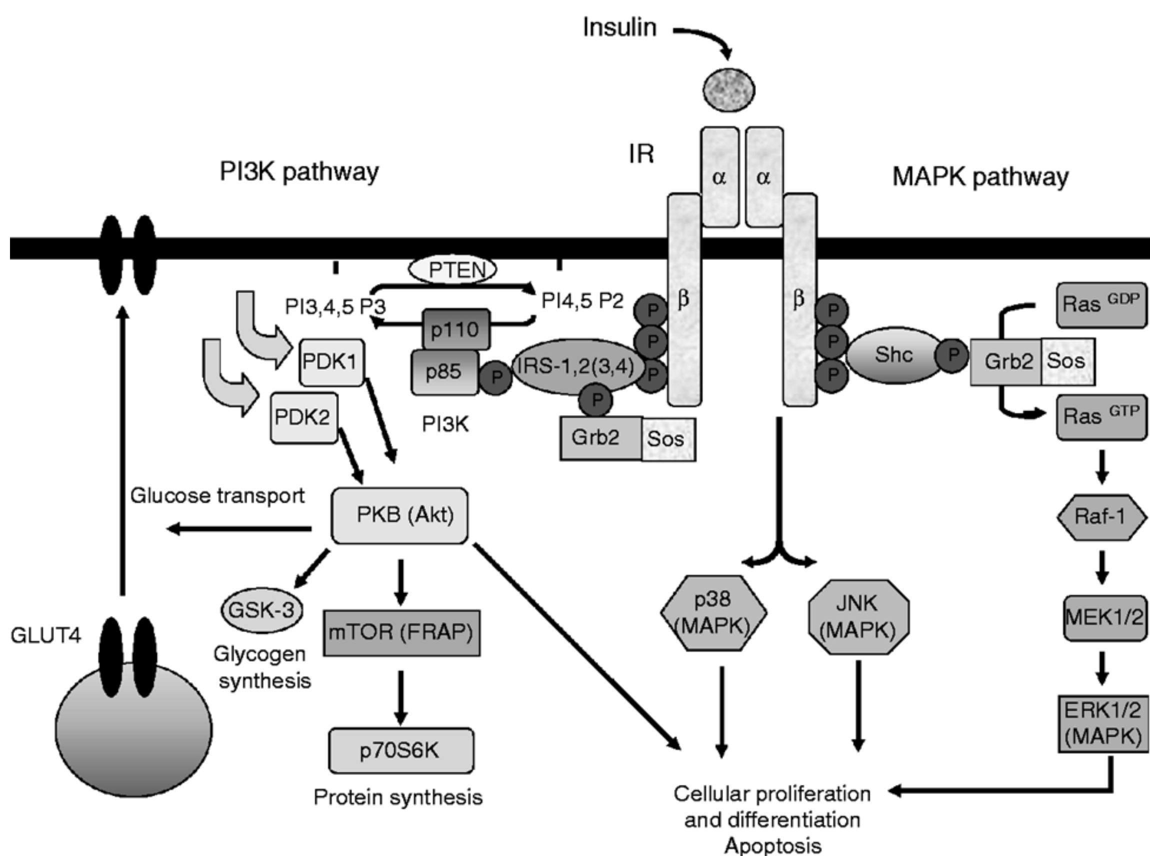

Figure 1A. Cellular function of insulin binding and glucose uptake.

Signaling Pathway in the Pancreatic β-cell and Insulin Secretion

Glucose enters the β-cell through facilitated diffusion which is accomplished through the intracellular GLUT2 transporter. The first rate limiting step is mediated by glucokinase which phosphorylates glucose to glucose-6-phosphate (Davis, et al., 1999, and Matschinsky, et al., 1998). Since glucokinase functions as a rate limiting step it can be considered a glucose sensor of the β-cell and its primary mechanism for insulin release and adaption to changes in serum glucose. When considering this theory as increased amounts of glucose enters the β-cell and glycolysis increases secondary to glucokinase stimulating the increased release of insulin. As serum glucose drops both

glucokinase and glycolysis drops thus reducing the amount of insulin released. Increased ATP production secondary to glucose metabolism blocks the KATP channels located on the β-cell membrane. Blockage of the KATP channels induces membrane depolarization increasing cytosol Ca+ triggering the exocytotic release of insulin. Pyruvate generated secondary to the glycolytic pathway has been suggested as a mechanism for the release of insulin (Maechler & Wollheim, 2001). Pyruvate enters the mitochondria and is metabolized in the TCA cycle through respiratory oxidation by NADH and FADH2, this reaction generates ATP which is expelled into the cytosol. ATP-sensitive K+ channels are closed due to the increase of ATP. This in turn depolarizes the β-cell membrane and opens Ca2+ voltage-dependent channels which increases calcium within the intracellular region. This increase of intracellular Ca2+ triggers the exocytosis of insulin secretory vacuoles on the membrane into the circulating plasma. Concentration changes of ATP and ADP within the cytoplasm cause closure of the channels and depolarization of the β-cell membrane. Changes or mutations of the β-cell KATP have been demonstrated to cause hyper-secretion of insulin.

