Novel Biomarkers and Genetic Variants for Type 2 Diabetes in Latinos

by

Joon Young Kim

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved April 2015 by the
Graduate Supervisory Committee:

Gabriel Shaibi, Chair
Lawrence J. Mandarino
Dawn K. Coletta
Elena A. De Filippis

ARIZONA STATE UNIVERSITY

May 2015

ABSTRACT

The shape of glucose response and one hour (1-hr) glucose during an oral glucose tolerance test (OGTT) are emerging biomarkers for type 2 diabetes. The purpose of this study was two-fold: (1) to investigate the utility of these novel biomakers to differentiate type 2 diabetes risk in Latino youth, and (2) to examine the genetic determinants in a Latino population.

Data from the ASU Arizona Insulin Registry (AIR) registry and the USC Study of Latino Adolescents at Risk for diabetes project were used to test the cross-sectional and prospective utility of novel biomarkers to identify youth at risk for type 2 diabetes. Pediatric and adult data from the ASU AIR registry were assessed to examine the association of single nucleotide polymorphisms (SNPs) with type 2 diabetes risk. Three KCNQ1 SNPs (rs151290; rs2237892; rs2237895) were examined as novel genetic variants for type 2 diabetes in Latinos.

Latino youth with a biphasic response in the AIR registry exhibited significantly better β-cell function ($P < 0.05$) compared to youth with a monophasic response. Additionally, Latino youth with a 1-hr glucose ≥155 mg/dL exhibited a significantly greater decline in β-cell function over 8 years compared with the <155 mg/dL group (β=-327.8±126.2, P = 0.01). Moreover, a 1-hr glucose ≥155 mg/dL was associated with a 2.5 times greater risk for developing prediabetes over time (P = 0.0001). 1-hr glucose was the most powerful predictor of prediabetes (area under the receiver operating characteristic curve=0.73) when compared to the traditional biomarkers including HbA1c (0.58), fasting (0.67), and 2-hr glucose (0.64). Two KCNQ1 SNPs (rs151290 and rs2237892) exhibited significant associations with type 2 diabetes risk factors. For the novel

glycemic markers, 15 SNPs were associated with the glucose response curve, while 18 SNPs were associated with 1-hr glucose.

These data suggest that glucose response curve and 1-hr glucose during an OGTT independently differentiate type 2 diabetes risk among Latino youth. Furthermore, it was successful to replicate the association of type 2 diabetes risk with 2 KCNQ1 SNPs in a Latino population. Data suggest that novel glycemic biomarkers are influenced by genetic background in this high-risk population.

DEDICATION

ad astra per aspera - to the stars through difficulties

I am thankful for vital support and encouragement of those whom I have encountered throughout my doctoral process, especially my family. I specifically dedicate this dissertation to my wife, Ja Youn Kwon. She has provided never-ending support and encouragement every step of the way through my doctoral training. Her words of encouragement and love have been instrumental to my success at ASU.

ACKNOWLEDGEMENTS

I would never have been able to successfully finish my dosctoral process without the guidance of my committee members, Dr. Gabriel Shaib, Dr. Dawn Coletta, Elena A. De Filippis, and Dr. Lawerence Mandarino. I would like to express my deepest gratitude to my mentor, Dr. Gabriel Shaibi, for his best mentorship. He has provided excellent guidance and patience that led me a better scientist. In addition to his support for my doctoral trainitng, I gratefully appreciate him for providing me with an excellent atmosphese for adjusting US culture and language. Hopefully, I will be able to repay his mentorship by achieving my career goals and providing same dedication to my future students. I would like to thank Dawn Coletta, for providing me many opportunities to participate in her studies. Her support and encouragement drived me to develop my scientific background. Thank you, Lawerence Mandarino, for sharing his expertise, mentorship, and knowledge that led me to develop my critical thinking skills. Thank you, Elena A. De Filippis, for being vital member of my dissertation committee. While I have not spent as much time with her, her input certainly made me having an insight into clinical practice. I would like to thank all of the graduate students and ASU/Mayo staffs. Without their efforts, many research progjects I have involved in couldn’t be managed effieciently. I would like to thank all the children and adults who gave their time and enery to participate in several clinical research projects.

TABLE OF CONTENTS

Page

CHAPTER Page

APPENDIX Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: GENERAL INTRODUCTION

Obesity has reached epidemic proportions in the US with approximately 32% of adolescents and two-thirds of adults classified as either overweight or obese (Ogden, Carroll, Kit, & Flegal, 2014). In the context of a widespread obesity epidemic, the burden of metabolic abnormalities (i.e., metabolic syndrome and type 2 diabetes) is of clinical and public health concern in both youth and adults (Fagot-Campagna, 2000; Ford, Li, & Zhao, 2010).

In order to diagnose disorders of glucose metabolism (i.e., prediabetes and type 2 diabetes), the oral glucose tolerance test (OGTT) has been used in the clinical practice. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (The Expert Committee on the, Diagnosis, & Classification of Diabetes, Mellitus, 1997; 2003) announced specific criteria for diagnosing new onset of type 2 diabetes based on the fasting and 2-hr glucose levels that are obtained from the OGTT. In addition, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were introduced as intermediate stages in the natural history of type 2 diabetes. Individuals with prediabetes (i.e., IFG and/or IGT) have been referred to as exhibiting relatively high risk for the future development of type 2 diabetes (The Expert Committee on the, Diagnosis, & Classification of Diabetes, Mellitus, 2003).

However, epidemiological studies in adults demonstrate the limitations of fasting and 2-hr post challenge glucose in predicting risk for type 2 diabetes, as only 50% of patients with prediabetes eventually convert to diabetes (Gerstein et al., 2007; Unwin, Shaw, Zimmet, & Alberti, 2002). These findings indicate that approximately half of individuals with new onset of type 2 diabetes are considered as maintaining their normal

glucose tolerance (NGT) status prior to the diagnosis of type 2 diabetes. In the pediatric populations, relatively rapid progression to overt type 2 diabetes has been observed compared to adults, as obese youth with IGT decompensate to frank type 2 diabetes over a mean follow-up of 20 months (Weiss et al. 2005). It has been clearly shown that impairment of β-cell function, which is a hallmark feature of type 2 diabetes and is considered one of the earliest indicators of diabetes risk (Bergman, Ader, Huecking, & Van Citters, 2002; DeFronzo, 2009; Tfayli, Lee, & Arslanian, 2010), starts even in NGT in both youth and adults. For this reason, in addition to the traditional glycemic markers (i.e., fasting and 2-hr glucose), there is a substantial interest in gaining individuals' metabolic information from the OGTT. This effort leads to further examine novel biomarkers to accurately identify high-risk individuals for the future development of type 2 diabetes.

In an effort to find an accurate identification tool for future type 2 diabetes, recent studies in adults recommended using a simple shape index during OGTT (Abdul-Ghani, Lyssenko, Tuomi, DeFronzo, & Groop, 2010; Fuchigami, Nakano, Oba, Metori, 1994; Kanauchi, M., Kimura, Kanauchi, K., & Saito, 2005; Trujillo-Arriaga & Roman-Ramos, 2008; Tschritter et al., 2003; Tura et al., 2011). They commonly found that individuals with a monophasic response (inverted U shape) during an OGTT exhibit greater insulin resistance and decreased β-cell function compared to individuals with a biphasic response (a second rise of plasma glucose after first decline). Further, Abdul-Ghani et al. (2010) revealed that prediabetic adults with a monophasic glucose response to an OGTT exhibited nearly double the risk of developing type 2 diabetes over a 7-8 year follow-up compared to prediabetics with a biphasic response. To our knowledge, whether the shape

of the glucose response curve is associated with type 2 diabetes risk in younger populations has not been determined. Therefore, our group tested the utility of this phenotype and confirmed that the biphasic phenotype is associated with lower risk of type 2 diabetes independent of traditional glycemic markers (i.e., fasting and 2-hr glucose) in Latino youth, potentially due to higher insulin sensitivity and better β-cell function (Kim, Coletta, Mandarino, & Shaibi, 2012).

In addition to glucose response shape index, one hour (1-hr) plasma glucose concentration during an OGTT has been shown an independent predictor of type 2 diabetes in adults (Abdul-Ghani, Williams, DeFronzo, & Stern, 2007; Abdul-Ghani M, Abdul-Ghani T, Ali, & DeFronzo, 2008; Abdul-Ghani, Lyssenko, Tuomi, DeFronzo, & Groop, 2009) and in youth (Tfayli, Lee, Bacha, & Arslanian, 2011). Especially, Abdul-Ghani et al. (2008) compared predictive power of glycemic indicators and found that 1-hr glucose of 155mg/dL was a better predictor of type 2 diabetes than either fasting or 2-hr glucose concentrations yielding the maximal sum of sensitivity (0.75) and specificity (0.79). However, little is known about longitudinal changes in metabolic health based on this cutoff value in younger population. Therefore, we examined the threshold of 1-hr glucose concentration (155 mg/dL) and confirmed this finding in Latino youth, suggesting that 1-hr glucose predicts the development of prediabetes and β-cell dysfunction over the 8 years follow-up period (Kim et al., 2013). Moreover, similar to the prospective study in adults, this emerging biomarker was more powerful to predict type 2 diabetes risk (i.e., development of prediabetes) than traditional glycemic indicators including HbA1c, fasting, and 2-hr glucose (Kim, Goran, Toledo‐Corral, Weigensberg, & Shaibi, 2014).

In order to understand the pathogenesis of type 2 diabetes, a growing number of studies have examined environmental factors such as diet or exercise (Ershow, 2009) and/or genetic factors, which contribute to the development of type 2 diabetes. Specifically, significant progress has been made with regards to identifying the genetic causes or single nucleotide polymorphisms (SNPs) since genome-wide association studies (GWAS) and large-scale meta-analysis have been widely performed. To date, GWAS have identified over 60 susceptibility loci which have been associated with type 2 diabetes risk (Brunetti, Chiefari, & Foti, 2014; Dupuis et al., 2010; McCarthy, 2010; Morris et al., 2012; Saxena et al, 2012). Moreover, ongoing efforts on the physiologic characterization of diabetes-related chronic disease risk factors such as obesity, dyslipidemia, hypertension, and glucose homeostasis have led to a better understanding of the pathogenesis of type 2 diabetes (Ingelsson et al., 2010).

Although previous data showed that similar physiological contributors (impairment of insulin release and action) seem to be involved in the monophasic glucose response and higher 1-hr glucose (Kim et al., 2012; Kim et al., 2013), it is still unknown whether there are genetic involvements in these novel phenotypic characteristics. To our knowledge, no studies have examined genetic determinants of glucose response curve or 1-hr glucose. Although we recently have replicated GWAS SNPs (n=28) that are related to type 2 diabetes risk in a Latino population (DeMenna et al., 2014), we did not examine genetic association with these emerging biomarkers. Therefore, in addition to the verification of the utility of novel biomarkers for predicting type 2 diabetes risk, it is necessary to examine the genetic association with these novel markers (e.g., glucose response curve and 1-hr glucose) to expand the understanding of novel markers.

To date, the majority of type 2 diabetes susceptibility loci have been associated with β-cell function while a limited number of genes related to insulin action have been identified (Saxena et al., 2012). For example, transcription factor 7-like 2 (TCF7L2) genetic variants have a substantially stronger effect on the impairment of insulin secretion as compared to insulin action (Grant et al., 2006; Morris et al., 2012). Since it is well established that obesity is also linked to the risk for future type 2 diabetes (Kahn, Hull, & Utzschneider, 2006), fat mass and obesity-associated (FTO) became a widely-replicated gene and numerous studies have confirmed the association between FTO SNPs and obesity-related phenotypes such as BMI and waist circumference in several populations (Dina et al., 2007; Kilpeläinen et al., 2011; Scuteri et al., 2007). However, these SNPs found via GWAS have shown modest effect sizes and collectively explain only 10% for the variance in type 2 diabetes risk (Imamura & Maeda, 2011; McCarthy & Zeggini, 2009; Morris et al., 2012; Voight et al., 2010). The limitations of current GWAS to date may be due to the involvement of novel (or not fully replicated) SNPs (Brunetti et al., 2014; Thomsen & Gloyn, 2014). It is possible that detailed physiological characterization of these SNPs may help clarify their associations with and/or role in type 2 diabetes risk (Ingelsson et al., 2010).

Compared to the genetic variants in TCF7L2 or FTO, some genes and SNPs were not widely replicated in various populations despite exhibiting a relatively high effect size. Moreover, the majority of genetic studies were performed on Europeans and little is known about the genetic influences on type 2 diabetes risk in Latino populations. For example, KCNQ1 has been associated with impaired β-cell function (Unoki et al., 2008; Yasuda et al., 2008) and its effect size is similar with that of TCF7L2 (Prokopenko,

McCarthy, & Lindgren, 2008). However, approximately 90% of genetic studies were performed among East Asian or Caucasian populations according to the meta-analysis of the effect of KCNQ1 SNPs on the type 2 diabetes risk (Liu et al., 2013). Therefore, it is necessary to replicate the study of genetic variants of KCNQ1 in Latino population. To our knowledge, two studies examined KCNQ1 SNP (rs2237892) in the Mexican population and exhibited significant association with susceptibility to type 2 diabetes (Gamboa-Meléndez et al., 2012; Parra et al., 2011). Further replication studies of KCNQ1 effects on type 2 diabetes risk are warranted in this population by recruiting more SNPs (e.g., rs2237895 and rs151290) in this gene, which have been studied in other populations. Collectively, more candidate SNPs along with non-traditional phenotypic markers for dysglycemia are critical to expand the genetic contributions to type 2 diabetes. Therefore, we examined genetic determinants of novel glycemic biomarkers (i.e., glucose response curve and 1-hr glucose) as well as included more SNPs (KCNQ1 genetic variants) in the genetic association analysis.

The overall purpose of this dissertation was two-fold; (1) to examine the utility of novel glycemic markers for predicting the type 2 diabetes risks, and (2) to examine genetic determinants of novel glycemic markers. To be specific, this dissertation explores: (1) the association of the glucose response curve during an OGTT with type 2 diabetes risk factors in Latino youth, (2) the utility of 1-hr glucose level to predict type 2 diabetes risk in obese Latino youth, (3) the predictive power of the 1-hr glucose level compared to traditional glycemic markers, and (4) genetic determinants of novel glycemic biomarkers as well as genetic influences of type 2 diabetes susceptibility genes including KCNQ1 in Latino population.

The explicit aims of the dissertation are outlined below.

Aim 1: To compare type 2 diabetes risk factors in Latino adolescents characterized by either a monophasic or biphasic glucose response during an OGTT.

Aim 2: To examine the utility of elevated 1-hr glucose levels to prospectively predict deterioration in β-cell function and the development of prediabetes in high-risk youth.

Aim 3: To compare the predictive power of 1-hr glucose to traditional glycemic markers (i.e., HbA1c, fasting, and 2-hr glucose) for prospectively identifying prediabetes in high-risk youth.

Aim 4: To examine genetic determinants of novel glycemic biomarkers (i.e., glucose response curve and 1-hr glucose level) and traditional clinical markers of type 2 diabetes risk (i.e., adiposity, lipid profile, fasting glucose, HbA1c, and OGTT-derived insulin release and resistance measures).

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CHAPTER 2: GLUCOSE RESPONSE CURVE AND TYPE 2 DIABETES RISK IN LATINO ADOLESCENTS.

Joon Young Kim, Dawn K. Coletta, Lawrence J. Mandarino, and Gabriel Q. Shaibi

Abstract

Objective

In adults, the shape of the glucose response during an oral glucose tolerance test (OGTT) prospectively and independently predicts type 2 diabetes. However, no reports have described the utility of this indicator in younger populations. The purpose of this study was to compare type 2 diabetes risk factors in Latino adolescents characterized by either a monophasic or biphasic glucose response during an OGTT.

Research Design and Methods

A total of 156 nondiabetic Latino adolescents completed a 2-hr OGTT. Monophasic and biphasic groups were compared for the following type 2 diabetes risk factors: fasting and 2-hr glucose, HbA1c, glucose area under the curve (AUC), insulin sensitivity (Matsuda index), insulin secretion (insulinogenic index), and β-cell function as measured by the disposition index (insulin sensitivity × insulin secretion).

Results

Of the participants, 107 youth were categorized as monophasic and 49 were biphasic. Compared with the monophasic group, participants with a biphasic response exhibited lower HbA1c (5.4 ± 0.3 vs. 5.6 ± 0.3%, $P < 0.01$) and lower glucose AUC (14,205 ± 2,382 vs. 16,230 ± 2,537 mg*dL^{-1}*h^{-1}, $P < 0.001$) with higher insulin

sensitivity (5.4 ± 3.2 vs. 4.6 ± 3.4, P ≤ 0.05), higher insulin secretion (2.1 ± 1.3 vs. 1.8 ± 1.3, P = 0.05), and better β-cell function (10.3 ± 7.8 vs. 6.0 ± 3.6, $P < 0.001$). Differences persisted after adjusting for age, sex, and BMI.

Conclusions

These data suggest that the glycemic response to an OGTT may differentiate risk for type 2 diabetes in youth. This response may be an early marker of type 2 diabetes risk among high-risk youth.

Introduction

In parallel with the current pediatric obesity epidemic, type 2 diabetes has emerged as a critical health concern among obese adolescents (Fagot-Campagna, 2000; Ogden, Flegal, Carroll, & Johnson, 2002). Although type 1 diabetes is more prevalent in the pediatric population, data from the SEARCH for Diabetes in Youth Study highlight a disproportionate distribution of type 2 diabetes among certain subpopulations of adolescents (Dabela et al., 2007). It is notable that for Hispanic females aged 15–19 years, the incidence of type 2 diabetes exceeds that of type 1 diabetes (Lawrence et al., 2009).

An important issue for the medical and research communities is to identify Latino youth at increased risk for premature type 2 diabetes so that appropriate prevention strategies may be initiated. In 1997, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (The expert committee on the diagnosis and classification of diabetes mellitus, 1997) introduced impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) as intermediate stages in the natural history of type 2 diabetes. In adults, pre- diabetes precedes frank type 2 diabetes by 5–10 years (Edelstein

et al, 1997; Saad et al, 1988); however, similar data are limited in younger populations. Weiss et al. (2005) have noted that obese youth with IGT decompensate to frank type 2 diabetes over a mean follow-up of 20 months. These data support the potential for a rapid progression to overt type 2 diabetes in youth, which may be exacerbated by pubertal insulin resistance (Goran & Gower, 2001; Gungor, Bacha, Saad, Janosky, & Arslanian, 2005). In contrast, Goran, Lane, Toledo-Corral, and Weigensberg (2008) have shown that obese Latino youth may vacillate between normal glucose tolerance (NGT) and IFG/IGT over time. Therefore, in addition to pre- diabetes, other markers of type 2 diabetes risk may be necessary.

Several recent studies in adults use the shape of the glucose curve during an oral glucose tolerance test (OGTT) to identify metabolic dysregulation and the potential risk for future type 2 diabetes (Abdul-Ghani, Lyssenko, Tuomi, DeFronzo, & Groop, 2010; Fuchigami, Nakano, Oba, & Metori, 1994; Kanauchi, M., Kimura, Kanauchi, K., & Saito, 2005; Morbiducci et al, 2014; Trujillo-Arriaga, & Roman-Ramos, 2008; Tschritter et al., 2003; Tura et al., 2011). Using a simple shape index, individuals with a monophasic response (inverted U shape) during an OGTT exhibit greater insulin resistance and decreased β-cell function compared with individuals with a biphasic response (a second rise of plasma glucose after first decline). A recent prospective study demonstrates that independent of fasting and/or post-challenge glucose concentrations, individuals with a monophasic response developed type 2 diabetes at a higher rate than those with a biphasic response (Abdul-Ghani et al., 2010).

Since our group tested the utility of the glucose response curve obtained from the OGTT for differentiating the risk for type 2 diabetes in a Latino youth, more attempts to

confirm previous findings in younger population have been made. Nolfe, Spreghini, Sforza, Morino, and Manco (2012) described the morphology of glucose response curve during an OGTT in Caucasian obese children and adolescents (N=553). More sophisticated classification criteria for the glucose response curve was used (i.e., monophasic, biphasic, triphasic, and upward monotonous). They found that, within a normoglycemic individuals (n=522), monophasic was most prevalent type of glucose response to an OGTT (n=285, 54%) and represented high risk for type 2 diabetes in terms of glucose intolerance, insulin resistance, and impairment of insulin secretion. Further, they discussed that more accurate metabolic information (i.e., type 2 diabetes risk) can be extracted when the glucose response curve was analyzed with morphologies of insulin curve or time of glucose peak. More recently, Bervoets, Mewis, and Massa (2014) examined the shape of plasma glucose response curve in relation to insulin sensitivity, insulin secretion, and other metabolic phenotypes in end-pubertal girls. A total 81 end-pubertal obese girls completed a standard 2-hr OGTT and divided into four types of the glucose response curves using a threshold of 2 mg/dL as follows: monophasic, biphasic, triphasic, and unclassified. Individuals with monophasic glucose response exhibited higher area under the curve for glucose, lower early-phase insulin secretion, and poorer β-cell function relative to insulin sensitivity compared to the participants with biphasic and triphasic glucose response. Collectively, aforementioned two studies further tested and confirmed the utility of the glucose response curve during an OGTT in the cross-sectional dataset. To our knowledge, our study was the first to examine the association of the glucose response curve with type 2 diabetes risk in younger populations. The purpose of this study was to compare diabetes risk factors in

Latino youth characterized by either a monophasic or biphasic glucose response during a 2-hr OGTT.

Research Design and Methods

Data from 156 nondiabetic Latino adolescents (aged 12–21 years) who participated in a community- based diabetes registry were used in the present analysis. Participants arrived at the Arizona State University Clinical Research Unit after an overnight fast. Anthropometric measurements included height, weight and BMI, waist and hip circumference, and seated blood pressure. A blood sample (~20 mL) was taken under fasting conditions to measure HbA1c and lipid profile, including total cholesterol, triglyceride, HDL, LDL, and VLDL. All laboratory tests were performed by a Clinical Laboratory Improvement Amendments–certified commercial laboratory (Sonora Quest Laboratories, Phoenix, AZ).

