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The Influence of Differing Characteristics upon the Potential for a Reversal from a State of Impaired Glucose Tolerance to One of Normal Glucose Tolerance

Rose Benedicte Gregoire

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By:
Rose Benedicte Gregoire
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Introduction: Diabetes is one of the leading causes of both morbidity and mortality worldwide and is a pressing concern since these figures are on the rise. The International Diabetes Federation indicated that approximately 463 million adults currently have diabetes globally, with an additional 374 million individuals living in prediabetic state (IDF, 2019). Diabetes is also at the forefront with regard to costs in that it has risen from $245 billion in 2012 (American Diabetes Association, 2018) to an estimated $327 billion in 2017—the latter of which is comprised of $237 billion in direct medical costs along with the remaining $90 billion incurred as a result of absenteeism, a reduced ability to work, and a general reduction in overall productivity (American Diabetes Association, 2018). While the transition from a state of prediabetes (i.e., impaired glucose tolerance) to overt diabetes has been heavily studied, there is a dearth of existing literature targeting the reversal from a state of impaired glucose tolerance (IGT) to one of normal glucose tolerance (NGT). Preventative measures undertaken to circumvent the likelihood of developing type 2 diabetes can more adeptly be accomplished by transitioning from a state of IGT back to a state of NGT before it is too late. Evaluating cardiovascular, glucoregulatory, lifestyle, and demographic characteristics along with urinary biomarkers, the baseline lipid profile, and anthropometric measures occurring amongst subjects in a state of IGT at baseline may shed light on potential predictors involved in the reversal back to NGT.

Aim: The goal of this study is to evaluate the possible predictors that may incite the regression from a state of impaired glucose tolerance (IGT) to one of normal glucose tolerance (NGT).

Methods: This study analyzed existing epidemiological data from the total insulin resistance atherosclerosis study (IRAS) cohort of 1,625 participants using an exploratory data analysis (EDA) approach. This study evaluated only participants with Impaired Glucose Tolerance (IGT) when measured at baseline (n=303). Subjects who transitioned from IGT to NGT (n=81) were compared to those who remained in a state of IGT during both visits (n=121) along with subjects who progressed from IGT to a state of overt type 2 diabetes at follow-up (n=101). Group differences for continuous and categorical variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis chi-square tests, respectively.

Results: Male participants were more likely to experience a successful reversal from a state of IGT to one of NGT compared to their female peers. Menopausal status was a statistically significant characteristic serving as an impediment in the transition from a state of IGT to one of NGT. Subjects with elevated BMIs were significantly less likely to reverse from IGT to NGT compared to those with lower BMIs.

Conclusion: The results suggest that female participants are less likely to experience a reversal from a state of impaired glucose tolerance to one of normal glucose tolerance compared to male participants. This finding appears to be contingent upon menopausal status when measured during both baseline and follow-up visits, respectively.

Keywords: normal glucose tolerance, impaired glucose tolerance, type 2 diabetes, prediabetes reversal.

Approval Page

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By:

Rose Benedicte Gregoire

Approved :

Dora Il'yasova, PhD
Committee Member

Ike S Okosun, MS, MPH, PhD
Committee Chair

July 16, 2021
Date

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The author of the thesis is:

Rose Benedicte Gregoire

3592 Garden Lakes Parkway NW

Rome, Georgia, 30165

The chair of the committee for this thesis is:

Dr. Ike Okosun

Georgia State School of Public Health

Diversion of Epidemiology & Biostatistics

Georgia State University

P.O. Box 3995

Atlanta, GA 30302-3995

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ABBREVIATIONS

BIE	Basal Insulin Effect
BMI	Body Mass Index
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DPP	Diabetes Prevention Program
EDA	Exploratory Data Analysis
F_2 -IsoPs	F_2 – Isoprostanes
FPG	Fasting Plasma Glucose
GEZI	Glucose Effectiveness at Zero Insulin
HDL	High Density Lipoprotein
HOMA	Homeostasis Model Assessment
HOMA-IR	Homeostasis Model Assessment for Insulin Resistance
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IL	Interleukin
IRAS	Insulin Resistance and Atherosclerosis Study
LDL	Low-Density Lipoprotein
MetS	Metabolic Syndrome
MHO	Metabolically Healthy Obesity
MUO	Metabolically Unhealthy Obesity
NGR	Normal Glucose Regulation
NGT	Normal Glucose Tolerance
NCEP/ATPIII	National Cholesterol Education Program Adult Treatment Panel III
NHANS	National Health and Nutrition Examination Survey
OGTT	Oral Glucose Tolerance Test
ox-LDL	Oxidized Low-Density Lipoprotein
P-value	Probability value (observed significant level)
RCT	Randomized Controlled Trial
ROS	Reactive Oxygen Species

S_G	Glucose Effectiveness
S_I	Insulin Sensitivity
TNF- α	Tumor necrosis factor alfa
T2D	Type 2 diabetes
2hrPG	Two-hour plasma Glucose