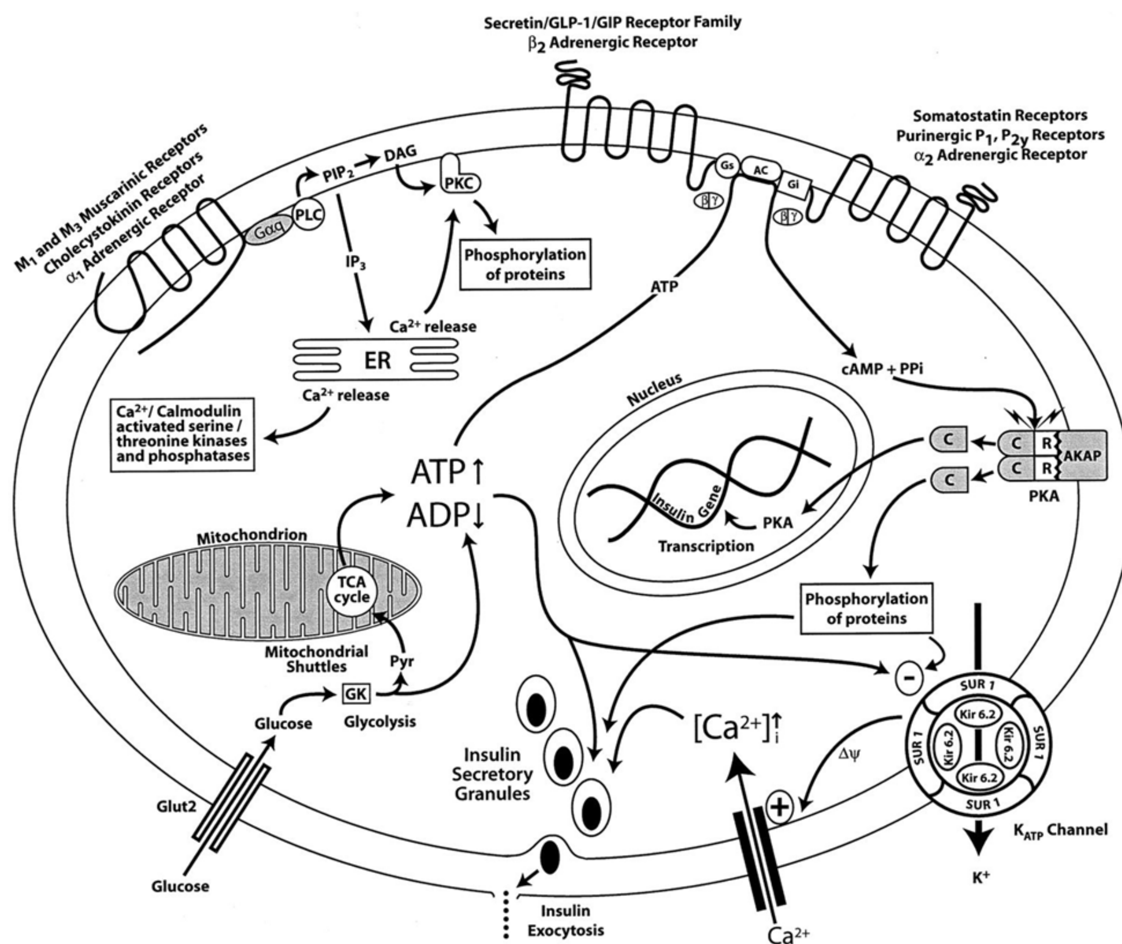

Figure 2A. Normal β-cell function.

Biphasic Insulin Secretion in Response to Glucose

Slightly more than 50% of an individual's insulin secretion occurs postprandial in response to a meal. The first-phase insulin secretion occurs rapidly after nutrient load and peaks within 2-4 minutes. Insulin is released rapidly from stored granules within the pancreatic β-cells in response to elevated serum glucose. Insulin has a very short half-life with serum insulin levels returning to normal (3.0 – 25.0mU/L) at 15 minutes (Gerich, 2002). This first-phase insulin response promotes the utilization of prandial glucose elevation by the peripheral tissue. The primarily tissue for glucose

uptake during the first phase is skeletal muscle which uptakes more than 80% of the prandial glucose (DeFronzo & Devjit, 2009). The first-phase insulin secretion also suppresses hepatic gluconeogenesis and maintains glucose homeostasis. The second phase insulin secretion increases after 30 minutes after prandial glucose elevation secondary to the digestion of nutrient load with subsequent elevation in serum glucose levels. Insulin levels gradually increase to a pseudo-steady state at about 120 minutes then returns to normal fasting levels.

Evaluating the first-phase insulin secretion is important in that the sensitivity of insulin can be evaluated along with pancreatic β-cell function. Reduced first-phase insulin secretion is an early sign of β-cell dysfunction and exhaustion leading to T2DM. This dysfunction in β-cell secretion can be seen long before elevation in fasting glucose is observed. The opposite can then be said in individuals with abnormal elevations of insulin during first-phase insulin secretion demonstrating reduced skeletal muscle sensitivity to insulin.

Definition of Type 2 Diabetes Mellitus (T2DM)

Williams and Louis, (1946) defined diabetes as "a metabolic disease characterized by a disturbance in the utilization and storage of the dextrose molecule by the body." Williams and Louis further stated "This basic disorder is generally accomplished by a train of events which is responsible for the derangement of other metabolic processes in the organism."

The National Institutes of Health through the National Diabetes Education Foundation defines diabetes as" a group of diseases marked by high levels of blood

glucose resulting from defects in insulin production, insulin action, or both, (National Diabetes Clearing House, 2013).

Type 2 diabetes mellitus is typically characterized as a heterogeneous group of metabolic abnormalities resulting from cellular resistance to insulin, impaired insulin production and or release which leads to eventual glucose overload and toxicity.

Pathogenesis and Natural Progression of T2DM

The pathogenesis of T2DM is complex and involves both genetic and environmental influences that result in hyperglycemia secondary to insulin resistance. Several different causes of T2DM have been identified which include specific mutations in the insulin molecule and insulin receptor genes, glucokinase genes, hepatic nuclear factors (HNF-1α and 4α) and mitochondrial DNA have been identified (DeFonzo & Abdul-Ghani, 2011). Peroxisomal proliferator-activated receptors (PPAR) have been discovered within the last twelve years which are responsible for regulating the metabolic activity of adipose tissue (Cruz-Garcia, et al., 2014). The normal history of T2DM begins with the patient having insulin resistance, normal glucose tolerance, with compensatory hyperinsulinemia. This progresses to a condition known as impaired glucose tolerance (IGT), glucose of 144-199 mg/dl after 2 hour OGTT, which stimulates an increase in basal insulin secretion and fasting hyperinsulinemia until β-cell exhaustion is reached resulting in reduced insulin production and continued elevation of both fasting plasma glucose (FPG) and glucose above 200 mg/dl after 2 hour OGTT (Gastaldelli, et al., 2004). The progression of insulin resistance to T2DM has been observed in a variety of populations to include Native Americans, Mexican

Americans, Black Americans, Caucasians, Pacific Islanders, and in the rhesus monkey animal model (Polonsky, et al., 1996; Saad, et al., 1989; Saad et al., 1888; Haffner, et al., 1995; & Weyner et al., 2000).

Review of Insulin Resistance

Insulin resistance is a condition defined as the tissues failure to respond to serum insulin causing the reduced cellular uptake and utilization of glucose and glucose homeostasis. Clinically the term "insulin resistance" suggests that an elevated level of insulin above the normal level is required to maintain normal glucose levels. On a cellular level insulin resistance consists of abnormal or reduced binding of insulin to the cellular insulin receptor and abnormal downstream signaling of the insulin molecule reducing the up-take and metabolism of glucose. This condition propagates compensatory over production and release of insulin by the pancreas resulting in hyperinsulinemia due to the reduced uptake and elevated serum levels of glucose. This over production and release of insulin by the pancreatic β-cells is to maintain euglycemia state post-prandially by triggering the uptake of glucose of skeletal muscle and adipose tissue. Insulin concomitantly suppresses hepatic glycogen release thus suppressing gluconeogenesis during periods of post-prandial insulin release.The over production and release of insulin secondary to insulin resistance is coupled to the eventual exhaustion and failure of the pancreatic β-cells to maintain glucose homeostasis and eventually type 2 diabetes.

Abnormalities in Insulin Release/Signaling Found in Insulin Resistance States

Insulin resistance has been hypothesized to develop from three mechanisms. First possibility is that the pancreatic β-cells may produce and release abnormal insulin molecules, the second possibility is that insulin resistance may result from an antagonist that reduces or blocks the insulin molecule on target cells, the third possibility is that a defect occurs in the target cells either at the insulin receptor or within the cells signaling pathways blocking the ability to uptake glucose for phosphorylation (Given, et al., 1980; & Shulman, 2000).