Oral Glucose Tolerance Test (OGTT)

Participants underwent a 2-hr OGTT following a 10-hr overnight fast. Subjects ingested a solution containing 75 g dextrose (1.75 g/kg), and venous blood samples were obtained at 0, 30, 60, 90, and 120 min for determination of plasma glucose and insulin concentrations. Plasma glucose was measured by the glucose oxidase method using a YSI 2300 STAT plus (YSI, Inc., Yellow Springs, OH), and insulin was measured in duplicate by ELISA (ALPCO Diagnostics, Windham, NH).

Classification of Response Curve

Glucose response phenotype (i.e., monophasic or biphasic) was classified according to previous studies (Abdul-Ghani et al., 2010; Kanauchi et al., 2005; Trujillo-Arriaga, & Roman-Ramos, 2008; Tschritter et al., 2003), with a glucose threshold of 4.5

mg/dL as described by Tschritter et al. (2013) to minimize fluctuations in glucose concentrations that may be caused by the method of glucose analysis rather than physiological reasons. A monophasic response was characterized by a gradual rise in plasma glucose concentrations until a peak was reached followed by a subsequent decrease until 120 min. A biphasic response was characterized by a gradual rise in glucose, followed by a ≥ 4.5 mg/dL fall, with a second rise of glucose of at least 4.5 mg/dL at a subsequent time point. Participants who exhibited a gradual increase in plasma glucose after glucose ingestion without a corresponding fall were deemed "unclassified" (n = 2) and were excluded for the present analysis (Tschritter et al., 2013).

Variables and Calculation

Type 2 diabetes risk factors included fasting plasma glucose and insulin, 2-hr plasma glucose and insulin, HbA1c, and glucose and insulin area under the curve (AUC). Total AUC for plasma glucose and insulin during the OGTT were calculated by the trapezoidal method using 30-min sampling time points (Matthews, Altman, Campbell, & Royston, 1990). In addition to these indicators, insulin action was estimated by the homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR) (Matthews et al., 1985) and the whole-body insulin sensitivity index of Matsuda and DeFronzo (1999), and insulin secretion was estimated by the insulinogenic index calculated using fasting and 30-min insulin and glucose concentrations (Phillips, Clark, Hales, & Osmond, 1994). β-cell function was estimated by the disposition index as the product of insulin action Matsuda index (Matsuda & DeFronzo, 1999) and insulin secretion insulinogenic index (Phillips et al, 1994).

Statistical Analysis

Independent sample t tests and χ^2 analyses were used to compare characteristics between glucose phenotypes. Two-way repeated-measures ANOVA was used to assess differences in the glucose and insulin levels at each time point during the OGTT. Analysis of covariance was used to compare phenotypes after adjusting for the potential confounding effects of age, sex, and BMI on type 2 diabetes risk factors. Data that did not meet the assumptions for normality (glucose values at 30 and 90 min and insulin values at each time point from the OGTT, HbA1c, and all indices for insulin sensitivity, secretion, and β-cell function) were $\log_{10}$ transformed; untransformed data are presented for ease of interpretation. Data were analyzed using PASW 18.0 statistical software package with significance set at $P \leq 0.05$.

Results

Descriptive characteristics of participants are presented in Table 2-1. No differences in sex, BMI categories (lean vs. overweight vs. obese), or glycemic status (NGT vs. prediabetes) were noted between glucose phenotypes. In addition, no significant differences were noted for age, anthropometrics (BMI and waist and hip circumference), lipids, or blood pressure.

Two-way repeated-measures ANOVA for plasma glucose concentrations during the OGTT demonstrated significant effects for group and time as well as group 3 time interaction, indicating differences between groups over the course of the OGTT (all $P < 0.0001$). Glucose and insulin concentrations for each OGTT time point are presented in Fig. 2-1.

Table 2-1. Descriptive Characteristics of Participants by Phenotype

Variables	Mean ± SD			p
	Monophasic (*n*=107)	Biphasic (*n*=49)	Total (*n*=156)	
Gender (Male/Female)	50(47%)/ 57(53%)	22(45%)/ 27(55%)	72(46%)/ 84(54%)	0.83
Lean/Overweight/Obese	56(53%)/ 24(23%)/ 26(24%)	26(53%)/ 12(25%)/ 11(22%	82(53%) /36(23%)/ 37(24%)	0.95
NGT/Prediabetes	86 (80%) / 21 (20%)	36 (73%) / 13 (27%)	122 (78%) / 34 (22%)	0.33
Age (year)	15.86 ± 2.74	16.37 ± 2.74	16.02 ± 2.74	0.28
BMI (kg/m^2)	26.43 ± 7.21	25.31 ± 5.84	26.08 ± 6.81	0.39
WC (cm)	89.57 ± 18.57	86.42 ± 14.32	88.58 ± 17.35	0.34
HC (cm)	103.3 ± 14.4	101.21 ± 11.86	102.64 ± 13.65	0.4
SBP (mmHg)	114.29 ± 11.63	115.42 ± 12.44	114.65 ± 11.86	0.59
DBP (mmHg)	70.06 ± 8.11	70.8 ± 11.03	70.29 ± 9.1	0.38
TRG (mg/dL)	99.69 ± 53.2	98.78 ± 51.15	99.4 ± 52.4	0.9
HDL (mg/dL)	43.19 ± 10.1	44.22 ± 7.77	43.51 ± 9.42	0.3
LDL (mg/dL)	86.71 ± 22.92	80.55 ± 25.16	84.78 ± 23.74	0.11
VLDL (mg/dL)	16.7 ± 8.88	16.59 ± 8.5	16.67 ± 8.74	0.96
Cholesterol (mg/dL)	152.33 ± 64.8	141.2 ± 30.57	148.83 ± 56.46	0.13

Note. NGT=normal glucose tolerance; IFG=impaired fasting glucose; IGT=impaired glucose tolerance; BMI=body mass index; WC=waist circumference; HC=hip circumference; SBP=systolic blood pressure; DBP=diastolic blood pressure; TRG=triglyceride; HDL=high-density lipoprotein; LDL=low-density lipoprotein; VLDL=very low-density lipoprotein

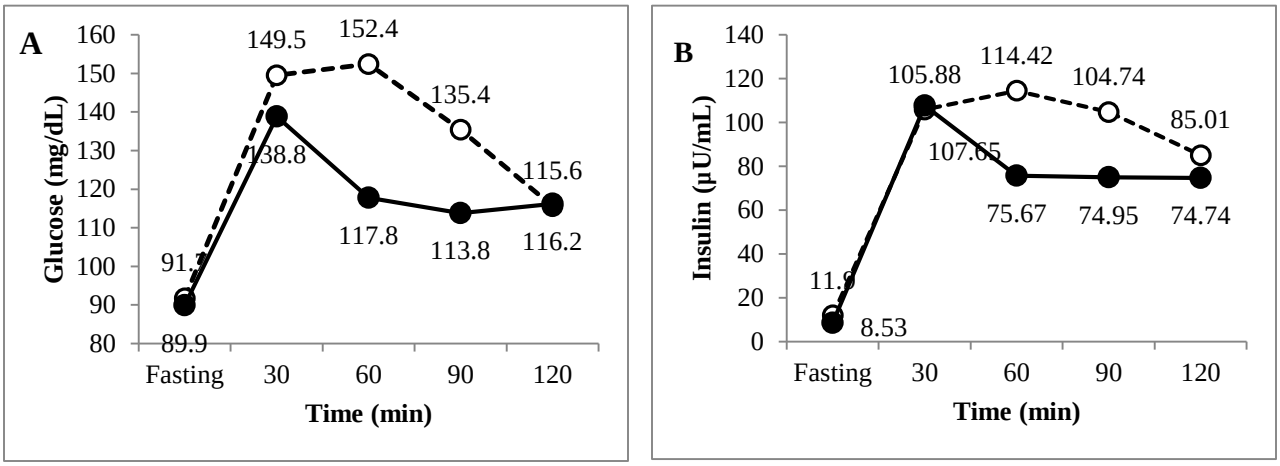

Figure 2-1. Glucose (A) and Insulin (B) Response Curves During OGTT in Monophasic (white circles and dashed line) and Biphasic (black circles and solid line).

Within each glucose phenotype, all time points across the OGTT (i.e., 0, 30, 60, 90, and 120) were significantly different from each other ($P < 0.0001$). The monophasic group exhibited significantly higher blood glucose levels at 30, 60, and 90 min compared with the biphasic group, while no differences were noted for either fasting or 2-hr glucose concentrations between groups. In terms of insulin response during the OGTT, there were significant effects for time ($P < 0.0001$) but not for group. The monophasic group had significantly higher insulin values at 60 and 90 min compared with the biphasic group.

Measures of glycemia are presented in Table 2-2. Participants with a monophasic response exhibited slightly but significantly higher HbA1c than biphasic participants, and these differences remained significant after adjusting for sex, BMI, and age. Glucose AUC in the monophasic group was 14.3% higher than in the bi- phasic group, and these differences were in- dependent of sex, age, or BMI (Table 2-2).

Table 2-2. Measures of Insulin and Glucose Homeostasis and β-cell Function

Variables	Mean ± SD	
	Monophasic (*n*=107)	Biphasic (*n*=49)
HbA1C (%)	5.55 ± 0.3	5.41 ± 0.27*
Glucose AUC ($mg*dL^{-1}*h^{-1}$)	16229.73 ± 2537.15	14205.31 ± 2382.49**
Insulin AUC($\mu U*mL^{-1}*h^{-1}$)	11113.33 ± 7280.4	9026.45 ± 5528.69
HOMA-IR	2.59 ± 2.08	2.03 ± 1.24
Matsuda Index	4.59 ± 3.38	5.43 ± 3.18*
Insulinogenic Index	1.75 ± 1.32	2.1 ± 1.32*
Disposition Index	6.03 ± 3.59	10.28 ± 7.8**

NOTE: *$P < 0.05$, **$P < 0.001$, All significances remained after adjusting gender and BMI or gender, BMI, and age. Insulin data were not available on 19 of the 156 participants (mono vs. biphasic, 93 vs. 44)

Insulin measures are presented in Table 2-2. No significant differences between groups were noted for insulin AUC or HOMA-IR. However, insulin sensitivity as measured by the Matsuda index and insulin secretion as measured by the insulinogenic index were both significantly higher in youth exhibiting the biphasic phenotype. β-cell function as measured by the disposition index was 42% higher in the biphasic group, and this difference remained significant after adjusting for covariates. Figure 2-2 displays the hyperbolic relationship between insulin sensitivity and insulin secretion for each group using the product of the Matsuda index and insulinogenic index. The best-fit line derived from the individual points of the monophasic group is shifted toward the origin (down and to the left) compared with the biphasic group (Fig. 2-2).

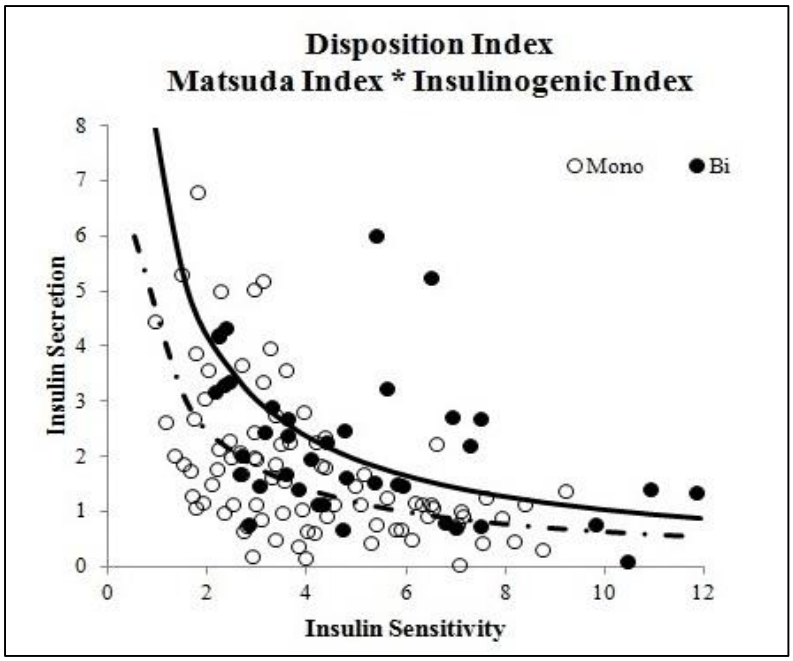

Figure 2-2. Hyperbolic Relationship Between Insulin Sensitivity and Insulin Secretion in Monophasic (white circles and dashed line) and Biphasic (black circles and solid line).

Conclusions

In the current study, we demonstrated that the shape of the plasma glucose response during an OGTT differentiates diabetes risk factors in Latino adolescents. Participants with a biphasic response exhibited lower glucose AUC and HbA1c, higher whole-body insulin sensitivity (Matsuda index) and insulin secretion, and better β-cell function compared with individuals with a monophasic response. These data extend previous studies in adults and suggest that the glucose response curve may be an early indicator of type 2 diabetes risk in adolescents.

Studies in adults have established that the shape of the glucose curve is related to both type 2 diabetes risk factors (Fuchigami et al., 1994; Kanauchi et al., 2005; Trujillo-Arriaga, & Roman-Ramos, 2008; Tschritter et al., 2003; Tura et al., 2011) and the development of type 2 diabetes (Abdul-Ghani et al., 2010). Tschritter et al. (2003)

studied glucose curves from 551 nondiabetic Caucasian adults and found that the bi-phasic response was associated with lower BMI, younger age, and higher insulin sensitivity and disposition index. The authors also reported that females and individuals with NGT were more likely to be characterized by a biphasic response. These findings were confirmed and expanded upon by Tura et al. (2011) who used glucose excursions during 3-hr OGTTs from nearly 600 Austrian women screened for gestational diabetes. The authors noted that a 3-hr OGTT captured even greater variations in glucose response, with some individuals exhibiting up to five phases. Greater complexity of the glucose curve (i.e., increasing number of phases) was associated with a healthier metabolic profile as indicated by higher insulin sensitivity and β-cell function as well as a lower prevalence of prediabetes and type 2 diabetes. These cross-sectional studies were confirmed prospectively where prediabetic adults with a monophasic glucose response during an OGTT exhibited nearly double the risk of developing type 2 diabetes during a 7- to 8-year follow-up compared with prediabetic subjects with a biphasic response (Abdul-Ghani et al., 2010). These studies suggest that the bi- phasic phenotype is associated with lower risk of type 2 diabetes potentially as a result of higher insulin sensitivity and better β-cell function.

In adults, insulin resistance and insulin secretory dysfunction are independently and interactively related to type 2 diabetes risk (Haffner, Miettinen, Gaskill, & Stern, 1995; Lillioja et al., 1993; Weyer, Bogardus, Mott, & Pratley, 1999). Specifically, the inability of the β-cell to compensate for insulin resistance is a primary determinant of type 2 diabetes (Bergman, Ader, Huecking, & Van Citters, 2002; Weyer et al., 1999). Compared with what is known in adults, the natural history of type 2 diabetes in youth is

less well understood. Recent studies support β-cell dysfunction as a key feature of type 2 diabetes in adolescents (Arslanian, 2002; Weiss & Gillis, 2008).

Not only does β-cell dysfunction contribute to type 2 diabetes in adolescents but it also was recently noted that Latino children and adolescents with prediabetes exhibit significantly lower β-cell function compared with their normoglycemic peers (Goran et al., 2004; Weigensberg, Ball, Shaibi, Cruz, & Goran, 2005). These data suggest that β-cell dysfunction contributes to prediabetes and type 2 diabetes in children and adolescents.

Taken together, our results extend findings on glucose response patterns in adults and type 2 diabetes pathophysiology in adults and youth to suggest that a monophasic glucose response may be associated with an increased risk for type 2 diabetes. This seemingly increased risk is evidenced by a lower disposition index that is due to significantly lower insulin sensitivity and secretion. When the disposition index of each group is plotted on the same graph (Fig. 2-2), the best-fit line representing the disposition index for the monophasic group is shifted closer to the origin (i.e., down and to the left) compared with that for the biphasic group. This shift is a hallmark feature of type 2 diabetes and is considered one of the earliest indicators of β-cell dysfunction (Bergman et al., 2002). It is important to note that the lower disposition index among the monophasic group was independent of BMI and, therefore, may confer additional type 2 diabetes risk beyond that of obesity. Furthermore, despite the lower disposition index observed in the monophasic group, neither the levels of fasting and 2-hr glucose nor the percentage of prediabetic subjects were significantly different between groups. When the dataset was restricted to only those participants with NGT, the disposition index in the monophasic

group remained significantly lower than that of the biphasic group ($P \leq 0.05$). Collectively, these findings suggest that the shape of the glucose response curve may be a very early marker of glucose dysregulation and type 2 diabetes risk and is detectable even before traditional indicators of hyperglycemia (The expert committee on the diagnosis and classification of diabetes mellitus, 2003). Whether the shape of the glucose curve is similarly predictive of the development of type 2 diabetes as traditional diabetes risk factors is an important question that should be addressed in future studies.

The physiological mechanisms responsible for the various glucose response curves are poorly understood. Although we found lower β-cell function (lower insulin sensitivity and secretion) in the monophasic group, we do not know whether this represents a cause or an effect of the phenotype or whether there are common biologic or genetic pathways linking these phenotypic characteristics. It may be that higher insulin sensitivity and secretion contribute to the biphasic response through more efficient and faster glucose clearance compared with the monophasic response. It is also possible that the timing of the insulin response may contribute to differences in the shape of the glucose response curve. Therefore, we divided individuals into either early (30-min) or late ($\geq$ 60-min) responders based on the timing of peak insulin concentrations. The biphasic group exhibited a higher percentage of "early responders" compared with the monophasic group (57 vs. 32%; $P < 0.01$); however, including insulin timing as a covariate in the final models did not change the results (data not shown). In addition, it is possible that an early return of plasma glucose concentrations toward baseline may stimulate a subtle counterregulatory response (Kanauchi, 2005; Trujillo-Arriaga, 2008), leading to a second rise in plasma glucose at 60 or 90 min. Another possible explanation

may be prolonged or delayed gastric emptying among monophasic individuals. Previous studies suggest that delayed gastric emptying is more common among adults with type 2 diabetes compared with control subjects, and prolonged gastric emptying is positively correlated with plasma glucose concentration (Horowitz et al., 1989). Among nondiabetic individuals, the incretin response following an oral glucose challenge is directly related to the rate of gastric emptying, which is inversely associated with postchallenge glucose and insulin concentrations (Horowitz, Edelbroek, Wishart, & Straathof, 1993). Taken together, it is possible that differences in gastric emptying as well as alterations in the incretin response may be associated with a monophasic glucose response and, ultimately, increases in type 2 diabetes risk with this phenotype.

To our knowledge, this is the first study to examine the shape of the glucose response curve in relation to type 2 diabetes risk among the pediatric population. We focused on Latino adolescents because this group represents a vulnerable population at increased risk for developing type 2 diabetes. We used defined glucose thresholds based on objective criteria to identify when and if more than one glucose peak was achieved. Very few researchers have published specific glucose thresholds for identifying differences between time points, and we believe using rigid criteria (i.e., a minimum of 4.5 mg/dL glucose excursion between a peak and a subsequent trough) rather than simply characterizing glucose curves through observation will minimize misclassification. When we analyzed our data using a previously published relative threshold of $\geq$ 2% difference between consecutive glucose time points (Tura et al., 2011), the overall results and interpretations were not affected. Despite these strengths, we acknowledge potential limitations in our data that should be considered.

First, we based our phenotype on the response to a single OGTT, which may have limited reproducibility in youth. Libman, Barinas-Mitchell, Bartucci, Robertson, & Arslanian (2008) demonstrated poor reproducibility of the OGTT in overweight youth in terms of identifying hyperglycemia. In addition, Kramer, Vuksan, Choi, Zinman, and Retnakaran (2014) evaluated the reproducibility of novel parameters of the insulin and glucose response during the OGTT. They reported 40% agreement on the shape of glucose response among three series of OGTT results. However, it is still unknown whether the shape of the glucose response is an inherent and, hence, reproducible biological process warrants further examination before using this assessment in longitudinal in youth. In addition, our classification of glucose phenotype was derived from the 2-hr OGTT. By using a longer OGTT (i.e., 3-hr OGTT) and/or more frequent sampling intervals (i.e., every 10 min) it is possible to capture more sophisticated curve types that will provide greater information on type 2 diabetes risk (Trujillo-Arriaga, 2008; Tura et al., 2011). Second, family history of diabetes, expo- sure to gestational diabetes in utero, and pubertal stage were not available for our analysis. It is well established that family history of diabetes is a strong risk factor for type 2 diabetes in both adults and youth (Arslanian, Bacha, Saad, & Gungor, 2005; Kelly et al., 2007; Kuo, C., Lin, Yu, Chang, & Kuo, H., 2010) and exposure to gestational diabetes in utero is a hypothesized risk factor for type 2 diabetes in youth (Fetita, Sobngwi, Serradas, Calvo, & Gautier, 2006). In addition, cross-sectional and longitudinal studies show that puberty is associated with insulin resistance, which may further contribute to type 2 diabetes risk (Ball et al., 2006; Goran, & Gower, 2001; Reinehr et al., 2009). Although we did not assess pubertal status in the current study, we did attempt to minimize the confounding

effects by adjusting for age. Nonetheless, age is not an ideal surrogate for pubertal stage, and we further acknowledge the relatively wide spectrum of age in our heterogeneous sample that should be addressed in future studies. Third, the utility of HbA1c as a potential type 2 diabetes risk factor in youth has not been well established (Lee, Wu, Tarini, Herman, & Yoon, 2011). It is also not clear whether this result is of clinical significance because differences were subtle. Lastly, the cross-sectional nature of our study precludes the ability to draw causal inferences about the shape of the glucose curve and type 2 diabetes risk. Given that type 2 diabetes is a progressive, chronic disease and typically presents in adulthood, examining markers that may identify risk in younger cohorts can offer temporal insight into the pathophysiological mechanisms of diabetes. It is interesting to note that previous studies suggest that adults with a biphasic response are characterized by younger age compared with those with a monophasic response (Fuchigami et al., 1994; Tschritter et al, 2003). In our cohort, .31% of the participants exhibited a biphasic response, which is slightly higher than the prevalence of biphasic in adult cohorts (AbdulGhani et al., 2010; Fuchigami et al., 1994; Kanauchi et al., 2005; Trujillo-Arriaga, & Roman-Ramos, 2008; Tschritter et al., 2003; Tura et al., 2011). Interestingly, similar prevalences of monophasic and biphasic glucose response were observed in recent two studies in younger population (Bervoets et al., 2014; Nolfe et al., 2012). To date, there are six studies in adults and two studies in youth (Table 2-3) describing the glucose response curve and suggest that the monophasic response is the dominant phenotype in adults (mean prevalence of monophasic response = 69%, range = 45 – 84%). From our data and those described above, it is difficult to determine whether this dominant state is indeed the "normal state" and whether the prevalence of different

glucose response curve phenotypes differs by age, gender, race/ethnicity or some other contributing factors (e.g., glycemic status). Given our findings and focus on a high-risk population, it is plausible that the proportion of youth with a monophasic response may be less in adolescents from a lower risk population (e.g., lean or Caucasian).