The pathological mechanisms which allow the development and release of an altered or mutated insulin molecule are extremely rare (Given, et al., 1980; & Olefsky, et al., 1980). This condition does not represent a common problem and incapable of producing the vast numbers of individuals with insulin resistance seen today. Counter-regulatory hormones that act as antagonists include glucocorticoids, catecholamines, glucagon, and growth hormones. Pheochromocytoma, glucagonoma, acromegaly, and Cushing's syndrome are disease processes that can result in insulin resistant states and significant increases of insulin but not nearly to the state that is normally seen in those patients with insulin resistance and are not diagnosed in the numbers of patients that are seen with insulin resistance. Target-tissue specific defects are responsible for insulin binding and signaling alterations. These problems are typically secondary to genetic mutations and are responsible for increased production and release of insulin in response to elevations in serum glucose (Taylor, 19;2, Okamoto & Accili, 2004). These target specific mutations can also affect insulin receptor binding capability and signal

transduction pathways. Defects in the insulin receptor have been found to also reduce the receptor numbers and activity (Taylor, 1992; & Okamoto & Accili, 2004). Rodent studies have demonstrated that mutagenesis of the insulin receptor and of the IRS1/2 lead to the development of insulin resistance and diabetes with age (Lei, et al., 1999). There have been different polymorphisms reported of the human IRS-1 gene to include decreased phosphorylation of IRS-1 resulting in reduction of PI-3 kinase activity (Yoshimura, et al., 1997). Post-transitional modifications resulting in abnormal protein expression in insulin signaling molecules have been reported in skeletal muscle and adipose tissue samples from type 2 diabetic patients (Hunter & Garvey, 1998). Type 2 diabetic patients have also demonstrated impaired autophosphorylation of insulin receptors from reduced receptor kinase activity (Obermaier-Kusser, et al., 1989), and impaired IRS-1 phosphorylation (Smith, et al., 1999, & Kim, et al., 1999).

Review of Current Tests for Insulin Sensitivity

Oral glucose tolerance test (OGTT): This test is one of the earliest tests for in vivo insulin sensitivity. The standard oral glucose load of 75g (calculated 1.75 g/kg body mass) is given to fasting patients over 5 minutes. Blood is then sampled every 15-30 minutes for 2-5 hours for serum glucose and insulin concentrations. The initial change in insulin/glucose ratio (Δinsulin/Δglucose) during a OGTT had been used as an index of insulin sensitivity. This insulin sensitivity calculation inspired the calculation of fasting and post load glucose and insulin values. The concept with this type of test is that the higher the increase in plasma glucose per unit of insulin then the lower of insulin sensitivity is observed. Pacini and Mari, (2003) suggested the use of a modified

OGTT for insulin sensitivity considering the area under the OGTT curve after 15 minutes. The standard clinical protocol for utilizing OGTT for diagnosing and treating diabetes has evolved to 2 hours with serum blood testing at fasting, 30 minutes, 60 minutes, and at 120 minutes.

Insulin tolerance test (ITT): This test is one of the earlier tests to evaluate in vivo insulin action. Fasting patients baseline serum glucose is calculated and are given a bolus of fast acting insulin (0.1 unit/kg/day) intravenously. Serum glucose levels are obtained every 5 minutes for 70 minute duration. The calculated insulin induced glucose metabolism index is expressed as the glucose disappearance rate [KITT = (0.693/t1/2) X 100 percent/min]. In this equation t 1/2 is equal to half-life of the plasma glucose decay. Normal values of KITT are greater than 2 percent/min with abnormal values being less than 1.5. Values between 1.5 and 2 are considered equivocal. The advantages of this test are that it is relatively simple as an intravenous test. There is a modified Short Insulin Tolerance Test (SITT) version that can be completed over 15 minutes and reduces the incidence of hypoglycemia. The test is reproducible and accurate both the standard version and the short version as Hirst, et al., (1993) demonstrated. The disadvantages of the test are hypoglycemia in patients during the test. Hypoglycemia stimulates hepatic glucose release and impairs peripheral glucose uptake. Inability of the test to allow insulin induced glucose metabolism secondary to the hypoglycemia/hyperinsulin response. The test is not indicated in pediatrics due to the problems associated with hypoglycemia.

Insulin suppression test (IST): is used to evaluate the disposal of an intravenous glucose load by a constant/fixed level of hyperinsulinemia. This test is completed to patients that have been fasting overnight and given 5mg of propranolol intravenously. Propranolol inhibits hepatic glucose release during the test. Intravenous epinephrine is given continuously at 6 mcg/min to suppress insulin secretion. Propranolol is given IV at a continuous rate of 0.08 mg/min with regular insulin 80mU/min, and glucose 6 mg/kg/min, all over 180 minutes. Steady state plasma glucose (SSPG) and steady state plasma insulin (SSPI) are observed after 90 minutes. Blood samples are obtained every 15 minutes for the first 90 minutes and then every 10 minutes for the last 90 minutes. The concentration of SSPG is proportional to the insulin mediated glucose metabolism with an increase in SSPG being proportional to an increase in insulin resistance. This test demonstrates a high correlation between insulin resistance and insulin suppression and the euglycemic clamp test $r = 0.092$, $p<0.001$ Greenfield, et al., (1981). The disadvantages of this test include: high amount of complexity rendering the test inappropriate for clinic use, propranolol may not be adequate to suppress hepatic glucose release particularly in the presence of insulin resistance and/or diabetes, SSPG levels can be different between individuals and the effects of hyperglycemia and hyperinsulinemia can cause additional metabolic changes that could alter the results, diabetic patients and those with insulin resistance could respond differently to epinephrine with the increase possibility of arrhythmias in the elderly.

The frequently sampled intravenous glucose tolerance test with minimal model analysis (FSIGTT): In this model fasting patients receive a bolus of 50% glucose

solution which is calculated at 0.3g/kg body weight over 1 minute. Basal blood samples are collected at -15, -10, -5 and -1 minutes with post bolusing samples being obtained at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes. This allows frequent sampling of glucose and insulin which are analyzed using a computer minimal model (MINMOD). There have been two modifications to the FSIGTT in an attempt to improve endogenous insulin response to the glucose bolus and enhance the computer models ability to analyze the data. The tolbutamide enhanced FSIGTT utilizes intravenous tolbutamide after 20 minutes with additional blood testing at 23, 24, and 27 minutes. Tolbutamide is typically given at 300 mg for normal individuals and 500 mg for overweight or obese patients. The second modified FSIGTT is the insulin-modified FSIGTT. This test utilizes insulin at 0.03 units/kg by intravenous bolus after the start of a standard FSIGTT protocol. This modification was established for the use of FSIGTT in diabetic patients to reduce the possibility of hyperglycemia.

Glucose clamp test (GCT): This type of test is considered the “Bench Mark Standard” to evaluate pancreatic insulin release, insulin induced glucose disposal, and insulin sensitivity. The glucose clamp test is designed based on the glucose/insulin feedback loop that regulates β-cell release of insulin and glucose uptake by the peripheral tissue. There are two variations of the GCT; euglycemic-hyperinsulinemic clamp test and hyperglycemic clamp test.

Euglycemic-hyperinsulinemic clamp test: The goal of this test is to raise and maintain the serum insulin levels while maintaining glucose levels at the basal level.