Table 2-3. Prevalence of Mono vs. Biphasic Glucose Response Curve From The Published Studies to Date in Adults and Youths

Study	Population	Total N (age)	Mono vs. Biphasic vs. (Unclassified)
Fuchigami (1994)	Japanese	70 (NA)	61 vs. 33% vs. (6%)
Tschritter (2003)	Caucasian	551 (36 yrs)	45 vs. 35% vs. (20%)
Kanauchi (2005)	Japanese	583 (62 yrs)	73 vs. 21% vs. (6%)
Trujillo-Arriaga (2008)	Mexican	100 (30 yrs)	84 vs. 16%
Abdul-Ghani (2010)	Finnish	2445 (46 yrs)	82 vs. 18%
Tura (2011)	Austrian women	475 (35 yrs)	69 vs. 31%
Nolfe et al (2012)	Caucasian	553 (4 to 18 yrs)	55 vs. 33% vs. (12%)
Bervoets et al. (2014)	Belgium (native and non-native)	81 (11 to 18 yrs)	35 vs. 37% vs. (28%)

Although our data supported that youth with a monophasic response are at higher risk for the type 2 diabetes, it is important to note that our data were cross-sectional and it is difficult to know whether those with a monophasic response will develop diabetes at either a higher or faster rate than those with a biphasic phenotype. If a monophasic response indicates a declining or insufficient β-cell response, it is physiologically reasonable to assume that those youth with a monophasic response will develop type 2 diabetes at either a higher rate or younger age (or both). Therefore, longitudinal data on the development of type 2 diabetes in younger populations should be analyzed in relation to the glucose response curve phenotypes and future studies focusing on the predictive

power of the shape of glucose response curve during the OGTT for identifying future type 2 diabetes are warranted.

In summary, the pattern of plasma glucose response during an OGTT may provide an early marker of type 2 diabetes risk in youth. We have demonstrated that participants with a biphasic response have significantly better β-cell function secondary to higher insulin sensitivity and secretion as well as lower glucose AUC and HbA1c. Moreover, our data suggest that the shape of the glucose curve may differentiate type 2 diabetes risk independent of obesity and before dysregulation of fasting or 2-hr glucose. Longitudinal studies to investigate whether glucose response phenotypes prospectively predict the development of type 2 diabetes in younger populations are warranted.

Acknowledgments

This work was supported by Health Research Alliance Arizona and the Center for Metabolic Biology at Arizona State University. Data management support was provided by Grant UL1-RR-024150 from the Mayo Clinic to use Research Electronic Data Capture (REDCap).

No potential conflicts of interest relevant to this article were reported.

J.Y.K. researched and analyzed data and wrote the manuscript. D.K.C. and L.J.M. researched data and reviewed and edited the manuscript. G.Q.S. researched data and wrote, reviewed, and edited the manuscript. G.Q.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study were presented in abstract form at the 71st Scientific Sessions of the American Diabetes Association, San Diego, California, 24–28 June 2011.

The authors are grateful to the children and their families who participated in this study. The authors thank Veronica Vital (Brookline College, Phoenix, AZ) and the staff of the Clinical Research Unit for their help with enrolling and testing participants.

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CHAPTER 3: ONE-HOUR GLUCOSE DURING AN ORAL GLUCOSE CHALLENGE PROSPECTIVELY PREDICTS β-CELL DETERIORATION AND PREDIABETES IN OBESE HISPANIC YOUTH

Joon Young Kim, Michael I. Goran, Claudia M. Toledo-Corral, Marc J. Weigensberg, Myunghan Choi, and Gabriel Q. Shaibi

Abstract

Objective

In adults, 1-hr glucose during an oral glucose tolerance test (OGTT) predicts the development of type 2 diabetes independent of fasting and 2-hr glucose concentrations. The purpose of the current investigation was to examine the utility of elevated 1-hr glucose levels to prospectively predict deterioration in β-cell function and the development of prediabetes in high-risk youth.

Research Design and Methods

Obese Latino youth with a family history of type 2 diabetes (133 male and 100 female; age 11.1 ± 1.7 years) completed a baseline OGTT and were divided into two groups based upon a 1-hr glucose threshold of 155 mg/dL (<155 mg/dL, n = 151, or ≥155 mg/dL, n = 82). Youth were followed annually for up to 8 years for assessment of glucose tolerance, body composition by dual-energy X-ray absorptiometry, and insulin sensitivity, insulin secretion, and the disposition index by the frequently sampled intravenous glucose tolerance test.

Results

Over time, the ≥155 mg/dL group exhibited a significantly greater decline in β-cell function compared with youth with a 1-hr glucose <155 mg/dL (β=-327.8 ± 126.2, P = 0.01). Moreover, this decline was independent of fasting or 2-hr glucose and body composition. When the data were restricted to only participants with normal glucose tolerance at baseline, a 1-hr glucose ≥155 mg/dL was independently associated with a 2.5 times greater likelihood of developing prediabetes during follow-up (95% CI 1.6–4.1, P = 0.0001).

Conclusions

These data suggest that a 1-hr glucose ≥155 mg/dL during an OGTT is an independent predictor of β-cell deterioration and progression to prediabetes among obese Latino youth.

Introduction

Once thought to be an adult disease, type 2 diabetes has emerged as an increasingly prevalent health condition in younger populations (Ogden, Flegal, Carroll, & Johnson, 2002). Estimates from the SEARCH for Diabetes in Youth Study suggest that the incidence rates of type 2 diabetes among adolescents are as high as 17.0–49.4/100,000 person-years and, among certain ethnic minority groups, may exceed rates of type 1 diabetes (Dabelea et al., 2007; Lawrence et al., 2009). Cohort studies of high-risk obese youth portray a more troubling picture where as many as 30% of these youth exhibit impairments in glucose regulation (Goran et al., 2004; Sinha et al., 2002). These data support the potential for a rapid progression to overt type 2 diabetes in youth, which may be exacerbated by pubertal insulin resistance (Goran & Gower, 2001; Gungor, Bacha, Saad, Janosky, & Arslanian, 2005). As such, identification of youth at highest risk

for premature type 2 diabetes is critical in order to initiate appropriate prevention strategies.

In 1997, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus introduced the term pre- diabetes to mean either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) to indicate intermediate stages in the natural history of type 2 diabetes (The expert committee on the diagnosis and classification of diabetes mellitus, 1997). However, prospective epidemiological studies in adults demonstrate the limitations of IFG and IGT in predicting risk, as only one-half of patients with prediabetes eventually convert to diabetes (Gerstein et al., 2007; Unwin, Shaw, Zimmet, & Alberti, 2002). These data are supported by pediatric studies where children and adolescents often vacillate between normal glucose tolerance (NGT) and prediabetes (Goran, Lane, Toledo-Corral, & Weigensberg, 2008; Weiss et al., 2005). Therefore, in addition to prediabetes, other markers may be necessary to accurately identify those at highest risk for developing type 2 diabetes.

Recently, 1-hr plasma glucose concentration during an oral glucose tolerance test (OGTT) has been shown to be an independent predictor of type 2 diabetes in adults. In a series of analyses, Abdul-Ghani, M., Abdul-Ghani, T., Ali, and DeFronzo, (2008), and Abdul-Ghani, Lyssenko, Tuomi, DeFronzo, and Groop (2009), and Abdul-Ghani, Williams, DeFronzo, and Stern (2007) found that a 1-hr glucose concentration of ≥155 mg/dL predicts the development of type 2 diabetes in two independent cohorts. Moreover, Abdul-Ghani et al. (2009) and Manco et al. (2010) found that 1-hr glucose of 155 mg/dL was a better predictor of type 2 diabetes than either fasting or 2-hr glucose concentrations yielding the maximal sum of sensitivity (0.75) and specificity (0.79).

Recently, Alyass et al. (2015) also supported previous findings as they reported the predictive power of 1-hr glucose (area under the receiver operating characteristic curve=0.80 in the Botnia study and 0.70 in the Malmo Prevention Project).

In addition, it is well established that a 1-hr glucose concentration of ≥155 mg/dL was associated with a high risk for (1) type 2 diabetes (Bardini, Dicembrini, Cresci, & Rotella, 2010; Bianchi et al., 2013; Cubeddu & Hoffmann, 2010; Joshipura, Andriankaja, Hu, & Ritchie, 2011; Marini et al., 2012), (2) metabolic syndrome (Cubeddu & Hoffmann, 2010), and (3) cardiovascular disease (Bianchi et al., 2013).

A recent cross-sectional study (Tfayli, Lee, Bacha, & Arslanian, 2011) of overweight/obese youth found that those with 1-hr glucose ≥155 mg/dL were more likely to exhibit IGT; however, independent of glucose tolerance status, those with 1-hr glucose ≥155 mg/dL exhibited lower insulin secretion relative to insulin sensitivity (i.e., disposition index [DI]) compared with those with 1-hr glucose<155 mg/dL. Unfortunately, the cross-sectional nature of that study limits the ability to draw predictive conclusions about the utility of this threshold over time. Given that conversion from prediabetes to overt type 2 diabetes in youth may occur rapidly (Weiss et al., 2005), the identification of sensitive and specific markers for type 2 diabetes is an important question that remains unanswered. Therefore, the purpose of this study was to examine whether a 1-hr glucose concentration ≥155 mg/dL can prospectively predict change in type 2 diabetes risk among high-risk youth. We tested the hypotheses that (1) obese youth with 1-hr glucose concentration ≥155 mg/dL exhibit a deterioration of β-cell function over time and (2) NGT obese youth with 1-hr glucose concentration ≥155 mg/dL have a greater likelihood of developing prediabetes over time.

Research Design and Methods

Data from 233 obese Latino children (133 male and 100 female; 11.1 ±1.7 years old at initial visit) who participated in the Study of Latino Adolescents at Risk (SOLAR) diabetes project at the University of Southern California (USC) were used in the present analysis. The SOLAR project is an ongoing longitudinal study in which participants are followed annually for determination of the natural history of type 2 diabetes in high-risk youth. To date, 201 participants had at least one follow-up visit, with some being followed for up to 8 years. Details of the study have previously been published (Goran et al., 2004). Briefly, children were required to meet the following study entry inclusion criteria: (1) age 8–13 years, (2) BMI ≥85th percentile for age and sex, (3) Latino ancestry (all four grandparents reporting to be Hispanic), and (4) a family history of type 2 diabetes (at least one parent, sibling, or grandparent). Participants were excluded if they were already diagnosed with type 1 or type 2 diabetes or if they were taking medications known to affect body composition or glucose homeostasis. Written informed consent and assent were obtained from parents and children, respectively. The institutional review board of the USC approved this study.

Outpatient Visit

Children arrived at the USC General Clinical Research Center (GCRC) at ~8:00 A.M. after an overnight fast. Weight and height were measured to determine BMI and BMI percentiles, waist circumference was assessed, and a physical examination including Tanner staging based on breast development in girls (Marshall & Tanner, 1969) and pubic hair in boys (Marshall & Tanner, 1970) was performed. A fasting sample was collected for determination of lipid profile (HDL, LDL, and VLDL, triglyceride, and total

cholesterol), and a 2-hr OGTT using a dose of 1.75 g glucose/kg body wt to a maximum of 75 g was performed. Blood samples were obtained at 0, 30, 60, and 120 min for determination of plasma glucose and insulin concentrations. Glucose tolerance was determined according to the American Diabetes Association (2010) as NGT (fasting glucose <100 mg/dL and 2-hr glucose <140 mg/dL), IFG (fasting glucose between 100 and 125 mg/dL), and IGT (2-hr glucose ≥140 mg/dL).

Inpatient Visit

Children were admitted to the GCRC for an overnight stay for determination of total body composition by dual-energy X-ray absorptiometry, body fat distribution by magnetic resonance imaging, and insulin sensitivity (SI) using an insulin- modified frequently sampled intravenous glucose tolerance test (FSIVGTT). Fasting samples were collected at 215 and 25 min prior to administration of glucose (25% dextrose, 0.3 g/kg body wt) at time 0. Subsequent blood samples were collected at time points 2, 4, 8, 19, 22, 30, 40, 50, 70, 100, and 180 min. Insulin (0.02 units/kg body wt, Humulin R [regular insulin for human injection]; Eli Lilly, Indianapolis, IN) was intravenously injected at 20 min. Values for glucose (glucose oxidase method Yellow Springs Instrument 2700 Analyzer; YSI, Yellow Springs, OH) and insulin (ELISA; Linco, St. Charles, MO) were entered into the MINMOD Millennium 2002 computer program (version 5.16) for determination of SI, insulin secretion using the acute insulin response (AIR), and DI as the product of SI and AIR (Bergman, Phillips, & Cobelli, 1981).

Statistical Analysis

Participants were divided into two groups based upon 1-hr glucose concentrations at their initial baseline visit (N = 233, <155 or ≥155 mg/dL). Independent-sample t tests

were used to compare anthropometry and body composition at baseline between the two groups (<155 group vs. ≥155 group). Baseline analysis included comparisons between groups for proportions of sex, Tanner stage, and prediabetes status using χ^2 tests and by ANCOVA for SI, AIR, and DI adjusting for age, sex, Tanner stage, body composition, and fasting and 2-hr glucose from the OGTT. Data that did not meet the assumptions for normality were $\log_{10}$ transformed; untransformed data are presented for ease of interpretation.

For longitudinal data analyses (n = 201), a hierarchical linear mixed model with a fixed-effects and a random-effects approach (Singer, 1998; West, 2009) was used to (1) evaluate the impact of 1-hr glucose ≥155 mg/dL at baseline on changes in DI over time and (2) estimate the main effects of group assignment (<155 vs. ≥155 group) after controlling for age, sex, Tanner stage, body composition, fasting and 2-hr glucose, and baseline DI on changes in DI over time. The grouping variable (<155 vs. ≥155 group) was modeled as a fixed predictor with adjustments made for the variation between individuals in the number of follow-up visits (i.e., random effects). In this model, "visit number" equals "follow- up years." β-Coefficients generated represent the unit changes of DI over time.

Generalized estimating equation model analysis (Zeger, Liang, & Albert, 1988) was used to predict the likelihood of developing prediabetes by group (<155 vs. ≥155 group) in only participants who were NGT at baseline (n = 125). Sequential models were developed to adjust for potential confounding effects of age, sex, Tanner stage, body composition, and fasting and 2-hr glucose. All data were analyzed using SPSS 20.0 with significance level set at $P \leq 0.05$.

Results

Cross-Sectional Analysis

Descriptive characteristics of the 233 participants at baseline were compared between those above or below 1-hr glucose of 155 mg/dL (Table 3-1). No differences in age, weight status (overweight vs. obese), or Tanner stage were noted. There was a significantly higher proportion of males in the <155 group compared with the ≥155 group (P = 0.007). Furthermore, prediabetes (IFG or IGT) was more commonly observed among those in the ≥155 group compared with those in the <155 group (P = 0.0002). Additionally, anthropometrics, lipids, and body composition and distribution measures were not different between groups.

Measures of glucose homeostasis and insulin dynamics from the baseline OGTT and FSIVGTT are presented in Table 3-1. Participants in the <155 group exhibited a healthier metabolic profile, as indicated by significantly lower HbA1c, 2-hr glucose, 2-hr insulin, area under the curve (AUC) for glucose and insulin, and higher DI compared with those in the ≥155 group. These differences persisted after adjustment for age, sex, Tanner stage, and body composition.

Longitudinal Analysis

A total of 201 participants had follow-up data and were included in the longitudinal linear mixed-model analysis. Participants were followed for up to 8 years (4.7 ± 2.7 years), accounting for a total of 1,145 observations. Those with 1-hr glucose ≥155 mg/dL at baseline exhibited a significantly lower β-coefficient for DI, indicating greater deterioration of β-cell function over time (model 1 [Table 3-2]).

Table 3-1. Characteristics of Participants by 1-hour Glucose at Study Entry

Variables	<155 (*n*=151)	≥155 (*n*=82)	*P*-value
Descriptive characteristics			
Sex (Male/Female)	96 (64%) / 55 (36%)	37 (45%) / 45 (55%)	0.007
Tanner stage			0.59
1	63 (42%)	33 (40%)	
2	45 (30%)	20 (25%)	
3	14 (9%)	7 (9%)	
4	18 (12%)	11 (13%)	
5	11 (7%)	11 (13%)	
Overweight/Obese	27 (18%) / 124 (82%)	12 (15%) / 70 (85%)	0.53
NGT/Prediabetes (IFG and/or IGT)	115 (76%) / 36 (24%)	42 (52%) / 39 (48%)	0.0002
Age (y)	11.1 ± 1.6	11.1 ± 1.8	1.00
BMI (kg/m^2)	28.9 ± 5.8	28.3 ± 4.8	0.52
BMI percentile (%)	97.1 ± 3.3	97.2 ± 2.9	0.82
Waist (cm)	89.7 ± 13.9	87.1 ± 12.2	0.19
SBP (mmHg)	109.4 ± 13.0	111.7 ± 11.7	0.18
DBP (mmHg)	62.5 ± 6.9	64.4 ± 6.2	0.04
SAAT (cm^2)	345.9 ± 157.4	333.1 ± 124.4	0.82
IAAT (cm^2)	49.8 ± 23.6	47.3 ± 17.6	0.56
Lean tissue mass (kg)	38.0 ± 10.3	35.8 ± 9.7	0.11
Fat mass (kg)	26.1 ± 11.0	24.1 ± 9.0	0.25
TAG (mg/dL)	110.3 ± 56.6	107.5 ± 61.3	0.57
HDL (mg/dL)	36.8 ± 8.8	38.3 ± 8.0	0.13
LDL (md/dL)	94.6 ± 21.9	93.4 ± 20.7	0.72
VLDL (mg/dL)	22.2 ± 11.3	21.5 ± 12.3	0.53
Cholesterol (md/dL)	153.5 ± 26.0	153.3 ± 26.0	0.96
HbA1c (%)	5.5 ± 0.3	5.6 ± 0.3	0.05
Fasting glucose (mg/dL)	89.3 ± 6.2	89.1 ± 6.4	0.85
1-hour glucose (mg/dL)	130 ± 15.9	171.5 ± 15.6	< 0.0001
2-hour glucose (mg/dL)	118.7 ± 15	132.8 ± 17.3	< 0.0001
Glucose AUC ($mg*dL^{-1}*h^{-1}$)	14948.3 ± 1266.1	17723.6 ± 1385.4	< 0.0001
Fasting insulin (μU/mL)	17.2 ± 10.2	15.7 ± 9.4	0.34
1-hour insulin (μU/mL)	161.4 ± 124.1	232.7 ± 149.6	0.02
2-hour insulin (μU/mL)	144.7 ± 129.4	186.7 ± 132.8	0.003
Insulin AUC ($\mu U*mL^{-1}*h^{-1}$)	17992.3 ± 11574.2	22248.8 ± 13173.7	0.003
SI ($\times 10^{-4} min^{-1}*\mu U*mL^{-1}$)	2.1 ± 1.5	2.1 ± 1.3	0.64
AIR (μU/mL)	1848.2 ± 1246.4	1572.9 ± 1292.7	0.03
DI ($\times 10^{-4} min^{-1}$)	2708.4 ± 1162.4	2321 ± 1034	0.006

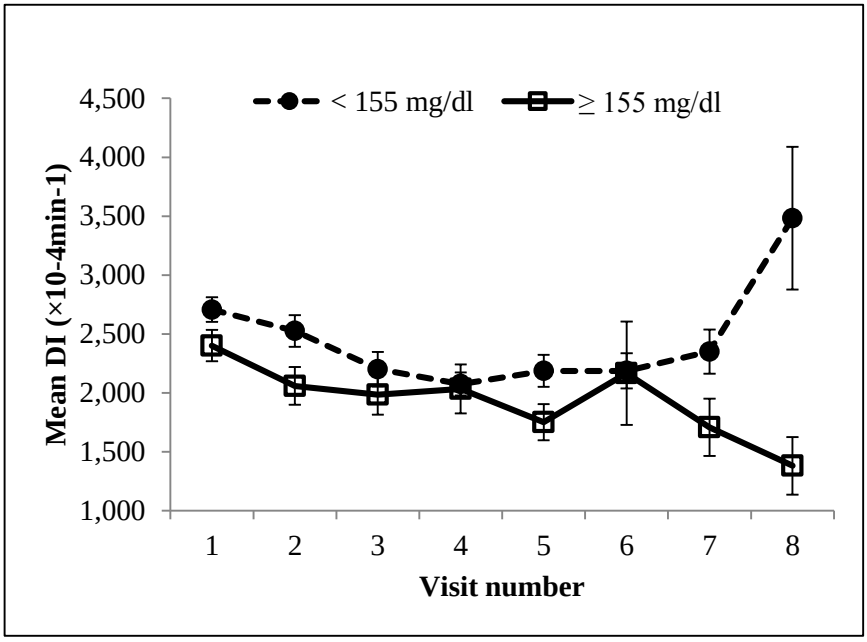
Data are means ± SD, n (%), NGT=normal glucose tolerance; IFG=impaired fasting glucose; IGT=impaired glucose tolerance; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; SAAT=subcutaneous abdominal adipose tissue; IAAT=intra-abdominal adipose tissue TAG=triglyceride; HDL=high-density lipoprotein; LDL=low-density lipoprotein; VLDL=very low-density lipoprotein; AUC=area under the curve; SI=insulin sensitivity; AIR=acute insulin response; DI=disposition index.