The euglycemic-hyperinsulinemic clamp test allows measurements of insulin sensitivity, insulin stimulated glucose uptake/metabolism (M), and the metabolic clearance rate of insulin (MCR). This test is complicated, invasive, and time consuming in that the test requires antecubital venous catheter for infusions of both glucose and insulin, arterial heated-catheter for frequently sampled blood testing, hand-held glucometer for rapid testing of blood glucose levels, intravenous dextrose 20% for infusion, and regular crystalline insulin for intravenous infusion. After an overnight fast patients are given regular insulin per IV at 5-120 mu/m2 /min to maintain a steady state insulin level. The average rate of insulin infusion is 40 mu/m2 /min, this will increase blood glucose levels above baseline by ~100 µu/ml. intravenous glucose is infused (milligrams/minute) and clamped to maintain basal level during the test while suppressing hepatic glucose production. Blood glucose readings are normally obtained every five minutes over the three hour test and samples are obtained every 15 minutes for insulin. The amount of glucose infused to maintain euglycemia then reflects the amount of glucose metabolized in the peripheral tissue and measured in milligrams/kilograms/minute (M value) reflecting an indirect index of peripheral tissue sensitivity (capability to bind) to the insulin molecule. The higher the M value the higher the sensitivity to insulin which is seen in individuals with normal metabolism. This is much different than patients with insulin resistance that require less glucose to maintain basal glucose levels. This is likely due to inability of insulin to bind and stimulate peripheral tissue cells to uptake and metabolize insulin.

Peripheral Insulin sensitivity index (ISI): Insulin sensitivity index is the ratio of M/I where (M) equals the rate of stabilized glucose levels during infusion and (I) equals the mean insulin level during the test. This measurement is useful when comparing different groups that have different steady-state concentrations of insulin from a euglycemic-hyperinsulinemic clamp study. The metabolic clearance rate of insulin (MCR) is calculated by (ml/kg/min) dividing the fusion rate of insulin by the change (or increase) in circulating insulin concentrations from the euglycemic-hyperinsulinemic clamp study.

Hyperglycemic Clamp: The hyperglycemic clamp test is designed to evaluate the pancreatic β-cell response to a controlled hyperglycemic condition. This test requires the patient to fast overnight and receive an intravenous bolus of glucose of 20% dextrose over 2 minutes increase basal serum glucose to 225 mg/dl. The amount of glucose give is calculated by (225 – the mean of three fasting serum glucose readings) then multiplied by the patient's weight (kg) with the results being glucose distribution glucose distribution factor which is 1 for normal adults, 1.1 for overweight children, and 1.5 for normal weight children. This calculation allows for the initial bolus calculation and is adjusted as needed during the test. Glucose measurements are obtained every 2.5 minutes with insulin and C-peptide during the first 15 minutes of the test and every 5 minutes during the remainder of the 120 minute test. Data from the hyperglycemic clamp test are used to calculate serum insulin response to glucose and whole body glucose metabolism.

Review of the Challenges with the Current Tests for Insulin Resistance

The current studies available to identify insulin resistance include the frequently sampled intravenous glucose tolerance test which is conducted over two hours after a bolus of 70 grams of glucose is ingested (Pacini, et al., 1986), hyperinsulinemic-euglycemic clamp technique conducted utilizing intravenous insulin and glucose concentrations in a hospital setting (DeFronzo, et al., 1979), and the insulin suppression test which is similar to the hyperinsulinemic-euglycemic test (Harano, et al., 1977; & Shen et al., 1970). These tests are all very expensive, demonstrate some risk for the patient, and are difficult or impossible to complete in the clinical setting. The HOMA-IR study has also been suggested for identifying individuals with insulin resistance and is based on calculations of the patient's height, weight, age, and cholesterol levels. This test is relatively inexpensive but is based on general numbers from groups of patient's results from insulin suppression and hyperinsulinemic-euglycemic clamp tests (Cheatham, et al., 1995). These tests are very time consuming and impractical for daily patient diagnosis and/or treatment of insulin resistance. These tests also lack the ability to provide accurate clinical identification and treatment evaluation of the disease (Matthews, et al., 1985).

Review of Metabolic Syndrome

Metabolic syndrome is a group of conditions secondary to the consequences of having insulin resistance without treatment or improvement of the condition. The Paris Prospective Study conducted in 1991 by Fontbonne & Eschwège demonstrated that patients with insulin resistance and central obesity had a significant increase in death

related to cardiovascular disease than those not diagnosed with insulin resistance. Diagnostic guidelines established by the National Cholesterol Program-Adult Treatment Panel (NCEP-ATP III) for metabolic syndrome include three confirmed factors from the five established by the NCEP-ATP III.

Reaven, (1988) first proposed a cluster of related metabolic conditions that were found to be secondary to hyperinsulinemia found in patients with insulin resistance. Reaven initially called this condition “syndrome X” from a cluster of metabolic abnormalities associated with insulin resistance. These conditions include glucose intolerance, increased serum triglycerides and very low density lipoproteins (VLDL), reduced serum high density lipoproteins (HDL), and hypertension. Reaven further summarized that these conditions determined to a large extent which patients would develop cardiovascular disease. Since Reaven first proposed the concept of “syndrome X” further studies have been completed and demonstrate similar conditions associated with insulin resistance to include the later development of diabetes type 2 as the patient ages (Expert Panel (NCEP), 2001; Hill, 2003; Reaven, 2003; & Srinivasan et al., 2003). Since “syndrome X” has first been described by Reaven in 1988, studies have identified this condition using various names to include: diabesity, deadly quartet, deadly pentad disease, dysmetabolic syndrome, polymetabolic syndrome, coronary risk syndrome, insulin-resistant syndrome, and hyperinsulinemia/insulin-resistant syndrome. Since the NCEP-ATP III Panel the term “metabolic syndrome” has been deemed appropriate to use for this condition.

NCEPT-ATP III Metabolic Risk Factors for Metabolic Syndrome

1. Abdominal obesity with a waist circumference in men greater than 102 cm (40 in) and greater than 88 cm (35 in) in women.

2. Serum triglyceride level equal to or greater than 150 mg/dl.

3. High density lipoprotein (HDL) level less than 40 mg/dl in men and 50 mg/dl in women.

4. Blood pressure equal to or greater than 130/85 mmHg.

5. Fasting blood glucose equal to or greater than 100 mg/dl/

(Three or more of the above conditions need to be present of the diagnosis of Metabolic Syndrome.)

Epidemiologic Studies Demonstrating Cardiovascular Complications from Metabolic Syndrome

Saely study (2005). Study design was prospective cohort utilizing 750 participants whom either had diagnosed or suspected coronary artery disease. The investigators used NCEP-ATP III and HOMA-IR criteria for their study. Study participants were evaluated for adverse cardiovascular changes to include stroke, MI, and thrombolytic changes such as coronary artery disease. Findings, The authors found that patients with insulin resistance and metabolic syndrome had higher rates of adverse vascular events and changes and was a good predictor of coronary artery disease.

Koren-Morag study (2005). Study design was prospective utilizing 14,284 participants whom had been previously diagnosed with coronary artery disease. The investigators used NCEP-ATP III for their study criteria. Study participants were

evaluated for ischemic stroke and trans-ischemic attacks (TIA) over and eight year period. The investigators concluded that the presents of metabolic syndrome predicts the onset of ischemic vascular disease and stroke in patients. The authors further suggested that impaired fasting glucose and hypertension were strong predictors of ischemic cerebrovascular event.

Millions study (2005). Study design was prospective case-controlled utilizing 392 adults 70 years of age and older. The investigators used NCEP-ATP III criteria for metabolic syndrome. Study participants were evaluated for diagnosis of ischemic nonembolic stroke during their adult life time over 65 years of age. The authors concluded that metabolic syndrome in the elderly was a significant contributor to acute ischemic nonembolic stroke.

Ray study (2005). Study design was retrospective cohort which included over 1 million medical records of pregnant women. The investigators used the National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association (AHA) criteria for metabolic syndrome for study inclusion. The investigators evaluated those women whom were diagnosed with placental dysfunction and metabolic syndrome that met the NHLBI and AHA criteria before pregnancy. The authors concluded that those women whom had features of metabolic syndrome before becoming pregnant had a higher incidence of placental dysfunction and fetal demise and that this risk increased with increased features of metabolic syndrome. The authors suggested that mechanism may be secondary to increased proinflammatory and thrombolytic factors associated with insulin resistance.

Ageno study (2006). Study design was prospective case-controlled utilizing 93 individuals with a confirmed diagnosis of deep venous thrombosis (DVT) were compared with 107 individuals that were confirmed not to have any idiopathic DVT in their prior medical history. Both groups were than clinical assessment for metabolic syndrome utilizing NCEP ATP III criteria. The authors found that metabolic syndrome was identified in 50.5 percent of the patients diagnosed with idiopathic DVT compared to 34 percent of the control group. The authors further concluded that metabolic syndrome was independently associated with idiopathic DVT.

Hu study (2006). Study design used by the authors was a retrospective case controlled study. The authors included 1280 patients that were diagnosed from the DESIRE (Drug-Eluting Stent Impact on Revascularization) study. These patients had met the criteria for metabolic syndrome utilizing the International Diabetes Federation worldwide definition of metabolic syndrome. The authors found that patients with metabolic syndrome had a significant higher amount of coronary artery disease compared to those individuals without metabolic syndrome. The authors also reported higher major adverse cardiac and cerebral events (MACCE) in those patients with metabolic syndrome. The authors concluded that individuals with metabolic syndrome had a higher amount of CAD with a higher risk of MACCE.

Porrini study (2006). Study design used by the authors was a prospective cohort to evaluate the possible impact metabolic syndrome has on renal transplantation and grafts. The authors used 230 patients that had underwent renal transplant with stable function at 1 year and at 18 months. Metabolic syndrome was defined by the Adult

Treatment Panel III criteria to identify the prevalence of metabolic syndrome in patients having undergone renal transplant and its impact on graft survival. The authors found that metabolic syndrome in patients who had undergone renal transplant had a significant increase in graft rejection, graft loss, and patient death.

Clinical Results of Insulin Resistance

Insulin resistance has been simply defined as the reduced biological effectiveness of insulin requiring larger amounts to maintain normal glucose levels (Wallace & Matthews, 2002). This condition evolves from hyperinsulinemia, after meal consumption, to glucose intolerance and finally full diabetes. Metabolic syndrome is the result of insulin resistance and results in lipid and lipoprotein dysregulation, hyperinsulinemia, hyperglycemia which act individually or together to progressively worsen the effectiveness of the disease state.

Inflammation and Endothelial Dysfunction

Insulin resistance is responsible for the proinflammatory state found in metabolic syndrome. This is secondary to up regulation of inflammatory adipokine tumor necrosis factor α, C-reactive protein, and interleukin 6 with a reduction in adiponectin (Avramoglu, et al., 2006). The increased concentrations of these inflammatory mediators are not due to infection, tissue injury or autoimmunity but secondary to the chronic release of inflammatory cytokines generated by adipocytes during oxidative stress form increased free fatty acids (FFA). Increased release of insulin drives serum glucose into the adipocyte for storage increasing the production of FFA which increases the over expression of inflammatory proteins (Hotamisligil, 2006).

The overexpression of inflammatory proteins contributes to the reduction of the signaling pathways of insulin and increases lipid peroxidation.

Hypertension

The mechanism of hypertension seen in patients with metabolic syndrome are thought to be secondary to obesity. Essential hypertension has been documented to be closely associated with abnormal metabolic effects of glucose intolerance, dyslipidemia, and obesity (Landsberg, 2001; & Scott, 2003). While obesity played the strongest role in uncontrolled hypertension studies have suggested that increased insulin activates the Renin-Angiotensin System (RAS) thus increasing the production and release of angiotensinogen, AII, and overexpression of the AT1 receptor which increases vasoconstriction and blood pressure (Landsberg et al., 2001; Scott, 2003).

Atherogentic Dyslipidemia

The atherogentic problem seen with metabolic syndrome is due to a combination of increased triglycerides, apolipoprotein B, small-dense low density lipoprotein (LDL), with reduced high-density lipoprotein (HDL) (Albertti, et al., 2006). These changes are secondary to increased triglyceride production stimulated by hyperinsulinemia (Deedwania, et al., 2006). Studies demonstrate that as the triglycerides are increased and not used they are converted to LDL cholesterol.

Thombogenicity

Insulin resistance increases platelet aggregation by increasing serum fibrinogen in addition to increasing plasminogen activator inhibitor 1. Thrombin generation is also increased during hyperinsulinemia which potentiates clot formation leading to increased

stroke and myocardial infarction found in patients with metabolic syndrome (Grundy, et al., 2002). Aso, et al., (2005) demonstrated that in the presents of metabolic syndrome and hyperlipidemia elevated plasma concentrations of thrombin-activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor (PAI)-1 while inhibiting fibrinolysis. This finding was confirmed by Kraja, et al., (2007) which also added that CRP, IL6 contributes with TAFI and PAI risk factors for the prothrombotic state in metabolic syndrome.

Sample Collection

Blood samples were collected in SST and EDTA tubes in the normal manner established by Joshua Medical Center laboratory protocol.

Insulin: whole blood sample was drawn into a SST tube then centrifuged after15 minutes. Serum was extracted after being centrifuged and stored in a standard transport tube at -18C. Samples were tested using chemiluminescent immunoassay; all specimens that were hemolyzed were rejected. Normal insulin levels were considered 3-19 µIU/ml, the conversion of pmol/L was made by multiplying µIU/ml by 6.0.

Proinsulin: whole blood sample was drawn into a SST tube then centrifuged after15 minutes. Serum was extracted after being centrifuged and stored in a standard transport tube at -18C. Serum levels were tested using immunchemiluminscent assay, specimens stored or allowed to reach ambient temperatures were rejected for study. Normal reference range of proinsulin was considered 20 pmol/L or less.

Hemoglobin A1c (HbA1c): was obtained in 3 ml EDTA tube as whole blood and stored at room temperature with analysis completed within three hours.

Colorimetreic/Immunoturbidimetric testing was the methodology used in this study. All specimens that had been frozen or clotted were rejected. Percent A1c interpretation included the following:

- ≤5.0 Indicates Absence of Diabetes
- 5.1-5.6 Indeterminate
- 5.7-6.4 Indicates Prediabetes
- ≥6.5 Indicates Presence of Diabetes

Intervals based on the American Diabetes Association position statement regarding glycated hemoglobin testing (American Diabetes Association, 2014).

C-Peptide: was obtained in 3 ml EDTA tube as whole blood and stored at room temperature with analysis completed within three hours. Testing methodology used for this study was an Automated Chemiluminescent Immunoassay. Sample rejection for this test included hemolyzed blood, heparinized plasma, or samples left at room temperature. Normal reference interval for C-peptide: 1.1-4.4 ng/mL.