These findings persisted after age, sex, Tanner stage, body composition, and fasting and 2-hr glucose were con- trolled for (models 2–4 [Table 3-2]). The pattern of change for the ≥155 group was characterized by a steady decline in DI resulting in a 54.8% decrease by year 8. In contrast, the <155 group was characterized by an initial decrease followed by a subsequent increase in DI, which resulted in a 28.6% higher DI than that at baseline (Fig. 3-1)

Table 3-2. Linear Mixed Models of Disposition Index (DI) Over Time by One Hour Glucose at Baseline

Dependent Variable	Effect	β ± SE	*P*-value
Model 1:	Intercept	2078.5 ± 111.3	< 0.0001
DI (unadjusted)	1-hour glucose (<155)	341.5 ± 137.9	0.01
Model 2:	Intercept	3563.3 ± 370.2	< 0.0001
DI (adjusted)	1-hour glucose (<155)	279.5 ± 130.0	0.03
	Age	-53.4 ± 27.6	0.05
	Sex	-201.8 ± 133.6	0.13
	Tanner stage	-146.2 ± 47.8	0.002
Model 3:	Intercept	3957.2 ± 395.6	< 0.0001
DI (adjusted)	1-hour glucose (<155)	338.8 ± 126.6	0.008
	Age	24.9 ± 31.2	0.43
	Sex	-334.6 ± 155.7	0.03
	Tanner stage	-85.2 ± 57.6	0.14
	Lean tissue mass (kg)	-0.022 ± 0.008	0.008
	Fat mass (kg)	-0.018 ± 0.006	0.009
Model 4:	Intercept	5672.7 ± 747.2	< 0.0001
DI (adjusted)	1-hour glucose (<155)	327.8 ± 126.2	0.01
	Age	19.8 ± 31.2	0.53
	Sex	-373.7 ± 155.8	0.02
	Tanner stage	-83.8 ± 57.9	0.15
	Lean tissue mass (kg)	-0.022 ± 0.008	0.007
	Fat mass (kg)	-0.014 ± 0.006	0.03
	Fasting glucose (mg/dL)	-14.5 ± 6.9	0.04
	2-hour glucose (mg/dL)	-2.9 ± 2.2	0.19

Figure 3-1. Changes in Disposition Index (DI) Over Time in Below155 and Above 155



Hierarchical generalized estimating equations were used to examine the odds of developing prediabetes (IFG or IGT) by group among participants with NGT at baseline (n = 125; 747 total observations). NGT participants with 1-hr glucose concentrations ≥155 mg/dL at baseline were 2.54 times more likely to develop prediabetes over time (model 1 [Table 3-3]). These findings persisted after controlling for age, sex, Tanner stage, body composition, and fasting and 2-hr glucose concentrations (models 2–4 [Table 3-3]). Fifty-eight percent of those in the <155 group maintained NGT status throughout follow-up compared with only 28% of those in the ≥155 group (P = 0.004).

Table 3-3. Multivariable Adjusted Odds Ratios and 95% Confidence Intervals (CI) for Developing Prediabetes for NGT at Baseline

	Odds Ratio (95% CI)	*P*-value
Model 1		
<155	1	
≥155	2.5 (1.6- 4.1)	0.0001
Model 2[a]		
<155	1	
≥155	2.6 (1.6 - 4.2)	0.0001
Model 3[b]		
<155	1	
≥155	3.1 (1.9 - 4.9)	< 0.0001
Model 4[c]		
<155	1	
≥155	2.4 (1.4 - 4.2)	0.0015

[a]Model 2 adjusted for age, sex, and Tanner stage
[b]Model 3 adjusted for age, sex, Tanner, stage, lean tissue mass and fat mass
[c]Model 4 adjusted for age, sex, Tanner, stage, lean tissue mass, fat mass, fasting glucose and 2-hour glucose.

Conclusions

In the current study, we demonstrate that a 1-hr glucose concentration during an OGTT differentiates diabetes risks and prospectively predicts deterioration in β-cell function and progression to prediabetes among obese Latino youth. These data extend previous cross- sectional studies in youth and support the potential prospective utility of 1-hr glucose concentrations during an OGTT to identify youth at highest risk for developing type 2 diabetes. Furthermore, these findings are independent of traditional risk factors for type 2 diabetes.

Longitudinal epidemiological studies in adults (Abdul-Ghani et al., 2008; Abdul-

Ghani et al., 2009; Abdul-Ghani et al., 2007) have established a cutoff value (155 mg/dL) for 1-hr plasma glucose concentration during an OGTT as a strong, independent predictor of type 2 diabetes. Abdul-Ghani et al. (2009) reported that the rate of conversion to diabetes over 8 years was significantly greater in NGT participants with 1-hr glucose concentrations ≥155 mg/dL compared with individuals whose 1-hr glucose concentration did not exceed 155 mg/dL (8.5 vs. 1.3%). Further- more, the predictive ability of 1-hr glucose concentrations was significantly stronger than either fasting or 2-hr glucose levels. The authors suggested that, while individuals with NGT are typically considered at low risk for the development of type 2 diabetes, a subgroup of those reaching a 1-hr threshold of 155 mg/dL during an OGTT may be at increased risk for future type 2 diabetes. Although the specific threshold identified by Abdul-Ghani et al. has been confirmed in two separate cohorts, others have identified alternative 1-hr glucose thresholds that may confer increase risk for type 2 diabetes. In a cross-sectional analysis, Manco et al. (2010) identified 161 mg/dL as a 1-hr threshold for differentiating type 2 diabetes risk factors including IGT, insulin resistance, and β-cell dysfunction among European adults. Only two cross-sectional studies in the pediatric population have tested the utility of 1-hr glucose concentration during an OGTT to identify diabetes risk (Manco et al., 2012; Tfayli et al., 2011). Tfayli et al. (2011) examined a biracial group (African American and Caucasian) of over- weight and obese youth and found that, independent of adiposity and glucose tolerance status, children with 1-hr glucose concentration ≥155 mg/dL exhibited 41% lower DI compared with those with a 1-hr glucose value below this threshold. A second cross-sectional study in youth by Manco et al. (2012) used receiver operating characteristic analysis to try to establish and validate the best 1-hr glucose

threshold for identifying diabetes risk. The authors reported that a cutoff value of 132.5 mg/dL identified IGT with 80.8% sensitivity and 74.3% specificity. Both of the aforementioned pediatric studies used cross-sectional designs, which have inherent limitations that are exacerbated by growth-related changes in children and adolescents. The present findings extend these previous studies to show that a 1-hr glucose concentration of ≥155 mg/dL does indeed predict diabetes risk over time and that the predictive ability is independent of other known risk factors. Of interest, when we modeled 1-hr glucose based on the threshold identified by Manco et al. (132.5 mg/dL), we observed a significant association with changes in DI that was similar in magnitude to the effect for the 155 threshold (β = -329.1, P = 0.02). However, this threshold was not associated with increased odds of developing prediabetes in our cohort (odds ratio 1.5, P = 0.19). It is plausible that population variation in terms of age, sex, or race/ethnicity may impact the predictive utility of various thresholds, as these factors have been shown to affect diabetes risk in youth (Goran & Gower, 2001; Lewy, Danadian, Witchel, & Arslanian, 2001; Goran, Bergman, Cruz, & Watanabe, 2002).

Little is known about the natural history of type 2 diabetes in youth. Most studies to date examining the pathophysiology of type 2 diabetes in youth have been cross-sectional in nature. Similar to findings in adult studies (Bergman, Ader, Huecking, & Van Citters, 2002; Weyer, Bogardus, Mott, & Pratley, 1999), β-cell dysfunction is thought to be a key feature in the development of type 2 diabetes (Elder, Woo, & D'Alessio, 2010; Gungor et al., 2005). Using cross-sectional data from this cohort, we previously observed that both IFG and IGT were associated with impaired β-cell function (Goran et al., 2004; Weigensberg, Ball, Shaibi, Cruz, & Goran, 2005). Furthermore, recent studies suggest

that obese youth with glucose levels toward the upper limit of the normal range (i.e., fasting glucose between 90 and 100 mg/dL and 2-hr glucose between 120 and 140 mg/dL) exhibited lower β-cell function compared with youth whose fasting and 2-hr glucose concentrations are <90 mg/dL and 120 mg/dL, respectively (Burns et al., 2011; Tfayli, Lee, & Arslanian, 2010). These findings have been confirmed longitudinally (Giannini et al., 2012), where obese NGT youth with 2-hr glucose concentrations between 120 and 139 mg/dL exhibited a significantly greater likelihood of developing IGT than obese NGT youth with 2-hr glucose levels between 100 and 119 mg/dL (42 vs. 21%, respectively). Collectively, these reports support impaired β-cell function as an important pathophysiologic process underlying prediabetes and overt diabetes in youth. The current results build upon these previous findings to indicate that independent of fasting or 2-hr glucose levels, a higher 1-hr glucose concentration is associated with β-cell dysfunction and the development of prediabetes.

Although it remains unclear whether the primary defect underlying type 2 diabetes in youth is related to insulin action or secretion, using β-cell function may offer the most robust risk measure. Recent studies in adults suggest that early defects in insulin secretion play a pivotal role in the pathophysiology of type 2 diabetes (Abdul-Ghani, Jenkinson, Richardson, Tripathy, & DeFronzo, 2006). A large prospective study reported that the impairment of first-phase insulin secretion (measured by the insulinogenic index during an OGTT) is a common characteristic of both IFG and IGT. Similarly, recent studies in youth (Bacha, Lee, Gungor, & Arslanian, 2010; Weiss et al., 2005) suggest that obese adolescents with prediabetes (IFG or IGT) exhibit primary defects in insulin secretion (commonly in first- phase insulin secretion) rather than insulin resistance.

However, these studies focused exclusively on obese adolescents who presumably already had some degree of insulin resistance. It is possible that higher 1-hr glucose reflects impairments in the first- phase insulin secretion and that elevation in 2-hr glucose reflects second- or late-phase insulin secretion. Our cross-sectional results suggest that differences in DI between the ≥155 group and the <155 group were the result of insulin secretion rather than SI, as the latter was not different between groups. If we model our longitudinal data with either SI or insulin secretion as the dependent variable, secretion rather than sensitivity appears to be the differentiating factor between groups over time. Independent of the mechanism, our data suggest that 1-hr glucose concentrations of at least 155 mg/dL during an OGTT may identify children at high risk for developing type 2 diabetes and who could benefit from focused and intensive prevention efforts. Moreover, the predictive ability of 1-hr glucose was independent of fasting markers of diabetes risk including IFG or an HbA1c ≥5.7%. Given that pediatricians often have to make clinical decisions about patients based upon a single visit, including a 1-hr glucose measure during a standard 2-hr OGTT may help identify those in need of more aggressive or closer follow-up.

To our knowledge, this was the first longitudinal study in youth to examine the threshold of 1-hr glucose concentration (155 mg/dL) in relation to changes in type 2 diabetes risk and development of prediabetes over time. We focused on a high-risk cohort, assessed diabetes risk using robust measures of insulin sensitivity and secretion from the FSIVGTT to estimate β-cell function, controlled for the potential confounding effects of maturation and body composition, and used powerful statistical modeling techniques to account for the variance component across time. Despite these strengths,

we acknowledge potential limitations that should be considered. First, we analyzed the data based on a single OGTT at baseline. Libman, Barinas-Mitchell, Bartucci, Robertson, and Arslanian (2008) demonstrated poor reproducibility of the OGTT in over- weight youth, with 2-hr glucose being less reproducible than fasting glucose. It would be worthwhile to examine whether the reproducibility of 1-hr glucose more closely resembles that of fasting or 2-hr measures and whether repeated measures of 1-hr glucose ≥155 mg/dL are more consistently associated with diabetes risk than is repeated IFG or IGT status. Second, given the longitudinal nature of the study, not all participants were available for every year of testing, so controlling for missing data by linear mixed modeling was necessary. Third, owing to the low conversion rate to overt type 2 diabetes, we opted to focus on changes in diabetes risk factors (β-cell dysfunction and prediabetes). There were only three cases of newly diagnosed type 2 diabetes over time (2 from the 1-hr glucose <155 group and 1 from the 1-hr glucose ≥155 group for overall conversion rates of 1.3% and 1.2%, respectively). The low overall conversion rate renders it difficult to make any reliable comparisons between two groups. When we separately examined the development of IFG and/or IGT over time, the results are outlined in Table 3-4.

Table 3-4. Change of Glycemic Status During The Follow-up Periods

Within NGT at baseline (*N*=125)	Maintain NGT n (%)	IFG n (%)	IGT n (%)	IFG + IGT n (%)	Total Prediabetes n (%)
Below 155 (*n*=93)	54 (58%)	10 (11%)	14 (15%)	15 (16%)	39 (42%)
Above 155 (*n*=32)	9 (28%)	5 (16%)	13 (40%)	5 (16%)	23 (72%)

Future studies will need to recruit much larger cohorts followed over longer periods to definitively test the utility of 1-hr glucose concentrations to predict the development of overt diabetes in youth. Lastly, we applied a single cutoff point of 1-hr glucose based upon adult studies to prospectively identify changes in diabetes risk factors. Future studies should use receiver operating characteristic analysis to identify the maximum sensitivity and specificity of a 1-hr glucose concentration to predict the development of type 2 diabetes across representative pediatric populations. These studies will not only allow for optimization of the best 1-hr glucose threshold but may also be used to compare the predictive power of this risk marker with other established diabetes risk factors such as fasting and postchallenge glucose concentrations as well as HbA1c.

In summary, a glucose concentration ≥155 mg/dL at 1-hr during an OGTT may be an early independent marker of future type 2 diabetes risk as measured by deterioration in β-cell function and progression to prediabetes in overweight and obese Latino youth with a family history of type 2 diabetes.

Acknowledgments

This study was sup- ported by National Institutes of Health Grant R01-DK-59211 (to M.I.G.) and by the GCRC, National Center for Research Resources Grant MO1-RR-00043.

No potential conflicts of interest relevant to this article were reported.

J.Y.K. analyzed data and wrote the manuscript. M.I.G., C.M.T.-C., and M.J.W. re- searched data and reviewed and edited the manuscript. M.C. analyzed data and reviewed and edited the manuscript. G.Q.S. researched data, reviewed and edited the manuscript, and assisted in writing the manuscript. G.Q.S. is the guarantor of this work

and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study were presented in abstract form at the 72nd Scientific Sessions of the American Diabetes Association, Philadelphia, Pennsylvania, 8–12 June 2012.

The authors are grateful to the children and their families who have participated in the USC SOLAR Diabetes Project. The authors thank the staff of the GCRC for their help with enrolling and testing participants.

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CHAPTER 4: COMPARING GLYCEMIC INDICATORS OF PREDIABETES: A PROSPECTIVE STUDY OF OBESE LATINO YOUTH

Joon Young Kim, Michael I. Goran, Claudia M. Toledo-Corral, Marc J. Weigensberg, and Gabriel Q. Shaibi

Abstract

Objective

One hour (1-hr) glucose during an oral glucose tolerance test (OGTT) is an emerging biomarker for type 2 diabetes. We compared the predictive power of 1-hr glucose to traditional glycemic markers for prospectively identifying prediabetes in youth.

Research Design and Methods

Obese normoglycemic Latino youth (N = 116) were assessed at baseline for glycated hemoglobin (HbA1c), fasting, 1-hr, and 2-hr glucose during an OGTT and were followed for up to 8 yr for the development of prediabetes. Receiver operating characteristic (ROC) curves were used and a multivariable prediction model was developed.

Results

The area under the 1-hr glucose ROC curve was the most powerful predictor of prediabetes over time [0.73, 95% confidence interval (CI) = 0.64 – 0.83]. However, combining all indicators into a single model was superior to individual marker models (0.77, 95% CI = 0.690.86). Conclusions: These results further support the utility of 1-hr

glucose during an OGTT as a prospective marker of diabetes risk in youth.

Introduction

Early identification of youth at greatest risk for developing type 2 diabetes is a critical step for delivering targeted prevention strategies. In addition to traditional markers of hyperglycemia [glycated hemoglobin (HbA1c), fasting glucose, and 2-hr glucose following an oral glucose tolerance test (OGTT)], several recent studies have shown that 1-hr glucose is an emerging biomarker for type 2 diabetes risk in children (Kim et al., 2013; Manco et al., 2012; Tfayli, Lee, Bacha, & Arslanian, 2011) and adults (Abdul-Ghani, M., Abdul-Ghani, T., Ali, & DeFronzo, 2008; Abdul-Ghani, Lyssenko, Tuomi, DeFronzo, & Groop, 2009; Abdul-Ghani, Williams, DeFronzo, & Stern, 2007; Manco et al., 2010). Recently, Alyass et al. (2015) compared the predictive power 1-hr plasma glucose with traditional risk factors for future type 2 diabetes. They revealed that 1-hr glucose concentraion from the OGTT showed fair/good predictive power (area under the receiver operating characteristic [ROC] curve=0.80), indicating that it was more powerful than HbA1c (area under the ROC curve=0.69). To our knowledge there were no longitudinal studies in younger population. Therefore, the purpose of this study was to prospectively compare the predictive power of 1-hr glucose to HbA1c, fasting, and 2-hr glucose for identifying progression to prediabetes in obese Latino youth. In addition, we combined predictors into a multivariate model to examine whether combining indicators improved the predictive power.

Research design and methods

Participants

Baseline data from 116 overweight/obese [body mass index (BMI) for age and

sex ≥85th percentile] Latino youth with a family history of type 2 diabetes (67 male/49 female; 11.5 ± 1.9 yr old) who participated in the USC Study of Latino Adolescents at Risk (SOLAR) diabetes project were used in this study. Data were restricted to participants with normal glucose tolerance (NGT) at baseline who were followed for at least 1 yr and up to 8 yr. The institutional review board of the USC approved this study.

Normoglycemic vs. Dysglycemic

Two groups were created based upon glycemic status during follow-up. Participants in the normoglycemic group maintained their NGT status over time while participants in the dysglycemic group met the American Diabetes Association (2010) criteria for prediabetes during at least one follow-up visit. Participants whose glycemic status fluctuated between NGT and pre- diabetes across follow-up periods (intermittent prediabetes), were classified as dysglycemic and data from their latest visit corresponding to a prediabetic state were used for analysis.

Measurements

Participants completed a fasting blood draw followed by a standard 2-hr OGTT. HbA1c as well as fasting, 1- hr, and 2-hr plasma glucose obtained during the OGTT was analyzed at the Los Angeles County-USC Medical Center Core Laboratory with the Hexokinase method (Dade Behring, Deerfield, IL, USA). HbA1c was measured by high-performance liquid chromatography (model 11c 2.2 HLC-723; Tosoh, Tokyo, Japan).

Statistical Analysis.

Independent sample *t*-tests and chi-square analysis were used to compare clinical features at baseline between glycemic groups. Non-normally distributed data were $\log_{10}$ transformed. PASW 20.0 statistical software package was used. Receiver operating

characteristic (ROC) curves were used to estimate the predictive power (i.e. area under the ROC curve) of each glycemic indicator to identify progression to prediabetes over time. In addition, a complete multivariable prediction model combining all glycemic indicators was developed and compared against the individual models. The algorithm developed by DeLong, E., DeLong, D., and Clarke-Pearson (1988) was used to compare the areas under the ROC curves. Statistical analyses related to predictive power of parameters were performed with the sas statistical software package.

Results

Of the 116 overweight/obese normoglycemic Latino youth at baseline, 57 participants (49.1%) experienced progression to prediabetes whereas 59 participants (52.9%) maintained their NGT status throughout follow-up. Among the dysglycemic group, 10 exhibited persistent prediabetes, 10 exhibited intermittent dysglycemia and were prediabetic at their last visit, and 37 exhibited prediabetes at least one time but reverted back to NGT at the last visit. No significant baseline differences in age, sex, BMI, waist circumference, visceral fat, total body fat mass, or HbA1c were noted between two groups. However, participants in the dysglycemic group exhibited significantly higher plasma glucose level at fasting (89.4±4.7 vs. 86.8±4.8mg/dL), 1- hr (149.8 ± 20.9 vs. 132.1 ± 21.2 mg/dL) and 2-hr (120.4 ± 12.3 vs. 114.2 ± 11.3 mg/dL), time points during the OGTT (all $P < 0.01$).

The comparison of area under the ROC curve was used to identify the model with the highest predictive powerful for prospectively identifying progression to prediabetes based on baseline glycemic measures (Table 4-1). Of the individual glycemic indicators, the highest predictive power was for 1-hr glucose (73.4%), followed by fasting glucose

(66.9%), 2-hr glucose (63.6%), and HbA1c (58.1%). Adding BMI as a covariate to each model had no effect on predictive power. When predictive powers were compared pair-wise, only 1-hr glucose and HbA1c were statistically different ($P < 0.05$). The area under the ROC curve for the complete model (HbA1c, fasting, 1-hr, and 2-hr glucose) was significantly better than any of the individual models for predicting progression to prediabetes with the exception of that for 1-hr glucose (77.0% vs. 73.0%, P = 0.26).

Table 4-1. Comparing Areas Under The ROC Curve Among Individual Parameters and A Multivariate Prediction Model

Parameter	ROC AUC	95 % CI	Compared to 1-hr glucose *P*-value	Compared to Full model[a] *P*-value
Fasting glucose	0.67	0.57 - 0.77	0.35	0.03
1-hr glucose	0.73	0.64 - 0.83	-	0.26
2-hr glucose	0.64	0.53 - 0.74	0.12	0.01
HbA1c	0.58	0.48 - 0.69	0.04	< 0.01
Full model [a]	0.77	0.69 - 0.86	0.26	-

[a] Full model: HbA1c, fasting, 1-hr, and 2-hr glucose
ROC, Receiver Operator Characteristic; AUC, Area Under the Curve; CI, Confidence Interval.