Glucose and Complete Metabolic Profile (CMP): whole blood sample was drawn into a SST tube then centrifuged after15 minutes. Serum was extracted after being centrifuged and stored in a standard transport tube at -18C. Colorimetric methodology used for testing the samples. Samples that were hemolyzed were unacceptable for testing and rejected. Normal reference range of glucose was considered 70-100 mg/dl (American Diabetes Association, 2014).

Those individuals with insulin resistance at 15 minutes should demonstrate elevated levels of insulin and glucose with elevated levels of C-peptide comparable to

the elevation of insulin. Since C-peptide is a waist product of the conversation of proinsulin to insulin and excreted slowly by the kidneys over 30 minutes these levels will be elevated to the proportion of proinsulin release. HbA1c is a calculated average level of glucose over 60 days, these levels should remain normal until the β-cells start to become exhausted due to over production and release of insulin with age. As the pancreatic β-cells become exhausted HbA1c is expected to be elevated with a reduction in insulin and proinsulin.

First phase insulin secretion will be determined by calculating the percentage of increase in insulin from the normal basal level after 15minutes and calculated as (100 x insulin level at 15minutes/Basal insulin level). The insulin secretion/insulin resistance index (IS/IR) will be calculated by [(Δ%I 0-15min/Δ%G 0-15)x100/ IxG] where I = insulin and G = glucose to evaluate β-cell function (Abdul-Ghani et al, 2006). Comparison analysis will be made with the QUICKI test calculated 1/[log(I0)+log(G0)], where I = insulin and G = glucose. The results from OGIST study will be compared with the above calculation. This comparison should increase validation of the test for insulin resistance since both the IS/IR index and the QUICKI are calculated from a mathematical calculation of fasting levels of both glucose and insulin where the OGIST results are patient serum results from insulin binding during the first phase insulin response.

Table 1A

Indices of First Phase Insulin Secretion and Insulin Sensitivity Index

1st Phase insulin Secretion during the first 15 minutes after oral glucose consumption	
Δ% Insulin 0-15 min	100 x (Peak insulin level at 15 min/Basal insulin level)
Insulin Sensitivity Index	First phase insulin level x First phase glucose level
Insulin Secretion/Insulin Resistance Index (IS/IR)	(Δ% insulin 0-30 min/Δ% Glucose 0-30 min)

Table 2A

T-Test for Gender, age, Ethnicity, BMI, Insulin, Proinsulin, C-peptide, Glucose, hbA1c, Triglycerides, HOMA, QUICKI, and McA

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Gender	0[a,b]	.	.	.
Age	224	49.21	14.63	.97
Ethnicity	0[a,b]	.	.	.
BMI	224	34.06	7.00	.467
Insulin (mU/L)	224	99.84	118.87	7.94
Proinsulin (pmol/L)	223	71.75	86.80	5.81
C-Peptide (ng/mL)	223	5.23	3.24	.217
Glucose (mg/dL)	224	147.46	53.13	3.55
HbA1c (%)	224	5.91	1.53	.10
Triglycerides (mg/dL)	224	186.92	109.00	7.28
HOMA	224	11.30	17.23	1.15
QUICKI	224	.25	.02	.00
McA	224	4.07	1.31	.08

a. t cannot be computed because the sum of caseweights is less than or equal 1.

b. t cannot be computed. There are no valid cases for this analysis because all caseweights are not positive.

Table 3A

One-Sample Test

					95% Confidence Interval of the Difference	
	t	*df*	Sig. (2-tailed)	Mean Difference	Lower	Upper
Age	50.34	223	.000	49.21	47.28	51.14
BMI	72.81	223	.000	34.06	33.14	34.99
Insulin (mU/L)	12.57	223	.000	99.84	84.18	115.49
Proinsulin (pmol/L)	12.34	222	.000	71.75	60.30	83.21
C-Peptide (ng/mL)	24.08	222	.000	5.23	4.81	5.66
Glucose (mg/dL)	41.54	223	.000	147.46	140.46	154.46
HbA1c (%)	57.78	223	.000	5.9170	5.715	6.119
Triglycerides (mg/dL)	25.66	223	.000	186.92	172.57	201.27
HOMA	9.82	223	.000	11.30	9.037	13.57
QUICKI	162.26	223	.000	.25	.25	.25
McA	46.30	223	.000	4.07	3.90	4.24

Table 4A

Descriptive Statistics

	Mean	Std. Deviation	*N*
Insulin (mU/L)	99.84	118.87	224
Age	49.21	14.63	224
BMI	34.06	7.00	224
Glucose (mg/dL)	147.46	53.13	224
HbA1c (%)	5.91	1.53	224
Triglycerides (mg/dL)	186.92	109.00	224
HOMA	11.30	17.23	224
QUICKI	.25	.023	224
McA	4.07	1.31	224