Conclusions

In the current longitudinal study, we showed that 1-hr glucose is the single best predictor for identifying future prediabetes among normoglycemic overweight/obese Latino youth. However, combining indicators into a multivariate model that included HbA1c, fasting, 1-hr, and 2-hr glucose was superior to any individual model.

Although standard clinical glycemic indicators (i.e. HbA1c, fasting, and 2-hr glucose) are currently used for the identification of prediabetes and diagnosis of type 2 diabetes, longitudinal epidemiological studies in adults show that these indicators do not

optimally predict future risk of type 2 diabetes (Gerstein et al., 2007; Unwin, Shaw, Zimmet, & Alberti, 2002). In addition, the utility of HbA1c as a screening tool for diabetes risk in youth remains controversial (Chan et al., 2014; Love-Osborne et al., 2013). For these reasons, studies continue to search for better biomarkers and/or models in youth and adults (Reinehr et al, 2009; Stern, Williams, & Haffner, 2002). Recent studies in adults support the utility of 1-hr glucose during an OGTT for identifying type 2 diabetes risk and Abdul-Ghani et al. verified that the predictive ability of 1-hr glucose was significantly better than traditional glycemic markers (Abdul-Ghani et al., 2008; Abdul-Ghani et al., 2009; Abdul-Ghani et al., 2007).

In the pediatric population, Tfayli et al. (2011) confirmed the previous findings in overweight/obese African American and Caucasian youth. Our group also reported that Latino youth with a higher 1-hr glucose exhibited greater deterioration of β-cell function and higher risk for developing prediabetes over time (Kim et al., 2013). Although both of the aforementioned studies tested the utility of 1-hr glucose for identifying type 2 diabetes risk, no study has compared this marker to traditional markers for predicting diabetes risk in youth.

To our knowledge, this is the first longitudinal study in youth to examine the predictive power of 1-hr glucose during the OGTT for predicting future prediabetes. We focused on Latino overweight/obese Latino youth with a family history of type 2 diabetes who experience disproportionate risk for developing type 2 diabetes relatively early in life. Our data support the utility of 1-hr glucose concentrations as a useful predictor for type 2 diabetes risk and suggest that this novel marker may be more powerful than traditional glycemic indicators. Interestingly, the complete multivariate model that

included fasting, 1-hr, 2-hr glucose, and HbA1c was not statistically better than 1-hr glucose alone. All other indicators were statistically less powerful than the combined model. When additional indicators (age, sex, BMI, visceral fat, total fat mass, fasting insulin, and 1-hr insulin) were added to the complete multivariate glycemic model the predictive power was slightly increased, but it was not significantly different (0.81 vs. 0.77, P = 0.10).

There are potential limitations of our study. Given the longitudinal nature of the study, not all participants were available for every year of testing. We attempted to define dysglycemia as those participants who experienced at least one instance of a prediabetes over time (Table 4-2). We acknowledge that this approach may overestimate the true incidence of prediabetes as the OGTT is not always reproducible and 65% of youth in the dysglycemic group did indeed convert back to NGT in subsequent years.

Table 4-2. Classification of Dysglycemic

	Baseline visit	Follow-up visits	Last visit	# of participants	Classification
Case 1	NGT	Prediabetes	Prediabetes	10 (17.5%)	Dysglycemic
Case 2	NGT	NGT	Prediabetes	10 (17.5%)	Dysglycemic
Case 3	NGT	Prediabetes	NGT	37 [20]* (65%)	Dysglycemic

Note. Case 2 and 3 are classified as participants who had intermittent prediabetes. In Case 3, latest visit (among "Follow-up visits") corresponding to a pediabetic state was used for our analysis. *Of 37 participants in the Case 3, while 17 youth experienced one instance of a prediabetes over time, 20 participants exhibited more than 2 times of development of prediabetes before the last visit (even though their glycemic status were reverted back to NGT at the last visit).

However, given that previous research in obese youth suggests that youth with intermittent prediabetes may be at greatest metabolic risk (Libman, Barinas-Mitchell, Bartucci, Robertson, & Arslanian, 2008), we included these youth as dysglycemic in our analyses. Longer follow-up periods and larger samples are needed to (1) definitively test

the predictive power of 1-hr glucose compared with traditional glycemic markers, and (2) identify a threshold for 1-hr glucose that best discriminates youth at highest risk for developing type 2 diabetes. In addition, the complex nature of type 2 diabetes warrants that other potential risk factors such as ethnicity, family history of diabetes, degree of obesity, or pubertal development should be considered in these analyses (Ek, Rossner, Hagman, & Marcus, 2014; Reiner et al., 2009).

In conclusion, this study highlights 1-hr glucose concentrations during an OGTT as an important biomarker of diabetes risk in youth. Among individual glycemic indicators, 1-hr glucose exhibited the highest predictive power for identifying future prediabetes.

Acknowledgements

We are grateful to the children and their families who have participated in the USC SOLAR Diabetes Project. We thank the staff of the GCRC for their help with enrolling and testing participants. This study has been supported by National Institutes of Health Grant R01-DK-59211 to Dr. Michael I. Goran and General Clinical Research Center, National Center for Research Resources Grant MO1-RR-00043.

J. Y. K. analyzed data and wrote the manuscript. M. I. G. researched data and reviewed/edited the manuscript. M. J. W. researched data and reviewed/edited the manuscript. C. T.-C. researched data and reviewed/edited the manuscript. G. Q. S researched data and reviewed/edited the manuscript, assisted in writing the manuscript. G. Q. S. is the guarantor of this work and, as such, takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study were presented in abstract from at the 73rd Scientific Sessions

of the American Diabetes Association, Chicago, IL, 21–25 June 2013.

Conflict of interest

The authors have declared no conflicting interests.

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CHAPTER 5: ASSOCIATION OF GENETIC VARIANTS FOR SUSCEPTIBILITY TO TYPE 2 DIABETES WITH NOVEL GLYCEMIC MARKERS IN LATINOS

Joon Young Kim, Dawn K. Coletta, Elena A. De Filippis, Lawrence J. Mandarino, and Gabriel Q. Shaibi

Abstract

Objective

Type 2 diabetes has emerged as a critical public health concern in youth and adults. Despite significant progress in identifying the genetic causes on type 2 diabetes, data related to new promising genes such as KQT-like subfamily member (KCNQ1) are lacking. Given that novel type 2 diabetes risk markers including glucose response curve and 1-hr glucose level were verified in both youth and adults, the purpose of our study was to examine whether these novel type 2 diabetes biomarkers are associated with common 59 SNPs (representing 31 genes including KCNQ1) in the Latino population.

Research Design and Methods

Data from the Arizona Insulin Resistance registry were used in this study. Metabolic, anthropometric, demographic and medical history information were obtained from 667 Latino youth and adults. Genotypes and cardiometabolic phenotypes were analyzed to examine the associations of genetic variants of interested susceptibility gene with phenotypes (i.e., adiposity, lipid profile, glycemic parameters, and measures of insulin dynamics) using software package Sequential Oligogenic Linkage Analysis Routines.

Results

Among 59 common SNPs, 15 SNPs (representing 8 genes/loci) were associated with the glucose response curve and 18 genetic variants (representing 9 genes/loci) were associated with 1-hr glucose. In both novel markers, rs11605924/CRY2 exhibited that the major allele was significantly associated with increased prevalence of the monophasic response and an elevated 1-hr glucose concentration. These findings indicate that these major alleles are risk alleles for early indicators of type 2 diabetes. The minor allele of rs4766415 and rs10735003/ADIPOR2 showed a significant association with increased prevalence of the monophasic response and an elevated 1-hr glucose level, indicating that these minor alleles were considered as risk alleles. For the KCNQ1 SNPs (n=3), fasting and 2-hr glucose, prediabetes, Matsuda Index, insulinogenic Index, and disposition index were significantly associated with both SNPs (rs151290 and rs2237892). SNP rs2237892 was significantly associated with 1-hr glucose concentration.

Conclusions

This study found that there were significant associations between KCNQ1 SNPs (rs151290 and rs2237892) and diabetes-related phenotypes. In addition, genetic associations of novel glycemic markers found multiple genetic variants that were associated with either the glucose response curve or 1-hr glucose level from an OGTT.

Introduction

Obesity has reached epidemic proportions in the US with approximately 32% of adolescents and two-thirds of adults classified as either overweight or obese (Ogden, Carroll, Kit, & Flegal, 2014). In the context of a widespread obesity epidemic, the burden of metabolic abnormalities (i.e., metabolic syndrome and type 2 diabetes) is of clinical

and public health concern in both youth and adults (Fagot-Campagna, 2010; Ford, Li, & Zhao, 2010). Due to the complex nature of metabolic impairments, the avenues by which type 2 diabetes has been studied are varied. In addition to environmental factors such as diet or exercise (Ershow, 2009), there is compelling evidence that genetic factors are also involved in the development of type 2 diabetes. To date, genome-wide association studies (GWAS) and large-scale meta-analysis have identified more than 60 single nucleotide polymorphisms (SNPs) that are associated with type 2 diabetes risk (Brunetti, Chiefari, & Foti, 2014; Dupuis et al., 2010; McCarthy, 2010; Saxena et al., 2012). However, these SNPs have shown modest effect sizes and collectively explain only 10% for the variance in type 2 diabetes risk (McCarthy & Zeggini, 2009; Morris et al., 2012; Imamura & Maeda, 2011; Voight et al., 2010). For this reason, ongoing efforts are needed to further identify genetic variants in relation to type 2 diabetes risk (Brunetti et al., 2014; Grarup, Sandholt, Hansen, & Pedersen, 2014; Thomsen & Gloyn, 2014).

It is important to note that, in addition to case-control analyses (Prokopenko, McCarthy, & Lindgren, 2008; Saxena et al., 2012, Scott et al., 2007; Zeggini et al., 2008), most susceptibility genes are examined in relation to traditional type 2 diabetes risk factors such as fasting and 2 hour (2-hr) glucose during an oral glucose tolerance test (OGTT) (Dupuis et al., 2010; Ingelsson et al., 2010; Saxena et al., 2010). However, longitudinal studies in adults demonstrated that these traditional type 2 diabetes risk markers do not optimally predict future development of type 2 diabetes (Gerstein et al., 2007; Unwin, Shaw, Zimmet, & Alberti, 2002). In order to more accurately identify individuals at highest risk for type 2 diabetes, clinical studies continue to search for novel biomarkers (Abdul-Ghani, Williams, DeFronzo, & Stern, 2007; Stern, Williams, &

Haffner, 2002). Our group observed that two different phenotypes of glucose response (mono- vs. bi-phasic) emerge by plotting glucose concentrations during an OGTT (Kim, Coletta, Mandarino, & Shaibi, 2012). Moreover, compared to a monophasic response, adults who exhibit a biphasic response may be at lower risk for developing type 2 diabetes as evidenced by Abdul-Ghani, Lyssenko, DeFronzo, and Groop (2010). In addition to this marker, we also observed that one hour (1-hr) glucose predicts the development of prediabetes and β-cell dysfunction among obese Latino youth (Kim et al., 2013). Moreover, 1-hr glucose may be more powerful than traditional glycemic indicators including fasting and 2-hr glucose for predicting type 2 diabetes risk (Kim, Goran, Toledo-Corral, Weigensberg, & Shaibi, 2014). Overall, our data suggest that the glucose response curve and 1-hr glucose concentration during an OGTT represent novel biomarkers for type 2 diabetes risk that are independent of traditional type 2 diabetes risk factors (Kim et al., 2012, 2013, 2014).

To our knowledge, no studies have examined genetic determinants for the glucose response curve or 1-hr glucose. Although we recently have replicated GWAS SNPs (n=28) that are related to type 2 diabetes risk in a Latino population (DeMenna et al., 2014), we did not examine genetic association with these emerging biomarkers. Collectively, more candidate SNPs along with non-traditional phenotypic markers for dysglycemia are critical to expand the current level of understanding of the genetic contributions to type 2 diabetes. Since these novel markers are associated with differential physiological risk in terms of insulin action and secretion, it is necessarily to identify unique SNPs in relation to insulin dynamics. In addition to our candidate SNPs study (DeMenna et al., 2014), we currently have included genetic variants in the

potassium voltage-gated channel, KQT-like subfamily member 1 (KCNQ1) that exhibit relatively higher effect size compared to other candidate SNPs in other ethnicities (Hu et al., 2009; Qi et al., 2009; van Vliet-Ostaptchouk et al., 2012). This gene is mainly expressed in a number of tissues, including heart, pancreas, kidneys, and intestine (Unoki et al., 2008; Yasuda et al., 2008) and thought to be related to β-cell dysfunction by regulating either glucose-stimulated (Ullrich et al., 2005) or incretin-stimulated insulin secretion (Mussig et al., 2009; Vallon et al., 2005). However, only two studies examined KCNQ1 SNP (rs2237892) in the Mexican population and exhibited significant association with susceptibility to type 2 diabetes (Gamboa-Melendez et al., 2012; Parra et al., 2011), while approximately 90% of genetic studies were performed among East Asian or Caucasian (Liu et al., 2013). Given that Latinos are disproportionately impacted by obesity and type 2 diabetes (Lawrence et al., 2009), further genetic studies related to KCNQ1 are warranted in this high-risk population.

Therefore, the overall purpose of this study was to examine the associations of KCNQ1 genetic variants with traditional and novel glycemic biomarkers (i.e., glucose response curve and 1-hr glucose level) in the Arizona Insulin Resistance (AIR) registry. Specific aims are two-fold: 1) to determine the association between KCNQ1 SNPs (rs2237892; rs2237895; rs151290) and traditional diabetes-related risk factors (i.e., adiposity, lipid profile, fasting glucose, HbA1c, and OGTT-derived insulin release and resistance measures) in 667 Latino population, and 2) to examine whether the novel biomarkers (i.e., glucose response curve and 1-hr glucose) are associated with 59 candidate SNPs including KCNQ1 SNPs from the AIR registry.

Research Design and Methods

Participants

A total of 667 Latino children and adults (aged 7-84 years) were enrolled with 97% consenting to the AIR registry. The primary purpose of this registry project was to examine cardiometabolic disease risk in the Latino community of Phoenix, Arizona (Shaibi, Coletta, Vital, & Mandarino, 2013). Of the 667 participants enrolled in the study, 365 were distributed across 92 families from the AIR registry. The 365 participants from 92 families generated 723 relative pairs that were distributed across fourteen relative-pair categories. The remaining 302 participants are represented as single individuals. Consent was obtained for banking of serum, DNA, and RNA for the examination of molecular mechanisms underlying type 2 diabetes.

Phenotypic Characterization and Measurements

Brief Medical History and Physical Examination. Participants arrived at the Arizona State University Clinical Research Unit after an overnight fast followed by screening of their medical history. Anthropometric measurements included height, weight, body mass index (BMI), hip and waist circumference (HC and WC, respectively), and systolic and diastolic blood pressure (SBP and DBP, respectively). Body composition (fat mass) was determined via bioelectrical impedance.

Metabolic Testing. The study included an OGTT in individuals with no known history of type 2 diabetes. Participants underwent a standard 2-hr OGTT following a 10-hr overnight fast. Subjects ingested a solution containing 75 g dextrose (1.75 g/kg), and venous blood samples were obtained at 0, 30, 60, 90, and 120 minutes for determination of plasma glucose and insulin concentrations. Plasma glucose was measured by the

glucose oxidase method using a YSI 2300 STAT plus (YSI, Inc., Yellow Springs, OH), and insulin was measured in duplicate by ELISA (ALPCO Diagnostics, Windham, NH). In addition, a blood sample (~20 mL) was taken under fasting conditions to measure hemoglobin A1C (HbA1c) and lipid profile (total cholesterol, triglyceride, high density lipoprotein [HDL], low density lipoprotein [LDL], and very low density lipoprotein [VLDL]), and liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]). All laboratory tests were performed by a Clinical Laboratory Improvement Amendments–certified commercial laboratory (Sonora Quest Laboratories, Phoenix, AZ).

Classification of Glycemic Status. Glucose tolerance was determined according to the American Diabetes Association (2010) as prediabetes (impaired fasting glucose [IFG]: fasting glucose between 100-125 mg/dL and/or impaired glucose tolerance [IGT]: 2-hr glucose between 140-199 mg/dL) and type 2 diabetes (fasting glucose ≥126 mg/dL and/or 2-hr glucose ≥200mg/dL).

OGTT-derived Indices. Insulin action was estimated by the homeostasis model assessment for insulin resistance (HOMA-IR) (Matthews et al, 1985) and the whole-body insulin sensitivity index of Matsuda and DeFronzo (1999). Insulin secretion was estimated by the insulinogenic index calculated using fasting and 30 minute insulin and glucose concentrations (Phillips, Clark, Hales, & Osmond, 1994). The β-cell function relative to the degree of insulin resistance was estimated by the disposition index as the product of insulin action and insulin secretion. Validation studies of these indices driven from OGTT by comparing to the gold standards (e.g., insulinogenic clamp or intravenous

glucose tolerance test) are presented in Table 5-1. It is important to note that all indices listed in Table 5-1 were validated to be used in Latino population across age groups.

Table 5-1. Validation of OGTT-derived Indices in Latinos

Index	Estimate	Validation	Reference
HOMA-IR	Insulin resistance	Inversely correlated with insulin sensitivity obtained from the IVGTT ($r=-0.81$ $p<0.01$)	Conwell, Trost, Brown, & Batch, 2004; Haffner, Kennedy, Gonzalez, Stern, & Miettinen, 1996; Matthews et al., 1985
Matsuda Index	Insulin sensitivity	Correlated with insulin sensitivity obtained from the hyperinsulinemic-euglycemic clamp ($r=0.78$, $p<0.0005$)	Matsuda & DeFronzo, 1999; Yeckel et al., 2004
Insulinogenic Index	Insulin secretion	Correlated with 1st phase insulin secretion obtained from the hyperglycemic clamp ($r=.068$, $p<0.001$)	Phillips et al., 1994; Wareham, Byrne, Hales &, Phillips, 1995; Weiss et al., 2005
Disposition Index	β-cell function relative to the degree of insulin resistance	Correlated with disposition index obtained from the IVGTT ($r=0.21$, $p=0.003$)	Retnakaran, Qi, Goran, & Hamilton, 2009

Classification of Glucose Response Curve. Glucose response phenotype (i.e., monophasic or biphasic) was classified according to previous study by Tschritter et al. (2003) with a glucose threshold of 4.5 mg/dL used to minimize fluctuations in glucose concentrations that may be due to the method of glucose analysis rather than

physiological reasons. A monophasic response was characterized by a gradual rise in plasma glucose concentrations until a peak is reached followed by a subsequent decrease over a 2-hr period. A biphasic response was characterized by a gradual rise in glucose, followed by a ≥4.5 mg/dL fall, with a second rise of glucose of at least 4.5 mg/dL at a subsequent time point. Participants who exhibit a gradual increase in plasma glucose after glucose ingestion without a corresponding fall was deemed "unclassified" and was excluded for this study.

SNP Genotyping

For the genotyping, a robust screening assay was designed for each SNP. Our method of choice was Taqman-based allelic discrimination polymerase chain reaction (AD-PCR), using Taqman SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). In this approach, two alternative probes were designed to anneal to the PCR product, covering the SNP of interest. The probe binded to the target template upstream from one of the primers and was displaced with cleavage when encountered by Taq polymerase during primer extension. Cleavage of the probe generated a signal (Gibson, 2006). Mismatched probes from energetically less stable duplexed with the target sequence and provided a greatly attenuated intensity signal. Reporter dye intensity was measured in a "real time" PCR-Fluorometer (Applied Biosystems; 7900HT sequence detector). Eventually, alleles were scored using the allelic discrimination software Sequence Detection System v2.1 (Applied Biosystems).

KCNQ1 SNPs Genotyping. Isolated genomic DNA from whole blood using the PAXgene Blood DNA procedure, per the manufacturer's instructions (Qiagen, CA, USA) were used in this study. Stored DNA samples were quantified and performed quality

check by using the ND-1000 Spectrophotometer (Nanodrop Technologies; Rockland, DE). KCNQ1 SNPs (rs2237892; rs2237895; rs151290) were obtained by the Assay-by-Design service (Applied Biosystems, CA, USA). Briefly, in a 384-well plate, 2 μL of purified genomic DNA (2 ng/ μL) was incubated with primers and probes with the KCNQ1 SNPs (0.09 μL), 3.5 μL of TaqMan Universal Polymerase Chain Reaction Master Mix–No AmpErase UNG, and 1.14 μL distilled water. Samples were polymerase chain reaction-amplified on the Applied Biosystems 9700HT Thermal Cycler under the following conditions; denatured for 10 minutes at 95℃, denatured, annealed, and extended for 40 cycles of 15 seconds at 92℃, and 1 minute at 60℃. The 384-well microplates were scanned for fluorescence emission using a 7900HT sequence detector (Applied Biosystems) and alleles were scored using the allelic discrimination software Sequence Detection System v2.3 (Applied Biosystems). For all SNPs genotyped in this study, our mean rate of success for genotyping was > 99%.

Statistical Genetic Analysis

The software package Sequential Oligogenic Linkage Analysis Routines (SOLAR) (http:// www.sfbr.org/solar) was used for testing our study hypotheses. A complete description of participants' characteristics after excluding outliers for each phenotype (if individual value is lower or greater than mean ± 4 x standard deviation) was described in Table 5-2. Heritability of the phenotypes (n=25) after controlling for age and sex effects which were used in this study was estimated and presented in Table 5-3. Association analysis using the measured genotype approach (MGA) within the variance components (VC) analytical framework was used and it allowed us to account for the non-independence among family members (Almasy & Blangero, 1998;

Boerwinkle, Chakraborty, & Sing, 1986). In this analytical framework, VCs were modeled as random effects (e.g., additive genetic effects and random environmental effects) and the mean effects of measured covariates (i.e., age and sex) were modeled as fixed effects on the trait mean. In MGA, generally, the marker genotypes were incorporated in the mean effects model as a measured covariate assuming additive allelic effects. The VCs, association parameters, and mean covariate effects were simultaneously estimated using maximum likelihood-based methods. Before performing MGA, the quantitative transmission disequilibrium test (QTDT) was performed to evaluate population stratification. With the presence of population stratification, the QTDT procedure was employed to assess association which is robust in the presence of stratification. A likelihood function based on multivariate normal density was numerically maximized to obtain parameter estimates. For the purposes of the exploration of the relationship between genetic variants (previously selected 56 SNPs and 3 KCNQ1 SNPs) and type 2 diabetes-related traits (traditional and novel biomarkers), we used a nominal *P* value of 0.05 as our threshold for statistical significance. Deviations from Hardy-Weinberg Equilibrium (HWE) was tested based on the calculated allele frequencies by the SOLAR program.

Phenotypes for Genetic Analysis. Twenty-five diabetes and metabolic phenotypes including indices of insulin action and secretion were used for the analysis: BMI, WC, HC, total cholesterol, HDL, LDL, VLDL, triglyceride, SBP, DBP, ALT, AST, fasting plasma glucose (FPG), 2-hr glucose, HbA1c, fasting plasma insulin (FPI), HOMA-IR, Matsuda index, insulinogenic index, disposition index, pre-diabetes status, and diabetes status. The formulas and references of indices of insulin action, secretion

and β-cell function, which are derived from the OGTT, were described previously (Kim et al., 2012). To normalize the trait distributions for genetic analyses, with exception of total cholesterol and SBP, all phenotypes were transformed using inverse normalization.

Results

The descriptive characteristics of the Arizona Insulin Resistance (AIR) subjects are shown in Table 5-2. Briefly, among 667 participants with mean age of 31.7 ± 13.4 (aged 7-85 years old), 61% (*n*=407) were female and 80% of the study population were adults (>18 years old). The prevalence of diabetes in our population was 12.3% (*n*=77) and 34% of the participants were classified with prediabetes (IFG or IGT; *n*=187). For the novel markers of type 2 diabetes risk, the prevalence of monophasic glucose response phenotype was 74.5% (*n*=435) while 25.5% (*n*=149) of our study population was characterized by having biphasic glucose response to an OGTT. The average of 1-hr glucose concentrations obtained from the OGTT was 150.2 ± 66.9 (mg/dL). Prior to determining genetic determinants of these novel glycemic markers, the utility of each biomarker was tested in youth, adults, and combined groups (youth and adults) in the AIR registry, respectively. For the glucose response curve, individuals with a monophasic response exhibited deleterious metabolic characteristics across age groups when compared to individuals with a biphasic response (Appendix A and B). In addition, a total of 609 participants had available data of 1-hr glucose level in the AIR registry and we confirmed the utility of this threshold (≥155 mg/dL). A glucose concentration ≥155 mg/dL at 1-hr during an OGTT exhibited higher risk for type 2 diabetes (Appendix C-E).

Heritability estimates (h^2) for the phenotypes after adjusting for age and sex were determined using SOLAR (Table 5-3). All of the phenotypes measured showed moderate

to high in magnitude (range 0.24-0.78) and were significant (all $P < 0.05$), with the exception of the glucose response curve, diabetes status, and prediabetes status.

Table 5-2. Characteristics of the Arizona Insulin Resistance (AIR) Registry Subjects

Phenotype	Sample Size	Mean ± SD or n (%)
Age (years)	667	31.7 ± 13.4
Sex (male/female)	667	260 (39.0%) / 407 (61.0%)
BMI (kg/m^2)	662	28.7 ± 6.5
WC (cm)	660	95.3 ± 16.1
HC (cm)	660	106.1 ± 13.5
Fat mass (kg)	662	22.6 ± 11.7
Total cholesterol (mg/dL)	657	168.5 ± 35.7
Triglycerides (mg/dL)	655	126.9 ± 75.6
HDL (mg/dL)	659	44.4 ± 11.0
LDL (mg/dL)	646	102.3 ± 29.2
VLDL (mg/dL)	644	20.4 ± 10.6
SBP (mmHg)	664	118.1 ± 14.8
DBP (mmHg)	666	74.5 ± 10.1
ALT (U/L)	647	24.7 ± 16.6
AST (U/L)	650	23.8 ± 10.2
HbAIc (%)	567	5.6 ± 0.3
FPI (uIU/mL)	491	9.2 ± 6.5
FPG (mg/dL)	626	94.7 ± 14.3
1-hr glucose (mg/dL)	609	150.2 ± 66.9
2-hr glucose (mg/dL)	551	121.8 ± 30.3
HOMA-IR	514	2.2 ± 1.6
Matsuda Index	480	5.1 ± 3.6
Insulinogenic Index	494	1.4 ± 1.1
Disposition Index	495	6.2 ± 5.4
Glucose response curve (mono/bi)	584	435 (74.5%) / 149 (25.5%)
Diabetes Status (non-diabetic/diabetic)	628	551 (87.7%) / 77 (12.3%)
Prediabetes Status (NGT/IFG and IGT)	551	54.6 (66.1%) / 187 (33.9%)

BMI , body mass index; WC, waist circumference; HC, hip circumference; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT; alanine aminotransferase, AST, aspartate transaminase; HbA1c, hemoglobin A1c; FPI, fasting plasma insulin; HOMA-IR, homeostatis assessment of insulin resistance; FPG, fasting plasma glucose; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

Table 5-3. Heritability (h^2) Estimates for Diabetes and Metabolic Phenotypes

Phenotype	$h^2 \pm$ SE (%)	*P*-value
BMI	37 ± 10	< 0.0001
WC	39 ± 10	< 0.0001
HC	34 ± 10	< 0.001
Fat mass	39 ± 11	< 0.0001
Total cholesterol	46 ± 12	< 0.0001
Triglycerides	58 ± 12	< 0.0001
HDL	78 ± 10	< 0.0001
LDL	31 ± 13	0.005
VLDL	46 ± 13	< 0.0001
SBP	36 ± 13	0.001
DBP	20 ± 11	0.024
ALT	34 ± 14	0.005
AST	29 ± 14	0.009
HbA1c	49 ± 11	< 0.0001
FPI	47 ± 14	< 0.001
HOMA-IR	51 ± 12	< 0.0001
Matsuda Index	50 ± 14	< 0.001
Insulinogenic Index	24 ± 14	0.042
Disposition Index	27 ± 13	0.019
FPG	41 ± 14	0.001
1-hr glucose	47 ± 12	< 0.001
2-hr glucose	28 ± 16	0.033
Glucose response curve	17 ± 25	0.256
Diabetes status	69 ± 51	0.100
Prediabetes status	39 ± 32	0.067

Phenotypes were inverse normalized for genetic analyses.

We genotyped 59 common SNPs (representing 31 genes/loci) and all were polymorphic and in HWE (P > 0.05). Minor allele frequencies of the SNPs ranged from 5% to 49% (Table 5-4), indicating that the SNPs were common in the AIR registry.

Table 5-4. Allele Frequency Distribution of Candidate SNPs (*n*=59) Studied in the AIR Registry

Gene or nearest gene	SNP ID	Chromosome	Major allele	Major allele frequency	Minor allele	Minor allele frequency
NOTCH2	rs10923931	1	G	0.9322	T	0.0678
PROX1	rs340874	1	T	0.6140	C	0.3860
GCKR	rs780094	2	C	0.6797	T	0.3203
G6PC2	rs560887	2	C	0.8726	T	0.1274
THADA	rs7578597	2	T	0.9450	C	0.0550
ADAMTS9	rs4607103	3	C	0.6647	T	0.3353
ADCY5	rs11708067	3	A	0.6902	G	0.3098
IGF2BP2	rs4402960	3	G	0.7187	T	0.2813
SLC2A2	rs11920090	3	T	0.8746	A	0.1254
WFS1	rs10010131	4	G	0.7187	A	0.2813
CDKAL1	rs10946398	6	A	0.7058	C	0.2942
GCK	rs4607517	7	G	0.7902	A	0.2098
JAZF1	rs864745	7	T	0.6609	C	0.3391
LOC100128217	rs2191349	7	G	0.5957	T	0.4043
SLC30A8	rs13266634[a]	8	C	0.7777	T	0.2223
SLC30A8	rs11558471[a]	8	A	0.7610	G	0.2390
CDKN2A/B	rs10811661	9	T	0.8740	C	0.1260
GLIS3	rs7034200	9	A	0.5685	C	0.4315
HHEX	rs1111875	10	C	0.6342	T	0.3658
LOC100420392	rs10885122	10	G	0.8548	T	0.1452
TCF7L2	rs7901695[b]	10	T	0.7413	C	0.2587
TCF7L2	rs4506565[b]	10	A	0.7392	T	0.2608
TCF7L2	rs7903146[b]	10	C	0.7610	T	0.2390
TCF7L2	rs12243326[b]	10	T	0.8094	C	0.1906
TCF7L2	rs12255372[b]	10	G	0.8048	T	0.1952
CRY2	rs11605924	11	A	0.5026	C	0.4974
FADS1	rs174550	11	C	0.6137	T	0.3863
KCNJ11	rs5215	11	T	0.6392	C	0.3608

MADD	rs7944584	11	A	0.8586	T	0.1414
MTNR1B	rs10830963	11	C	0.7689	G	0.2311
KCNQ1	rs151290	11	C	0.6369	A	0.3631
KCNQ1	rs2237892	11	C	0.7226	T	0.2774
KCNQ1	rs2237895	11	A	0.5235	C	0.4765
ADIPOR2	rs12342[c]	12	G	0.5164	A	0.4836
ADIPOR2	rs767870	12	T	0.8916	C	0.1084
ADIPOR2	rs929434[c]	12	C	0.5148	T	0.4852
ADIPOR2	rs1029629[c]	12	A	0.5459	C	0.4541
ADIPOR2	rs1044471	12	C	0.5837	T	0.4163
ADIPOR2	rs1058322	12	C	0.6848	T	0.3152
ADIPOR2	rs1468491	12	G	0.9381	C	0.0619
ADIPOR2	rs2058033	12	A	0.7808	C	0.2192
ADIPOR2	rs2068491	12	A	0.5825	G	0.4175
ADIPOR2	rs2286380	12	A	0.9361	T	0.0639
ADIPOR2	rs3809266[c]	12	C	0.5164	A	0.4836
ADIPOR2	rs4140992	12	C	0.6155	T	0.3845
ADIPOR2	rs4766415	12	T	0.6061	A	0.3939
ADIPOR2	rs9805042	12	C	0.9068	T	0.0932
ADIPOR2	rs10735003	12	C	0.5994	T	0.4006
ADIPOR2	rs10848569[c]	12	G	0.5148	A	0.4852
ADIPOR2	rs12582624[c]	12	G	0.5583	C	0.4417
IGF1	rs35767	12	G	0.7888	A	0.2112
TSPAN8	rs7961581	12	T	0.8237	C	0.1763
FTO	rs8050136[d]	16	C	0.7435	A	0.2565
FTO	rs3751812[d]	16	G	0.7704	T	0.2296
FTO	rs9939609[d]	16	T	0.7435	A	0.2565
FTO	rs17818902	16	T	0.7456	G	0.2544
FTO	rs7203051	16	G	0.5962	C	0.4038
FTO	rs1075888	16	T	0.5212	G	0.4788
HNF1B	rs4430796	17	A	0.5927	G	0.4073

[a]Two SNPs in the SLC30A8 gene are in strong linkage disequilibrium with r2=9.0

[b]Five SNPs in the TCF7L2 gene are in strong linkage disequilibrium with r2 ranging from 0.88 to 0.99

[c]Six SNPs in ADIPOR2 gene are in strong linkage disequilibrium with r2 ranging from 0.81 to 0.89

[d]Three SNPs in the FTO gene are in strong linkage disequilibrium with r2 ranging from 0.86 to 0.98

Association Between Candidate SNPs (*n*=39) and Traditional Clinical Phenotypes

The Associations of candidate SNPs with diabetes and metabolic phenotypes have been reported in our previous study (DeMenna et al., 2014), with the exception of genetic variants in KCNQ1 and ADIPOR2 genes. Briefly, our previous study found that there were 28 SNPs (representing 19 genes/loci) that exhibited nominal associations with either anthropometrics/lipids or glucose regulation/insulin dynamics-related phenotypes. Of them, 5 SNPs from 5 genes exhibited the most powerful and significant association with anthropometrics, lipids, and glucose/insulin dynamics (rs3751812/FTO: BMI, HC; rs13266634/ SLC30A8: BMI; rs4607517/GCK: FPG, HbA1c; rs10830963/MTNR1B: VLDL; rs7578597/THADA: Cholesterol, LDL).

Association Between KCNQ1 SNPs and Traditional Clinical Phenotypes

Three KCNQ1 SNPs (rs151290, rs2237892, rs2237895) were included in this analysis. Of them, 2 SNPs (rs151290 and rs2237892) exhibited nominal ($P < 0.05$) associations with multiple phenotypes, while rs2237895 was not associated with any of the phenotypes. Specific mean values for the phenotypes that were associated with each KCNQ1 SNP are shown in Table 5-5 and 5-6. Specifically, rs151290 was significantly associated with total cholesterol, LDL, DBP, FPG, prediabetes status, Matsuda index, insulinogenic index, and disposition index (all $P < 0.05$). Consistent direction of increased risk in each phenotype was observed with the exception of FPI and Matsuda index, indicating that major allele C was considered as a risk allele while minor allele A was protective. In addition, there were trends toward significance in the association between rs151290 and FPI and 2-hr glucose. Similarly, rs2237892 was significantly associated with SBP, FPG, 2-hr glucose, HbA1c, prediabetes status, Matsuda index,

insulinogenic index, and disposition index (all $P < 0.05$). Direction of increased risk in the SNP rs2237892 was consistent except Matsuda index. For the rs2237892, major allele C was considered as a risk allele while minor allele T was protective.

Table 5-5. Mean Values for the Phenotypes Associated with KCNQ1 SNP rs151290

SNP	Phenotype	Major/major (C/C)	Major/minor (C/A)	Minor/minor (A/A)	Dir.	*P*-value
rs151290	Total cholesterol	170.89 ± 35.98	168.86 ± 35.04	160.94 ± 39.54	↓	0.02
	LDL	104.29 ± 30.01	102.82 ± 28.43	94.25 ± 30.78	↓	0.02
	DBP	75.50 ± 9.71	74.20 ± 10.03	73.93 ± 10.95	↓	0.05
	FPI	8.59 ± 5.81	9.6 ± 6.34	10.43 ± 7.93	↑	0.05
	FPG	95.82 ± 17.25	95.04 ± 13.27	92.69 ± 10.18	↓	0
	2-hr glucose	135.69 ± 46.92	132.92 ± 45.91	132.86 ± 48.48	↓	0.06
	Pre-Diabetes n, (%)	115 / 78 (40%)	173 / 82 (32%)	53 / 16 (23%)	↓	0
	Matsuda Index	5.41 ± 3.76	4.70 ± 3.04	4.92 ± 48.48	↓	0.04
	Insulinogenic Index	1.23 ± 1.01	1.56 ± 1.18	1.68 ± 1.20	↑	0.01
	Disposition index	5.12 ± 3.63	6.64 ± 3.02	7.74 ± 6.60	↑	0.02

Note. Dir. direction of change

Table 5-6. Mean Values for the Phenotypes Associated with KCNQ1 SNP rs2237892

SNP	Phenotype	Major/major (C/C)	Major/minor (C/T)	Minor/minor (T/T)	Dir.	*P*-value
rs2237892	SBP	118.40 ± 14.48	118.40 ± 14.81	116.26 ± 15.73	↓	0.005
	FPG	96.25 ± 16.87	94.29 ± 12.18	91.29 ± 8.51	↓	< 0.001
	2-hr glucose	135.65 ± 47.27	133.66 ± 45.17	127.28 ± 49.59	↓	0.019
	HbA1c	5.59 ± 0.34	5.55 ± 0.31	5.56 ± 0.33	↓	0.01
	Pre-Diabetes n, (%)	157 / 102 (39%)	140 / 68 (33%)	44 / 6 (12%)	↓	< 0.001
	Matsuda Index	5.27 ± 3.56	4.78 ± 3.23	4.47 ± 3.51	↓	0.026
	Insulinogenic Index	1.22 ± 1.01	1.57 ± 1.13	2.15 ± 1.47	↑	< 0.0001
	Disposition index	5.24 ± 6.75	6.75 ± 5.98	9.13 ± 7.08	↑	< 0.001

Note. Dir. direction of change

Association Between Candidate SNPs (*n*=59) and Glucose Response Curve

Among 59 genetic variants (representing 31 genes/loci), 15 SNPs (representing 8 genes/loci) were associated with the glucose response curve (Table 5-7). Major allele of rs10923931/NOTCH2, rs780094/GCKR, rs13266634/SLC30A8, rs12243326/TCF7L2, rs11605924/CRY2, and rs2058033/ADIPOR2 exhibited significant association with increased prevalence of the monophasic response, indicating that these major allele were considered as risk allele. Moreover, minor allele of rs10830963/MTNR1B, rs3809266, rs3809266, rs4766415, rs767870, rs10735003/ADIPOR2, and rs8050136, rs1075888, rs9939609/FTO showed significant association with increased prevalence of the monophasic response, indicating that these minor allele were considered as risk alleles.

Association Between Candidate SNPs (*n*=59) and 1-hr Glucose

Among 59 candidate SNPs (representing 31 genes/loci), 18 genetic variants (representing 9 genes/loci) were associated with 1-hr glucose (Table 5-8). Major allele of rs4402960/IGF2BP2, rs4607517/GCK, rs11605924/CRY2, rs2237892/KCNQ1, and rs12342, rs1058322/ADIPOR2 exhibited a significant association with an elevated 1-hr glucose concentration, indicating that these major alleles were considered as risk alleles. Moreover, minor allele of rs340874/PROX1, rs1111875/HHEX, rs2191349/LOC100420392, rs274550/FADS1, and rs929434, rs1029629, rs2068491, rs4766415, rs10735003, rs10848569, rs12582624/ADIPOR2 showed a significant association with an elevated 1-hr glucose level, indicating that these minor alleles were considered as risk alleles.

Table 5-7 Associations of Candidate SNPs With Glucose Response Curve (Mono (%) vs. Bi (%))

Gene or [illegible]	SNP ID	Major/major	Major/minor	Minor/minor	Dir.	*P*-value
NOTCH2	rs10923931	365 (76%) vs. 116 (24%)	46 (68%) vs. 22 (32%)	0 (0%) vs. 2 (100%)	↓	0.034
GCKR	rs780094	193 (75%) vs. 66 (25%)	178 (77%) vs. 52 (23%)	42 (68%) vs. 20 (32%)	↓	0.02
SLC30A8	rs13266634	232 (73%) vs. 86 (27%)	131 (78%) vs. 38 (22%)	18 (62%) vs. 11 (38%)	↓	0.043
TCF7L2	rs12243326	278 (76%) vs. 88 (24%)	115 (75%) vs. 38 (25%)	15 (52%) vs. 14 (48%)	↓	0.044
CRY2	rs11605924	120 (82%) vs. 26 (18%)	200 (72%) vs. 77 (28%)	92 (71%) vs. 37 (29%)	↓	0.025
MTNR1B	rs10830963	238 (72%) vs. 91 (28%)	143 (76%) vs. 44 (24%)	29 (88%) vs. 4 (12%)	↑	0.048
ADIPOR2	rs2058033	260 (72%) vs. 101 (28%)	100 (78%) vs. 29 (22%)	49 (84%) vs. 9 (16%)	↑	0.017
ADIPOR2	rs2068491	259 (76%) vs. 82 (24%)	136 (73%) vs. 50 (27%)	18 (69%) vs. 8 (31%)	↓	0.027
ADIPOR2	rs3809266	228 (70%) vs. 96 (30%)	165 (80%) vs. 42 (20%)	19 (90%) vs. 2 (10%)	↑	0.003
ADIPOR2	rs4766415	159 (73%) vs. 58 (27%)	191 (75%) vs. 62 (25%)	62 (75%) vs. 21 (25%)	↑	0.022
ADIPOR2	rs767870	311 (72%) vs. 118 (28%)	96 (81%) vs. 22 (19%)	5 (83%) vs. 1 (17%)	↑	0.029
ADIPOR2	rs10735003	157 (74%) vs. 56 (26%)	188 (75%) vs. 64 (25%)	67 (76%) vs. 21 (24%)	↑	0.024
FTO	rs8050136	209 (69%) vs. 93 (31%)	180 (80%) vs. 44 (20%)	23 (85%) vs. 4 (15%)	↑	0.018
FTO	rs1075888	110 (69%) vs. 49 (31%)	202 (75%) vs. 68 (25%)	101 (81%) vs. 23 (19%)	↑	0.024
FTO	rs9939609	209 (70%) vs. 91 (30%)	179 (80%) vs. 45 (20%)	22 (81%) vs. 5 (19%)	↑	0.007

Note. Dir., direction of change

Table 5-8 Associations of Candidate SNPs With 1-hr glucose

Gene or nearest gene	SNP ID	Major/major	Major/minor	Minor/minor	Dir.	*P*-value
PROX1	rs340874	168.12 ± 55.52	166.42 ± 61.29	172.05 ± 50.16	↑	0.042
IGF2BP2	rs4402960	167.40 ± 49.35	168.73 ± 62.74	165.20 ± 63.57	↓	0.005
GCK	rs4607517	163.71 ± 61.29	175.03 ± 52.45	167.03 ± 41.38	↑	0.002
HHEX	rs1111875	164.38 ± 59.31	169.04 ± 57.80	173.61 ± 51.90	↑	0.002
LOC100420392	rs2191349	167.15 ± 49.94	167.61 ± 59.36	169.09 ± 68.66	↑	0.001
CRY2	rs11605924	174.87 ± 53.92	164.90 ± 61.65	166.56 ± 52.50	↓	0.009
FADS1	rs274550	163.31 ± 51.67	168.53 ± 55.52	175.81 ± 74.38	↑	0.039
KCNQ1	rs2237892	152.21 ± 72.01	152.24 ± 59.73	148.37 ± 58.19	↓	0.013
ADIPOR2	rs12342[c]	167.02 ± 47.78	165.83 ± 56.64	171.87 ± 68.23	↑	< 0.0001
ADIPOR2	rs929434[c]	171.87 ± 68.23	165.88 ± 56.73	166.91 ± 47.63	↓	< 0.0001
ADIPOR2	rs1029629[c]	170.52 ± 67.97	168.50 ± 58.62	165.53 ± 45.65	↓	0.028
ADIPOR2	rs1058322	165.95 ± 59.41	169.66 ± 56.40	167.22 ± 57.08	↑	0.029
ADIPOR2	rs2068491	166.86 ± 60.77	169.69 ± 53.05	165.84 ± 51.73	↓	0.05
ADIPOR2	rs4140992	172.49 ± 68.43	165.53 ± 56.51	166.91 ± 47.63	↓	< 0.0001
ADIPOR2	rs4766415	167.48 ± 48.61	168.67 ± 62.99	165.37 ± 63.82	↓	0.004
ADIPOR2	rs10735003	167.42 ± 49.00	169.06 ± 63.01	164.55 ± 62.37	↓	0.004
ADIPOR2	rs10848569	171.87 ± 68.23	165.88 ± 56.73	166.91 ± 47.63	↓	< 0.0001
ADIPOR2	rs12582624	168.13 ± 65.36	168.26 ± 57.67	165.94 ± 46.71	↓	< 0.001

Note. Dir., direction of change

Conclusions

This study described the genetic contributions to novel markers of dysglycemia (i.e., glucose response curve and 1-hr glucose) in the AIR registry. In the present study, we first demonstrated that there were significant associations between KCNQ1 SNPs (rs151290 and rs2237892) and diabetes-related phenotypes including β-cell function, a hallmark feature of type 2 diabetes, which is considered one of the earliest indicators of diabetes risk. Second, we described the genetic determinants of novel glycemic markers and found multiple genetic variants that were associated with either the glucose response curve or 1-hr glucose level from an OGTT.