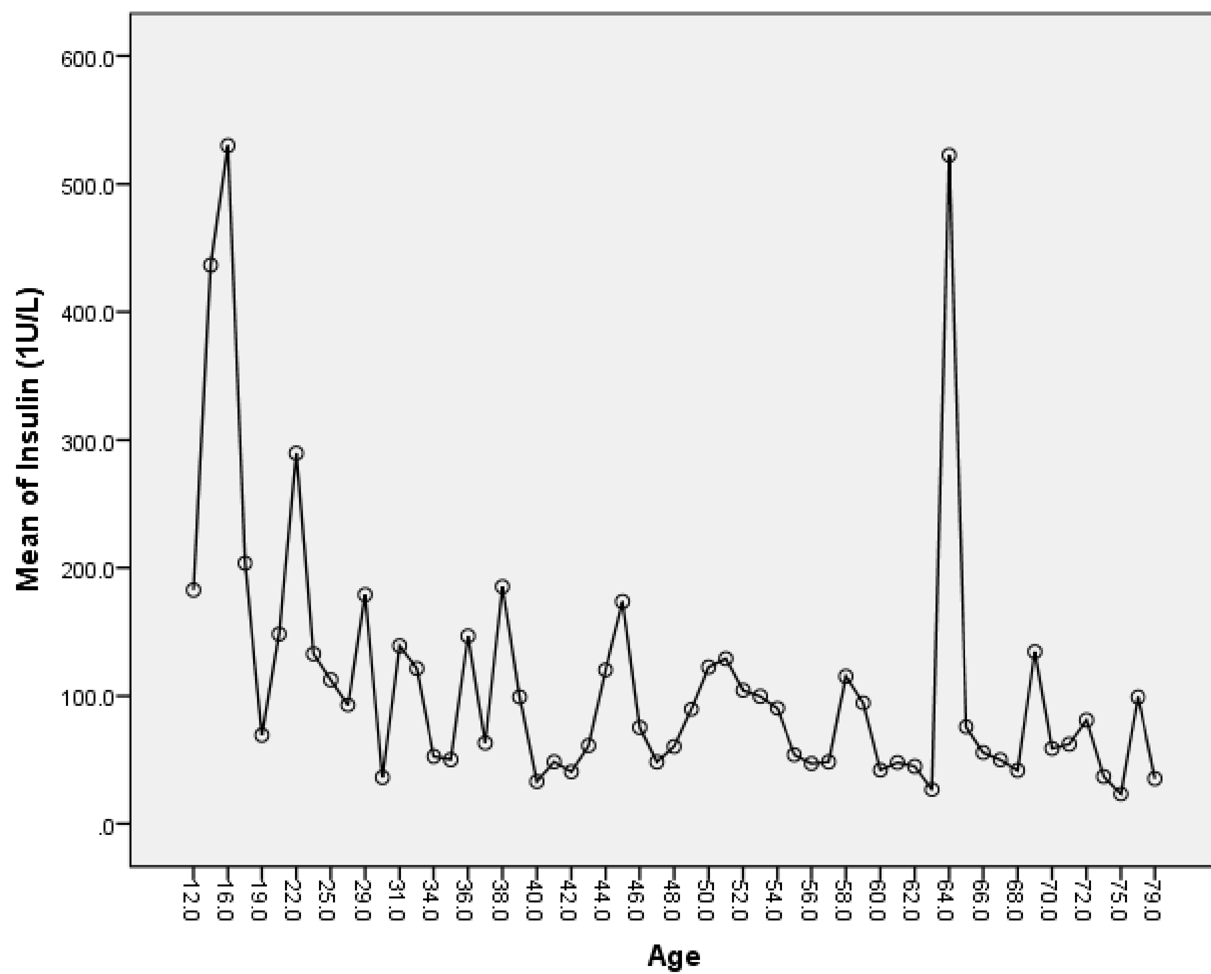

Figure 3A. Comparison mean insulin/age.

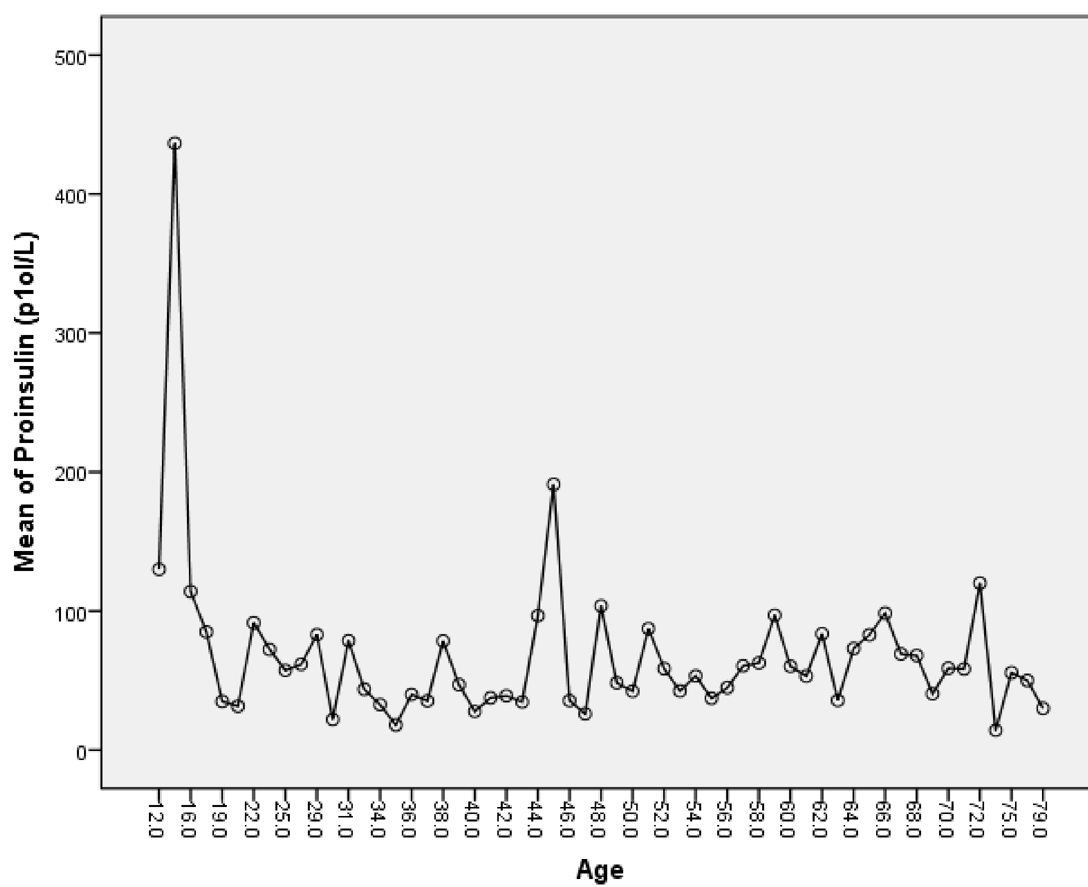

Figure 4A. Comparison proinsulin/age.

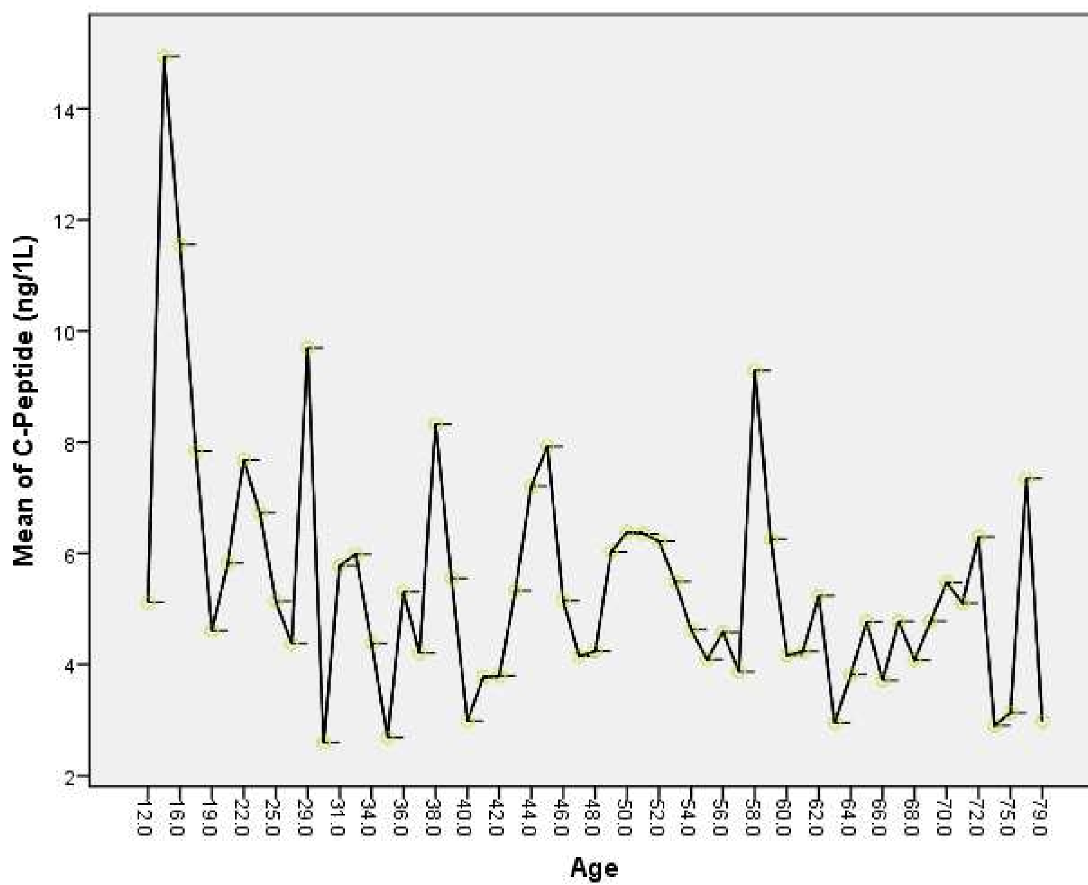

Figure 5A. Comparison C-peptide/age.