KCNQ1 is a gene encoding the pore-forming subunit of a voltage-gate K+ channel that is expressed in a number of tissues, including heart, pancreas, kidneys, and intestine (Unoki et al., 2008; Yasuda et al., 2008). Since it is well established that this encoded protein plays an important role in the electrical depolarization of the cell membrane in the heart and presumably in pancreatic β-cells, an increasing number of studies have examined its polymorphisms in relation to type 2 diabetes risk (Liu et al., 2013; Morris et al., 2012). Although it is not clear whether the genetic variants of KCNQ1 affect its gene expression, functional investigations have shown that selective blockades of this K+ channel stimulates insulin secretion through alterations in the membrane repolarization potential of the pancreatic β-cells (Ullrich et al., 2005). Furthermore, KCNQ1 is involved in hormone and electrolyte transport, suggesting that it may affect incretin secretion in the gastrointestinal tract (Vallon et al., 2005). Müssig et al. (2009) confirmed its effects on incretin-stimulated insulin secretion by measuring

differences in plasma GLP-1 and GIP levels along with insulin secretion during the OGTT among 3 genotyping groups in rs151290.

The association between KCNQ1 genetic variants and type 2 diabetes risk has been increasingly replicated, but the majority of these studies (around 90% of KCNQ1 SNP studies) have focused on those of Caucasian or East Asian descent (Liu et al., 2013). A growing number of studies have provided evidence that KCNQ1 genetic variants (mostly rs2237892 and rs2237895) were associated with clinical phenotypes including fasting glucose, first and second phases of insulin secretion, and homeostatic model assessment of β-cell function (Hu et al., 2009; Qi et al., 2009; van Vliet-Ostaptchouk et al., 2012). To our knowledge, two studies examined KCNQ1 SNP (rs2237892) in the Mexican population and exhibited significant association with susceptibility to type 2 diabetes, as odds ratio for developing type 2 diabetes ranged from 1.20 to 1.36 (Gamboa-Melendez et al., 2012; Parra et al., 2011).

Our data from a Latino cohort exhibited that 2 (rs151290 and rs2237892) out of the 3 KCNQ1 SNPs examined were associated with type 2 diabetes risk factors including prediabetes status, insulin resistance, impairment of insulin secretion, and β-cell function. These data suggest that major allele C for both SNPs is considered a risk allele and our findings were concordant with previous findings (Hu et al., 2009; Qi et al., 2009; van Vliet-Ostaptchouk et al., 2012). Unexpectedly, for both rs151290 and rs2237892, the major allele C (i.e., risk allele) was associated with a slightly higher Matsuda index (indicates insulin sensitivity) than the minor allele (rs151290 [A] and rs2237892 [T], i.e., protective allele). However, when the disposition index (i.e., insulin secretion x insulin sensitivity) was examined across genotypes, risk allele C was associated with lower β-

cell function relative to the degree of insulin resistance. It is because major allele C exhibited significant association with the impairment of insulin secretion (i.e., lower insulinogenic index). Given the fact that the prevalence of overweight and obesity was 50% in children and 81% in adults in the AIR registry, we expected that our study participants were already affected by a certain degree of insulin resistance (e.g., regardless of their given genotypes). In this respect, it may physiologically explain that the protective allele in these SNPs were associated with higher insulin secretion (to compensate given certain degree of insulin resistance), which leads to a higher disposition index. It is important to note that consistent patterns of protective or deleterious allele for the risk variables were detected for two SNPs (rs151290 and rs2237892) and they were found to replicate the same association direction (i.e., same risk allele) as the one identified in the original GWAS and candidate gene studies (Hu et al., 2009; Qi et al., 2009; van Vliet-Ostaptchouk et al., 2012).

More importantly, although continuous efforts have been conducted to further physiologically characterize candidate SNPs, the majority of type 2 diabetes susceptibility loci have been replicated with traditional clinical markers such as fasting and 2-hr glucose (Ingelsson et al., 2010). To our knowledge, no studies have examined genetic determinants of either the glucose response curve or 1-hr glucose concentration from an OGTT. We first reported the heritability estimates of the novel glycemic makers in this study. It is important to note that there is considerable loss of power observed for discrete variables due to the low prevalence of the biphasic response (Williams & Blangero 2004). Moreover, since it is also possible that heritability can be age dependent, the heritability estimate of the glucose response curve may be underestimated (i.e., age

effect was significant (P = 0.0023) when the heritability was estimated in the model of the glucose response curve). Interestingly, the heritability estimate of 1-hr glucose was significant and it was higher than that of either fasting or 2-hr glucose.

We identified 15 SNPs nominally associated with the glucose response curve. Of these, associations of rs3809266 in adiponectin receptor 2 (ADIPOR2) gene and rs9939609 in the fat mass and obesity-associated (FTO) gene with the glucose response curve exhibited the most powerful and significant effect in our dataset (all $P < 0.01$). ADIPOR2 was first cloned by Yamauchi et al. (2003) and it has been characterized as enhancing fatty acid oxidation, increasing glucose uptake, increased adenosine 5'-monophosphate-activated protein kinase activity, and interacting with peroxisome proliferator-activated receptor pathways (Yamauchi et al., 2002). The studies of adiponectin receptor gene expression in relation to type 2 diabetes risk further supported above function (Civitarese et al., 2004; Debard et al., 2004). Interestingly, out of the total 15 SNPs in ADIPOR2 in our dataset, 6 SNPs were found to be associated with the glucose response curve. It is important to note that all 6 SNPs were not in strong linkage disequilibrium, indicating that they are not sharing the same genetic information. Moreover, minor alleles for the 5 SNPs were consistently associated with a higher prevalence of monophasic, indicating that those are risk alleles. However, these trends of deleterious alleles were not concordant with previous findings from the San Antonio Family Diabetes Study, exhibiting that the majority of the minor alleles for the ADIPOR2 SNPs were associated with decreased fasting triglyceride levels (Richardson et al, 2006). Given that a handful of studies have reported genetic associations of adiponectin receptors with cardiometabolic risk and these results were also inconsistent in various

populations (Broedl et al., 2006; Cohen et al., 2011; Hara et al., 2005; Peters et al., 2013; Vaxillaire et al., 2006), further population specific examination of this relationship is still warranted.

Another strong association with the glucose response curve was observed in rs9939609 in the FTO gene. FTO is the fat mass and obesity-associated protein and physiological function of FTO has been continuously and widely examined. Recent studies have demonstrated that the FTO gene may play an important role in energy homeostasis by regulating either energy expenditure or energy intake in humans (Cecil, Tavendale, Watt, Hetherington, & Palmer, 2008; Fredriksson et al, 2008; Haupt et al., 2009) and animals (Church et al., 2009; Fischer et al., 2009). In addition, FTO has been described as a regulator of adipose tissue metabolism (i.e. lipolysis), as it contributes to the regulation of fat mass (Jacobsson, Schioth, & Fredriksson, 2012; Wåhlén, Sjölin, & Hoffstedt, 2008). Based on our data, 3 SNPs in FTO exhibited a consistent direction of increased prevalence of monophasic glucose response (i.e., minor alleles were associated with increased type 2 diabetes risk). It is important to note that these results are concordant with our previous findings, which showed that minor alleles for these 3 SNPs were associated with increased BMI, waist circumference, hip circumference, FPI, and HOMA-IR (DeMenna et al., 2014).

In addition to the aforementioned SNPs/genes, we have further nominally identified genetic variants in neurogenic locus notch homolog protein 2 (NOTCH2), glucokinase regulator (GCKR), solute carrier family 30 (zinc transporter), member 8 (SLC30A8), transcription factor 7-like 2 (TCF7L2), cryptochrome circadian clock 2 (CRY2), and melatonin receptor 1B (MTNR1B) that were found to be associated with the

glucose response curve. Briefly, NOTCH2 is a type 1 transmembrane receptor and a genetic association study exhibited an odds of 1.13 (95% CI=1.08–1.17) for type 2 diabetes (Zeggini et al., 2008). From the Botnia study focused on the Finnish population, major allele G in NOTCH2 was associated with islet function measured by elevated fasting and 2-hr glucagon concentrations obtained from the OGTT (Jonsson et al., 2013). GCKR is an important regulator of glucokinase activity, which is a key glucose phosphorylation enzyme responsible for the first rate-limiting step in the glycolysis pathway and regulates glucose-stimulated insulin secretion from pancreatic β-cells and glucose metabolism in the liver (Chu et al., 2004; Matschinsky, 1996). Dupuis et al. (2010) observed that the major allele C in GCKR was associated with elevated fasting glucose and fasting insulin or HOMA-IR. SLC30A8 gene is exclusively expressed in pancreas (mainly in β-cells) and it is localized to insulin secretory granules, implicating that it plays an important role in the storage and maturation of insulin in the granules of the β-cell (Chimienti, Devergnas, Favier, & Seve, 2004; Chimienti et al., 2006). Commone genetic varitant (rs13266634) in SLC30A8 was associated with odds of 1.15 for type 2 diabetes (Dupuis et al., 2010) TCF7L2 codes for a transcription factor involved in the Wnt signaling pathway and genetic variants have been associated with type 2 diabetes risk (Grant et al., 2006; Saxena et al., 2007; Dupuis et al., 2010). Major allele T in TCF7L2 was associated with impaired insulin secretion, insulin sensitivity, and enhanced rate of hepatic glucose production in various ethnic groups (Damcott et al., 2006; Elbein et al., 2007, Lyssenko et al., 2007; Musso et al., 2009). Lastly, CRY2 and MTNR1B regulate circadian rhythm regulation (Kume et al., 1999) and the odds of type 2 diabetes were 1.04 (95% CI=1.02–1.06) and 1.09 (95% 1.06-1.12), respectively

(Dupuis et al., 2010). Taken together, we confirmed that directions of increased prevalence of monophasic response for aforementioned SNPs are concordant with either Dupuis et al. (2010), or our previous study (DeMenna et al., 2014).

Compared to the genetic determinants of the glucose response curve, the majority of associations with 1-hr glucose was stronger. This result may be affected by the moderate and significant heritability estimate of 1-hr glucose (h^2=47±12). A total of 20 SNPs were found to be associated with 1-hr glucose level. Notably, 10 SNPs in ADIPOR2 were observed in the genetic association analysis and 8 SNPs exhibited a consistent direction of changes, suggesting that minor alleles were associated with a decreased 1-hr glucose. It is notable that 3 out of 10 SNPs in the ADIPOR2 gene were in strong linkage disequilibrium with r^2 ranging from 0.81 to 0.89. However, rs12342 which was in strong linkage disequilibrium with rs929434 and rs1029629, exhibited a conflict in direction of increased 1-hr glucose when compared to the associations of rs929434 and rs1029629. Although we are not fully able to explain this discrepancy among the SNPs which were in strong linkage disequilibrium, it may be due to the wide variability of phenotype within individuals. When genotypes (i.e., major/major, major/minor, minor/minor) were analyzed by two groups (i.e., non-carrier of risk allele [major/major] vs. carrier of risk allele [major/minor and minor/minor]) in the SNP rs12342, a significant association with 1-hr glucose was not observed. For this reason, it is possible that the current association is false. Collectively, in contrast to the glucose response curve, these trends with 1-hr glucose were concordant with the changes in traditional phenotypes from a previous study (Richardson et al., 2006).

An interesting finding from our current study was an association between KCNQ1 SNP (rs2237892) and the 1-hr glucose. In addition to the associations between rs2237892 and traditional diabetes risk factors including prediabetes status, insulin resistance, impairment of insulin secretion, and β-cell function, this SNP was associated with a novel glycemic marker, 1-hr glucose. Consistent with associations of traditional clinical markers, major allele C in rs2237892 was associated with elevated 1-hr glucose levels. Based on the physiological characterization of the candidate SNPs we selected, it is likely that genetic variants in KCNQ1 impact various physiological features including glycemic controls by insulin efficiency and secretion.

In addition, we have further identified genetic variants in insulin-like growth factor 2 mRNA binding protein 2 (IGF2BP2), glucokinase (GCK), CRY2, prospero homeobox 1 (PROX1), hematopoietically expressed homeobox (HHEX), and fatty acid desaturase 1 (FADS1). Briefly, IGF2BP2 is a paralog of IGF2BP1, which regulates translation of insulin-like growth factor 2. A cluster of IGF2BP1 genetic variants were found to be associated with type 2 diabetes risk including decreased first-phase insulin secretion and reduced β-cell function (Grarup et al., 2007). Minor allele T in rs4402960 was considered a risk allele, as odds exhibited 1.11-1.14 (Scott et al., 2007). GCK gene plays an important role in regulating glucose cycling in several tissues, including the liver and pancreas (Li et al., 2009). For the SNP rs4607517, odds of type 2 diabetes was 1.07 (95% CI=1.05-1.10), suggesting that the minor allele A was the risk allele (Dupuis et al., 2010). PROX1 gene plays a crucial role in the development of β-cell and acts as a novel co-regulator of bile acid synthesis and gluconeogenesis (Song, Li, & Chiang, 2006). Dupuis et al. (2010) showed that the major allele C of rs340874 was associated with

development of type 2 diabetes. Note that allele C was considered a minor allele in our study. HHEX gene encodes a transcription factor that is involved in Wnt signaling, which is an important pathway for cell growth and development (Foley & Mercola, 2005). In general, the major allele C of rs1111875 is considered a risk allele by exhibiting odds of 1.13 for type 2 diabetes (Saxena et al., 2007). Lastly, FADS1 encodes fatty acid desaturase 1, which catalyzes the biosynthesis of highly unsaturated fatty acids, suggesting that increased activity of these enzymes may lower circulating triglyceride concentrations (Keane & Newsholme, 2008). For the SNP 174550 in FADS1, the major allele T was associated with increased risk for type 2 diabetes (Dupuis et al., 2010). Note that allele T was considered a minor allele in our study. Taken together, we confirmed consistent patterns of protective or deleterious alleles for elevated 1-hr glucose in the aforementioned SNPs when compared to previous findings.

To our knowledge, this is the first study to examine genetic determinants of novel glycemic markers, which include the glucose response curve and 1-hr glucose during an OGTT. Moreover, we explored the genetic associations of 3 KCNQ1 SNPs (rs151290, rs2239892, and rs2239895) with diabetes-related phenotypes. The AIR registry is composed of Latino participants who reside in the Phoenix-Arizona area. We observed that the prevalence of prediabetes and type 2 diabetes (35% and 12%, respectively) is similar to the reported U.S prevalence in the Latino population (Cowie et al., 2009). Given that Latinos are disproportionately impacted by obesity and type 2 diabetes (Lawrence et al, 2009), our previous and current studies are crucial since we provided heritability estimates of diabetes-related phenotypes including novel glycemic markers (i.e., glucose response curve and 1-hr glucose) in the AIR registry as well as performed

physiological characterization of candidate genes/SNPs that were previously associated with type 2 diabetes (Dupuis et al., 2010; Gloyn et al., 2003; Grarup et al., 2007; Saxena et al., 2007; Zeggini et al., 2007, 2008). In order to expand our previous study and broaden our knowledge of the pathogenesis of type 2 diabetes in this population, current study included 3 SNPs in the KCNQ1 gene which has shown relatively higher effect size for the development of type 2 diabetes compared to other potential genetic variants (Hu et al., 2009; Qi et al., 2009; van Vliet-Ostaptchouk et al., 2012). In addition, we have attempted to characterize candidate SNPs measured by examining the association with novel glycemic biomarkers (i.e., glucose response curve and 1-hr glucose). Despite these strengths, we acknowledge potential limitations that should be considered. First, the number of subjects (≤ 626 participants' DNA samples are currently available for genotyping) for the genetic analysis in this study may not be enough to examine the stringently significant association between candidate SNPs and phenotypic markers of type 2 diabetes risk (Lin & Sullivan, 2009). Therefore, we previously mentioned that, for the purpose of exploration, we used a nominal *P* value of 0.05 as our threshold for statistical significance in this study. Larger cohorts of Latino population are warranted to confirm theses SNPs associated with novel glycemic markers (glucose response curve and 1-hr glucose). Second, our key outcome measures related to type 2 diabetes risk were obtained from the OGTT including glucose response curve, fasting, 1-hr, 2-hr glucose concentrations, and OGTT-derived indices (HOMA-IR, Matsuda index, insulinogenic index, and disposition index). For this reason, we acknowledge that poor reproducibility of the OGTT is a limitation (Libman, Barinas-Mitchell, Bartucci, Robertson, & Arslanian, 2008; Mooy et al., 1996) and further studies will need to include more

comprehensive and sophisticated phenotyping measure to determine insulin sensitivity/secretion and β-cell function.

In summary, we set out to determine whether there are associations between candidate genetic variants in type 2 diabetes susceptibility genes and clinical phenotypes including both traditional and novel markers. Moreover, we included KCNQ1 SNPs that were strongly associated with type 2 diabetes risk mainly in Caucasian and East Asian in order to replicate/confirm the findings in a Latino population. Although there remain some discrepancies in the direction of increased risk in multiple SNPs/genes (i.e., ADIPOR2), the majority of associations with the glucose response curve and 1-hr glucose were concordant with changes in the traditional clinical risk markers from the previous GWAS and/or candidate study. These discrepancies may stem from the power of the analysis (i.e., sample size matter), or inherent population differences. Our data lead us towards a better understanding of the genetic background of novel glycemic markers as well as KCNQ1 genetic influences on the risk of type 2 diabetes in the Latino population.

Acknowledgements

This work was supported by Health Research Alliance Arizona and the Center for Metabolic Biology at Arizona State University. Data management support was provided by Grant UL1-RR-024150 from the Mayo Clinic to use Research Electronic Data Capture (REDCap).

No potential conflicts of interest relevant to this article were reported.

J.Y.K. researched and analyzed data and wrote the manuscript. D.K.C. and L.J.M. researched data and reviewed and edited the manuscript. E.A.D reviewed and edited the

manuscript. G.Q.S. researched data and wrote, reviewed, and edited the manuscript. G.Q.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study were presented in paper form at the Interdisciplinary Research Symposium at Arizona State University. 17 May 2015.

The authors are grateful to the children and their families who participated in this study. The authors thank the staff of the Clinical Research Unit for their help with enrolling and testing participants.

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CHAPTER 6: CONCLUSION

In this dissertation, we introduced novel glycemic markers, which included the glucose response curve and 1-hr glucose level during an OGTT, as efficient and accurate tools for identifying type 2 diabetes risk in Latino youth. Since we did not know whether these novel glycemic markers were influenced by environmental or genetic factors, we further examined the associations between type 2 diabetes susceptibility genetic variants including KCNQ1 SNPs and novel glycemic markers.

First, we demonstrated that the shape of the plasma glucose response during an OGTT differentiates type 2 diabetes risk factors in Latino adolescents (Kim et al., 2012). Our data showed that youth with a biphasic response harbors metabolically healthier characteristics including lower glucose area under the curve and HbA1c, higher whole-body insulin sensitivity (Matsuda index) and insulin secretion (insulinogenic index), and better β-cell function relative to the insulin sensitivity (disposition index) than individuals with a monophasic response. Interestingly, two types of glucose response curves exhibited no difference in fasting and 2-hr glucose levels, which have traditionally been used as a diagnosis tool for the development of prediabetes or type 2 diabetes (The Expert Committee on the, Diagnosis, & Classification of Diabetes, Mellitus, 1997; 2003). These data extend previous studies in adults (Abdul-Ghani, Lyssenko, Tuomi, DeFronzo, & Groop, 2010; Fuchigami, Nakano, Oba, & Metori, 1994; Kanauchi, M., Kimura, Kanauchi, K., & Saito, 2005; Trujillo-Arriaga, & Roman-Ramos, 2008; Tschritter et al., 2003; Tura et al., 2011) and suggest that the glucose response curve may be an early indicator of type 2 diabetes risk in youth. Recently, one study tested the reproducibility of emerging parameters of the insulin and glucose response on the OGTT, including the glucose response curve, and reported that agreement on the shape classification within

individuals between three separate OGTTs occurred in around 40% (Kramer et al., 2014). Given the fact that OGTT harbors poor reproducibility in terms of identifying hyperglycemia, future studies need to test whether the shape of the glucose response curve is an inherent (i.e., reproducible biological process). Further, longitudinal studies to examine whether the shape of glucose response during an OGTT prospectively predicts the development of type 2 diabetes by comparing with traditional glycemic markers such as HbA1c, fasting and 2-hr glucose are warranted.