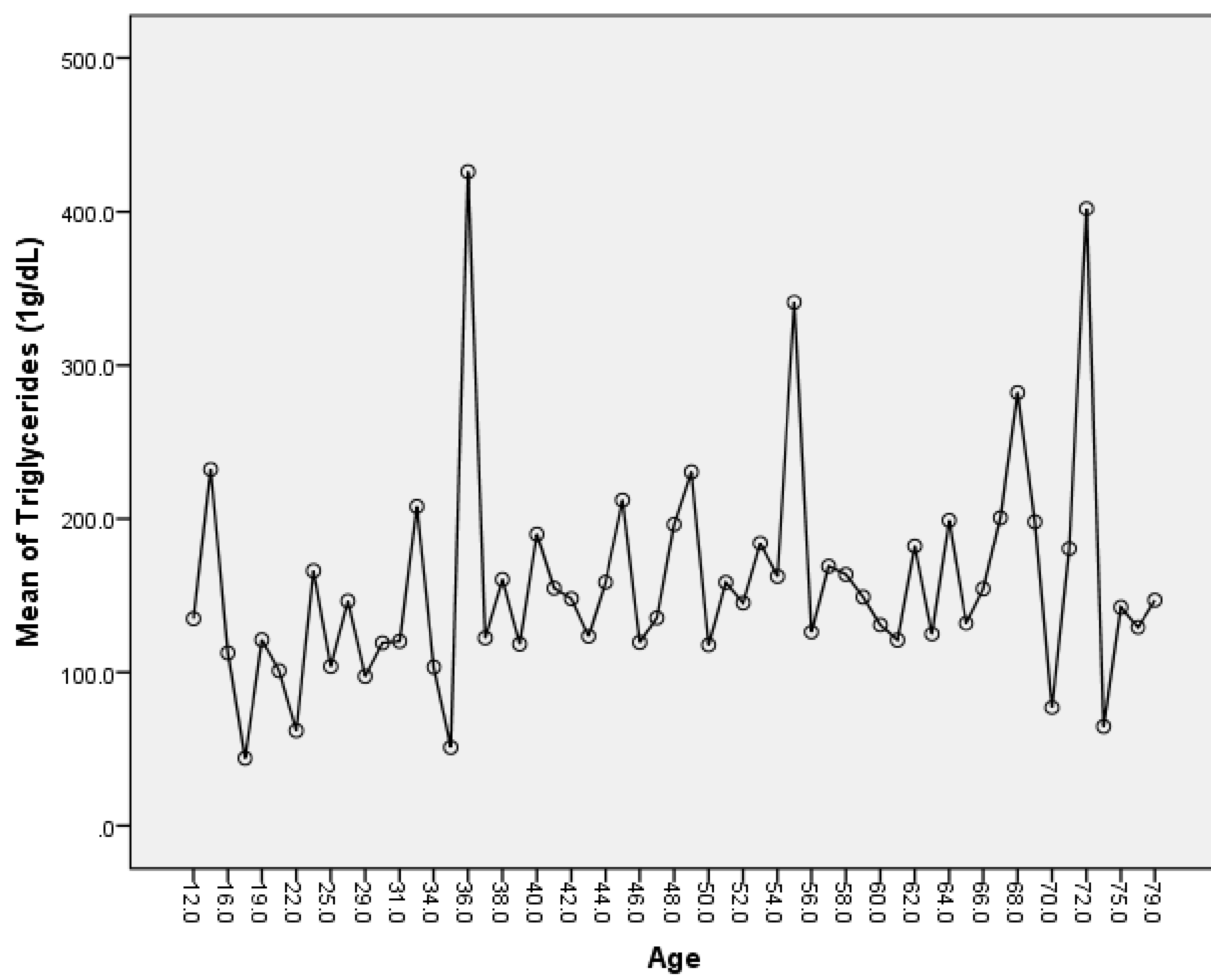

Figure 6A. Comparison triglycerides/age.

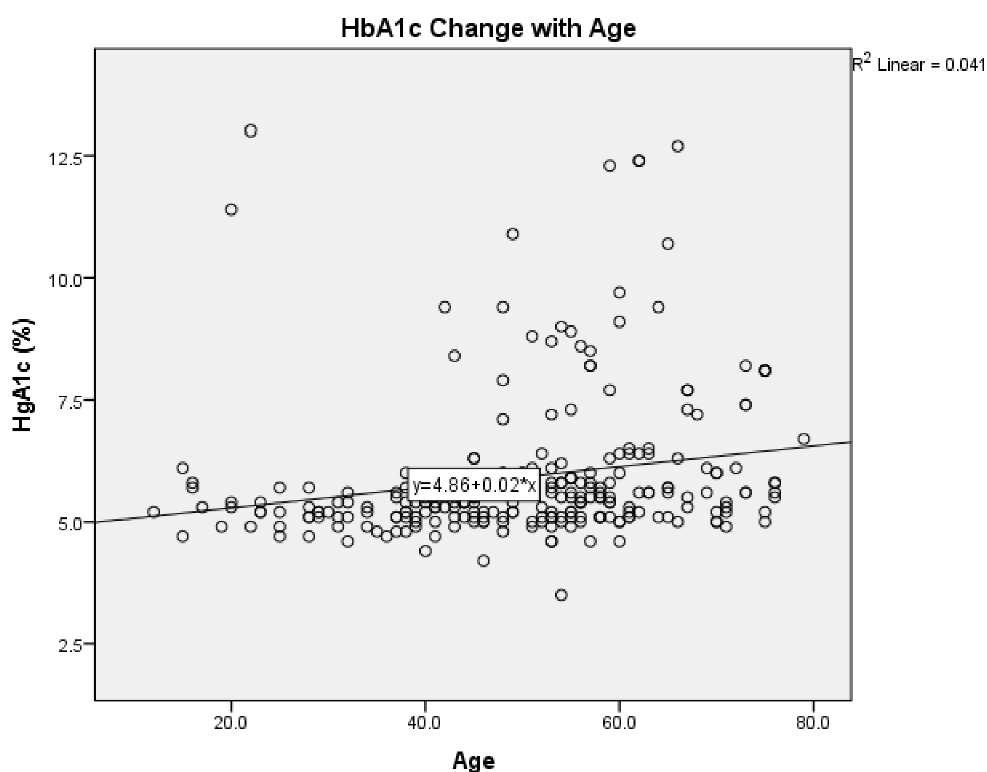

Figure 7A. Comparison HbA1c/age.

This demonstrates the increase in HbA1c levels with increased age. The higher HbA1c levels found in patients over 40 years of age is due to advanced β-cell exhaustion due to insulin resistance. The higher HbA1c levels observed in younger patients was observed with severe insulin resistance. Those patients having elevated insulin levels above 500 mU/L at the end of the first phase insuling response to glucose.