In order to examine whether there are common biologic or genetic pathways linking the phenotypic characteristics of the glucose response curve, we further performed the genetic association analysis of this novel markers in conjunction with type 2 diabetes susceptibility SNPs. In order to improve statistical power for the genetic association analysis, we included a total of 667 participants (20.4% children, and 79.6% adults) who participated in the AIR registry. It is important to note that we confirmed the utility of this marker for differentiating type 2 diabetes risk across age groups in the Latino population prior to testing genetic associations (data not published). Briefly, a total of 584 participants were available to be classified by either monophasic or biphasic glucose response curve phenotypes. A monophasic glucose response curve (n=435) was the preponderant phenotype compared to the biphasic glucose response curve (n=149). Similar with our published youth study (Kim et al., 2012), a monophasic glucose response was associated with a more deleterious anthropometric and metabolic profile, including higher BMI, fat mass, waist and hip circumferences, triglyceride, blood pressures, HbA1c, and lower HDL, Matsuda index, insulinogenic index, and disposition index (all $P < 0.05$). Overall, our data revealed that the pattern of plasma glucose

response during an OGTT differentiates the risk for type 2 diabetes in the Latino population across age groups.

When the associations between candidate genetic variants (n=59; representing 31 genes/loci) and the glucose response curve were examined, we identified 15 SNPs nominally associated with this marker. These identified SNPs represented 8 unique genes as follows: ADIPOR2, FTO, NOTCH2, GCKR, SLC30A8, TCF7L2, CRY2, and MTNR1B. We further confirmed that directions of increased prevalence for the monophasic response (i.e., increased risk for type 2 diabetes) of these SNPs were concordant with either previous GWAS findings or our own (DeMenna et al., 2014; Dupuis et al., 2010; Richardson et al., 2006; Saxena et al., 2007; Scott et al., 2007; Zeggini et al., 2008, 2007). Therefore, based on the associations between the glucose response curve and multiple SNPs, we obtained the information about the biological contributions to this marker. Our data suggests that the shape of the mono phasic response is linked with energy homeostasis, regulation of glucose and lipid metabolism, and β-cell function.

In addition to the glucose response curve, we further tested the utility of the 1-hr glucose level during an OGTT as another novel glycemic marker among 201 obese Latino youth who were followed for up to 8 years (mean 4.7 $\pm$ 2.7 years) (Kim et al, 2013). We demonstrated that participants with 1-hr glucose ≥155 mg/dL at baseline exhibited a significantly lower β-coefficient for disposition index obtained from the FSIVGTT, indicating greater deterioration of β-cell function over time. Moreover, when data was restricted to NGT participants, we also observed that those with 1-hr glucose concentrations ≥155 mg/dL at baseline were 2.5 times more likely to develop prediabetes

over time. Importantly, our findings were also independent of traditional glycemic indicators (i.e., fasting and 2-hr glucose). These data extend previous cross-sectional studies in youth (Tfayli et al., 2011) and support the potential prospective utility of 1-hr glucose concentrations during an OGTT to identify youth at a higher risk for developing type 2 diabetes.

Furthermore, we also compared the predictive power of 1-hr glucose to traditional glycemic indicators including HbA1c, fasting, and 2-hr glucose for identifying progression to prediabetes in obese Latino youth (Kim et al., 2014). Of the 116 obese normoglycemic Latino youth at baseline, 49.1% of the study participants experienced progression to prediabetes at least more than one time while 52.9% maintained their NGT status throughout each follow-up. When the area under the ROC curve (i.e., predictive power) was estimated, we observed that 1-hr glucose is the single best predictor for identifying future prediabetes compared to HbA1c, fasting, and 2-hr glucose. In a series of studies involving the 1-hr glucose level, we also attempted to find which genetic components were involved in this biomarker. Much larger cohorts followed over longer periods to definitively test the utility of 1-hr glucose concentrations to predict the development of overt type 2 diabetes in youth are warranted.

Prior to testing genetic determinants of 1-hr glucose, we confirmed the utility of 1-hr glucose level for differentiating type 2 diabetes risk across age groups in the AIR registry, which were used in the genetic association analysis (data not published). In order to improve statistical power to detect the genetic associations, we analyzed this marker as a continuous variable (instead of dichotomously; above or below 1-hr glucose of 155 mg/dL). Briefly, 1-hr concentrations obtained from the OGTT were found to

increase alongside BMI, fat mass, waist and hip circumference, blood pressures, lipid profile, HbA1c, fasting and 2-hr glucose, and HOMA-IR (all $P < 0.05$). Additionally, 1-hr glucose concentrations were negatively and significantly correlated with Matsuda index, insulinogenic index, and disposition index (data not published). Overall, our data confirmed the utility of 1-hr glucose level during an OGTT to identify Latino youth and adults at a higher risk for type 2 diabetes.

When the genetic determinants of 1-hr glucose were tested by using 59 genetic variants which represented 31 genes/loci, we identified 18 SNPs nominally associated with this marker. The identified SNPs represented 9 unique genes as follows: IGF2BP2, GCK, CRY2, KCNQ1, ADIPOR2, PROX1, HHEX, LOC100420392, and FADS1. We further confirmed that directions of the elevated 1-hr glucose level (i.e., increased risk for type 2 diabetes) for these SNPs were concordant with previous findings from GWAS or candidate SNP studies (Dupuis et al., 2010; Morris et al., 2012; Richardson et al., 2006; Saxena et al., 2007). Therefore, based on the associations between the 1-hr glucose and multiple SNPs/genes, we were able to gather the biological information on this marker. Our data suggests that an elevated 1-hr glucose during an OGTT was affected by the genes which were known as type 2 diabetes risk including energy homeostasis, regulation of glucose (including glucose cycling in liver or pancreas tissues) and lipid metabolism, β-cell function, and cell growth and development.

Lastly, in order to expand our knowledge of the pathogenesis of type 2 diabetes in the Latino population, we further genotyped 3 KCNQ1 SNPs (rs151290, rs2237892, rs2237895) and explored genetic association analysis with diabetes-related phenotypes in the AIR registry. Of them, 2 SNPs (rs151290 and rs2237892) exhibited nominal ($P <$

0.05) associations with multiple phenotypes. Consistent direction of increased risk in total cholesterol, LDL, DBP, FPG, prediabetes status, insulinogenic index, and disposition index was observed when the SNP rs151290 was analyzed. Similarly, rs2237892 was also found to be associated with consistent direction of elevated risk in SBP, FPG, 2-hr glucose, HbA1c, prediabetes status, insulinogenic index, and disposition index. In general, our data suggest that major allele C for both SNPs is considered a risk allele and our findings were concordant with previous findings (Hu et al., 2009; Qi et al., 2009; van Vliet-Ostaptchouk et al., 2012). The results of this study are promising since genetic variants in the KCNQ1 are not fully understood in relation to type 2 diabetes risk in Latino individuals despite its relatively high effect size compared to other candidate SNPs in other ethnicities (Hu et al., 2009; Qi et al., 2009; van Vliet-Ostaptchouk et al., 2012). Larger cohorts of the Latino population are warranted to replicate theses SNPs associated with the susceptibility to type 2 diabetes.

In summary, in order to find more accurate identification parameters for the risk of type 2 diabetes in a Latino population, we tested the utility of novel glycemic markers, which include the glucose response curve and 1-hr glucose level during an OGTT. Furthermore, we described the genetic determinants of these novel glycemic markers and explored the biological pathways that are possibly involved in the glucose response curve and 1-hr glucose level.

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APPENDIX A

DESCRIPTIVE CHARACTERISTICS OF ADULT PARTICIPANTS IN THE AIR REGISTRY BY GLUCOSE RESPONSE PHENOTYPE

Variables	Monophasic (*n*=327)	Biphasic (*n*=101)	*P*-value
Gender (Male/Female)	131 (40%) / 196 (60%)	31 (31%) / 70 (69%)	0.101
NGT/Prediabetes	170 (59%) / 118 (41%)	68 (73%) / 25 (27%)	**0.019**
Age (year)	38.04 ± 9.79	34.77 ± 7.51	**<0.001**
BMI (kg/m^2)	30.12 ± 5.81	28.84 ± 4.78	0.061
Fat mass (kg)	24.75 ± 10.96	22.90 ± 9.06	0.145
WC (cm)	99.18 ± 13.97	96.23 ± 11.81	0.069
HC (cm)	108.56 ± 11.97	107.38 ± 9.27	0.467
SBP (mmHg)	120.41 ± 14.64	116.27 ± 14.74	**0.009**
DBP (mmHg)	76.86 ± 9.21	74.17 ± 9.76	**0.008**
TRG (mg/dL)	140.07 ± 83.64	124.11 ± 71.09	0.074
HDL (mg/dL)	43.68 ± 10.99	46.86 ± 12.21	**0.014**
LDL (mg/dL)	108.81 ± 28.57	108.06 ± 27.40	0.973
VLDL (mg/dL)	22.02 ± 10.93	20.29 ± 11.06	0.122
Cholesterol (mg/dL)	176.51 ± 34.81	174.50 ± 33.51	0.652
ALT (U/L)	27.80 ± 18.05	23.21 ± 14.80	**0.007**
AST (U/L)	23.89 ± 9.92	23.76 ± 10.62	0.817
FPI (uIU/mL)	9.12 ± 6.62	8.61 ± 4.96	0.526
FPG (mg/dL)	95.34 ± 13.79	91.63 ± 9.45	**0.010**
HbA1c (%)	5.63 ± 0.33	5.48 ± 0.29	**<0.001**
2-hr glucose (mg/dL)	136.89 ± 46.12	124.35 ± 35.10	**0.025**
Glucose AUC (mg*dL-1*h-1)	18782.29 ± 5650.37	15521.07 ± 4847.83	**<0.001**
Insulin AUC (μU*mL-1*h-1)	9156.63 ± 5569.05	8161.23 ± 5954.13	**0.043**
Matsuda index	4.97 ± 3.45	5.90 ± 3.87	**0.037**
HOMA-IR	2.20 ± 1.66	2.02 ± 1.37	0.377
Insulinogenic index	1.19 ± 1.01	1.48 ± 1.01	**0.005**
Disposition index	5.07 ± 4.52	7.70 ± 5.95	**<0.001**

Data are means ± SD, n (%), NGT=normal glucose tolerance; Prediabetes, impaired fasting glucose and impaired glucose tolerance; BMI=body mass index; WC=waist circumference; HC=hip circumference; SBP=systolic blood pressure; DBP=diastolic blood pressure; TRG=triglyceride; HDL=high-density lipoprotein; LDL=low-density lipoprotein; VLDL=very low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FPI, fasting plasma insulin; FPG, fasting plasma glucose; AUC, area under the curve; HOMA-IR, homeostatis assessment of insulin resistance;

APPENDIX B

DESCRIPTIVE CHARACTERISTICS OF YOUTH AND ADULT PARTICIPANTS IN THE AIR REGISTRY BY GLUCOSE RESPONSE PHENOTYPE

Variables	Monophasic (n=435)	Biphasic (n=149)	P-value
Gender (Male/Female)	181 (42%) / 254 (58%)	53 (36%) / 96 (64%)	0.209
NGT/Prediabetes	253 (64%) / 141 (36%)	103 (73%) / 38 (27%)	0.061
Age (year)	32.63 ± 12.74	28.98 ± 10.56	**0.001**
BMI (kg/m^2)	29.19 ± 6.41	27.63 ± 5.35	**0.012**
Fat mass (kg)	23.42 ± 11.73	20.58 ± 9.71	**0.018**
WC (cm)	96.66 ± 15.83	92.81 ± 13.36	**0.013**
HC (cm)	107.18 ± 12.81	105.21 ± 10.49	0.127
SBP (mmHg)	118.79 ± 14.17	116.05 ± 14.02	**0.034**
DBP (mmHg)	75.16 ± 9.41	73.05 ± 10.31	**0.012**
TRG (mg/dL)	130.01 ± 79.17	115.01 ± 65.60	**0.023**
HDL (mg/dL)	43.51 ± 10.78	45.95 ± 11.02	**0.011**
LDL (mg/dL)	103.21 ± 28.87	98.95 ± 29.65	0.115
VLDL (mg/dL)	20.67 ± 10.71	18.93 ± 10.30	0.072
Cholesterol (mg/dL)	169.12 ± 35.63	163.30 ± 35.97	0.069
ALT (U/L)	25.64 ± 17.29	21.16 ± 13.06	**0.001**
AST (U/L)	23.52 ± 9.79	22.90 ± 10.18	0.355
FPI (uIU/mL)	9.51 ± 6.83	8.76 ± 5.20	0.210
FPG (mg/dL)	94.49 ± 12.44	91.08 ± 8.61	**0.001**
HbA1c (%)	5.61 ± 0.32	5.46 ± 0.29	**<0.001**
2-hr glucose (mg/dL)	132.40 ± 43.23	121.54 ± 32.09	**0.007**
Glucose AUC (mg*dL-1*h-1)	18189.20 ± 5183.01	15099.44 ± 4253.96	**<0.001**
Insulin AUC (μU*mL-1*h-1)	9645.36 ± 6085.70	8383.93 ± 5755.68	**0.016**
Matsuda index	4.94 ± 3.51	5.76 ± 3.63	**0.006**
HOMA-IR	2.24 ± 1.65	2.02 ± 1.32	0.706
Insulinogenic index	1.32 ± 1.11	1.68 ± 1.16	**<0.001**
Disposition index	5.27 ± 4.32	8.61 ± 6.76	**<0.001**

APPENDIX C

DESCRIPTIVE CHARACTERISTICS OF YOUTH PARTICIPANTS IN THE AIR REGISTRY BY 1-HOUR GLUCOSE

Variables	<155 mg/dl (*n*=101)	≥155 mg/dl (*n*=57)	*P*
Gender (Male/Female)	47 (47%) / 54 (53%)	26 (46%) / 31 (54%)	0.522
NGT/Prediabetes	91 (90%) / 10 (10%)	29 (53%) / 26 (47%)	**<0.001**
Mono/Biphasic response	57 (58%) / 42 (42%)	51 (59%) / 6 (11%)	**<0.001**
Age (year)	16.61 ± 2.67	16.12 ± 2.66	0.276
BMI (kg/m^2)	25.30 ± 6.33	27.15 ± 7.40	0.118
Fat mass (kg)	17.17 ± 11.38	20.43 ± 13.01	0.151
WC (cm)	87.01 ± 16.86	89.81 ± 17.53	0.321
HC (cm)	101.58 ± 12.98	103.53 ± 14.40	0.407
SBP (mmHg)	113.74 ± 11.01	115.02 ± 13.31	0.601
DBP (mmHg)	69.79 ± 9.03	70.75 ± 9.15	0.509
TRG (mg/dL)	90.08 ± 46.18	113.67 ± 57.21	**0.003**
HDL (mg/dL)	43.88 ± 8.82	42.61 ± 10.58	0.303
LDL (mg/dL)	81.28 ± 24.13	91.04 ± 21.48	**0.006**
VLDL (mg/dL)	15.14 ± 7.71	19.00 ± 9.53	**0.003**
Cholesterol (mg/dL)	140.16 ± 28.72	153.05 ± 25.76	**0.003**
ALT (U/L)	17.06 ± 7.91	21.13 ± 15.21	0.113
AST (U/L)	21.25 ± 8.08	23.33 ± 10.89	0.216
FPI (uIU/mL)	9.72 ± 7.02	10.45 ± 6.47	0.380
FPG (mg/dL)	89.96 ± 6.12	93.42 ± 6.33	**0.001**
HbA1c (%)	5.47 ± 0.30	5.55 ± 0.29	0.095
2-hr glucose (mg/dL)	105.84 ± 19.51	139.22 ± 26.97	**<0.001**
Glucose AUC (mg*dL-1*h-1)	14116.07 ± 1723.98	18533.16 ± 1945.73	**<0.001**
Insulin AUC (μU*mL-1*h-1)	8874.23 ± 5158.57	13016.87 ± 8360.60	**0.001**
Matsuda index	5.72 ± 3.78	3.91 ± 2.65	**<0.001**
HOMA-IR	2.10 ± 1.47	2.44 ± 1.59	0.213
Insulinogenic index	1.94 ± 1.34	1.53 ± 1.22	**0.020**
Disposition index	8.99 ± 6.47	4.32 ± 2.93	**<0.001**

DESCRIPTIVE CHARACTERISTICS OF ADULT PARTICIPANTS IN THE AIR REGISTRY BY 1-HOUR GLUCOSE

Variables	<155 mg/dL (*n*=189)	≥155 mg/dL (*n*=262)	*P*-value
Gender (Male/Female)	64 (34%) / 125 (66%)	101 (39%) / 161 (61%)	0.179
NGT/Prediabetes	166 (88%) / 22 (12%)	73 (37%) / 127 (63%)	**<0.001**
Mono/Biphasic response	109 (59%) / 77 (41%)	214 (90%) / 24 (10%)	**<0.001**
Age (year)	34.27 ± 8.32	40.20 ± 10.01	**<0.001**
BMI (kg/m^2)	28.91 ± 5.20	30.98 ± 5.96	**<0.001**
Fat mass (kg)	23.13 ± 9.72	26.11 ± 11.32	**0.005**
WC (cm)	96.09 ± 13.08	101.31 ± 13.74	**<0.001**
HC (cm)	107.41 ± 11.47	109.93 ± 11.77	**0.021**
SBP (mmHg)	115.91 ± 12.70	123.01 ± 15.90	**<0.001**
DBP (mmHg)	73.82 ± 8.37	78.67 ± 9.99	**<0.001**
TRG (mg/dL)	116.84 ± 65.03	151.57 ± 85.54	**<0.001**
HDL (mg/dL)	46.70 ± 12.61	42.56 ± 9.82	**<0.001**
LDL (mg/dL)	104.43 ± 25.84	111.82 ± 28.55	**0.020**
VLDL (mg/dL)	18.91 ± 9.03	24.10 ± 11.90	**<0.001**
Cholesterol (mg/dL)	170.47 ± 31.03	180.52 ± 35.09	**0.003**
ALT (U/L)	23.04 ± 14.46	30.40 ± 19.54	**<0.001**
AST (U/L)	23.06 ± 9.81	25.14 ± 10.81	**0.023**
FPI (uIU/mL)	7.91 ± 5.24	10.27 ± 7.26	**0.006**
FPG (mg/dL)	89.09 ± 6.73	100.43 ± 17.04	**<0.001**
HbA1c (%)	5.49 ± 0.27	5.70 ± 0.35	**<0.001**
2-hr glucose (mg/dL)	107.37 ± 21.43	162.70 ± 52.07	**<0.001**
Glucose AUC (mg*dL-1*h-1)	14111.26 ± 1722.69	21092.66 ± 4917.30	**<0.001**
Insulin AUC (μU*mL-1*h-1)	7515.18 ± 5059.99	10281.22 ± 5907.72	**<0.001**
Matsuda index	6.37 ± 3.75	4.10 ± 3.07	**<0.001**
HOMA-IR	1.75 ± 1.78	2.55 ± 1.83	**<0.001**
Insulinogenic index	1.58 ± 1.16	1.00 ± 0.76	**<0.001**
Disposition index	8.57 ± 5.77	3.15 ± 2.03	**<0.001**

APPENDIX E

DESCRIPTIVE CHARACTERISTICS OF YOUTH AND ADULT PARTICIPANTS IN THE AIR REGISTRY BY 1-HOUR GLUCOSE

Variables	<155 mg/dL (*n*=290)	≥155 mg/dL (*n*=319)	*P*-value
Gender (Male/Female)	111 (38%) / 179 (62%)	127 (40%) / 192 (60%)	0.380
NGT/Prediabetes	257 (89%) / 32 (11%)	102 (40%) / 153 (60%)	**<0.001**
Mono/Biphasic response	166 (58%) / 119 (42%)	265 (90%) / 30 (10%)	**<0.001**
Age (year)	28.12 ± 10.89	35.90 ± 12.99	**<0.001**
BMI (kg/m^2)	27.65 ± 5.87	30.29 ± 6.40	**<0.001**
Fat mass (kg)	21.05 ± 10.70	25.09 ± 11.83	**<0.001**
WC (cm)	92.92 ± 15.12	99.25 ± 15.12	**<0.001**
HC (cm)	105.38 ± 12.31	108.78 ± 12.51	**0.001**
SBP (mmHg)	115.16 ± 12.16	121.57 ± 15.75	**<0.001**
DBP (mmHg)	72.41 ± 8.81	77.26 ± 10.29	**<0.001**
TRG (mg/dL)	107.52 ± 60.43	144.71 ± 82.38	**<0.001**
HDL (mg/dL)	45.72 ± 11.50	42.57 ± 9.94	**0.001**
LDL (mg/dL)	96.31 ± 27.54	108.00 ± 28.52	**<0.001**
VLDL (mg/dL)	17.59 ± 8.76	23.16 ± 11.66	**<0.001**
Cholesterol (mg/dL)	159.91 ± 33.48	175.67 ± 35.19	**<0.001**
ALT (U/L)	20.96 ± 12.88	28.74 ± 19.15	**<0.001**
AST (U/L)	22.43 ± 9.28	24.81 ± 10.83	**0.003**
FPI (uIU/mL)	8.56 ± 5.99	10.31 ± 7.08	**0.011**
FPG (mg/dL)	89.39 ± 6.53	99.14 ± 15.86	**<0.001**
HbA1c (%)	5.48 ± 0.28	5.67 ± 0.34	**<0.001**
2-hr glucose (mg/dL)	106.85 ± 20.77	158.34 ± 49.22	**<0.001**
Glucose AUC (mg*dL-1*h-1)	14112.94 ± 1720.15	20633.88 ± 4633.32	**<0.001**
Insulin AUC (μU*mL-1*h-1)	8008.43 ± 5127.97	10837.62 ± 6555.15	**<0.001**
Matsuda index	6.13 ± 3.77	4.06 ± 2.98	**<0.001**
HOMA-IR	1.88 ± 1.30	2.53 ± 1.78	**<0.001**
Insulinogenic index	1.71 ± 1.24	1.11 ± 0.89	**<0.001**
Disposition index	8.72 ± 6.02	3.38 ± 2.28	**<0.001**