Chapter I

Introduction

1.1 Background

Prediabetes is an intermediate stage between normal glucose regulation and the onset of type 2 diabetes, which is problematic in that it leads to increased risks for the development of diabetes mellitus along with the potential onset of vascular complications [1]. More specifically, worldwide estimates obtained by the International Diabetes Federation indicate that while nearly 463 million adults are currently living with diabetes, another 374 million adults are also prediabetic [2]. While this figure is already high, the anticipated global diabetes prevalence is projected to rise to 700 million by 2045 alone, which is why it is imperative that the precursors and biomarkers that may lead up to this are discovered and addressed. Despite the existence of randomized controlled trials (RCTs) that have demonstrated relative success in preventing the progression from prediabetes to type 2 diabetes, they were nonetheless limited in that they considered lifestyle factors or pharmacological interventions only. Unfortunately, these two methods alone are problematic in that medications lose efficacy upon discontinuation, and lifestyle modification is effective solely upon the condition that it becomes an ingrained habit that is interwoven into the fabric of one's daily routine.

Moreover, while lifestyle intervention protocols have been more effective in terms of preventing or mitigating the progression from prediabetes to type 2 diabetes, this is not the case with respect to the reversal of a state of impaired glucose tolerance (IGT) back to one of normal glucose tolerance (NGT) instead. This poses a challenge due to the lack of extant studies that have deliberately focused upon the pre-specified reversal of prediabetes and the restoration of normal glucose regulation as a primary outcome of interest. The lack of research in this area is of

concern due to the emerging evidence indicating that the reversal of prediabetes to a state of NGT is highly important. This reversal confers benefits that include protection from the future development of diabetes, the minimization of complications stemming from this state, along with the provision of an increased likelihood of longevity during the long-term follow-up of subjects.

Given that prediabetes is an intermediary step towards the onset of type 2 diabetes, understanding the complex interplay involved in the transition is necessary to tailor interventions to those at risk before this rare window of opportunity closes. Extant research has found that some of the notable predictors that are accountable for the transition from normal glucose regulation to prediabetes include variables such as being male, older in age, having a higher BMI, living with a larger waist circumference, along with having a higher conglomeration of total fat and abdominal adipose tissue [3, 4, 5, 6, 7, 8]. Moreover, participants with higher fasting plasma glucose (FPG) and two-hour plasma glucose (2hrPG) levels are also more prone towards the eventual onset of prediabetes; this is in accompaniment to factors such as having lower levels of insulin sensitivity, insulin secretion, and disposition index [5].

Moreover, additional variables affiliated with the transition from normal glucose regulation to prediabetes include higher baseline plasma levels of LDL, cholesterol, and of triglycerides occurring in tandem with a lower level of HDL cholesterol [6, 7, 8]. The implication of these findings similarly resonates with respect to the reversal of prediabetes and the restoration of normal glucose regulation as well. More specifically, participants with lower baseline levels of FPG and of 2hrPG, who were younger in age, experienced greater insulin secretion, and who encountered weight loss were more likely to regress from a prediabetic state to one of NGT instead [9]. The primary body of research in this area is composed of RCTs that have investigated lifestyle and/or medication interventions with varying degrees of success. As a

result, this study goes one step further by evaluating the changes occurring over time with respect to measures of adiposity, the lipid profile, cardiovascular characteristics, glucoregulatory mechanisms, metabolites, along with anthropometric, lifestyle, and demographic characteristics of interest. In addition, the inclusion of F_2 – Isoprostanes into this comprehensive list of factors is a novelty that will hopefully shed light upon that which may lead to the successful regression from prediabetes back to a state of NGT.[1] Increasing our knowledge base on the underpinnings of a reversal from IGT to NGT can hopefully aid by increasing awareness, reducing the proliferation of diabetic comorbidities, and can hopefully save lives through a reduction of future risks, such as (but not limited to) cardiovascular disease (CVD) and premature mortality.

1.2 Purpose of the Study

The existing landscape illustrates that while a large amount of research has been conducted with respect to the evaluation of the progression from a state of IGT to the eventual onset of type 2 diabetes, there is a dearth of studies that examine the reversal of prediabetes to a state of normal glucose regulation. Despite the existence of several RCTs that demonstrate the utility behind lifestyle interventions and of pharmacological agents, they nonetheless focus specifically upon preventing the progression from prediabetes to type 2 diabetes [1]. As a result, few studies have evaluated the pre-specified reversal of prediabetes as a primary outcome of interest.

While the review undertaken by Sallar and Dagogo-Jack in 2020 is the most current and comprehensive analysis of the existing body of research in this area [1], this study takes it one step further by addressing some of the incongruous findings emerging with respect to participant characteristics, such as demographic, adiposity, and glucoregulatory measures along with

[1] amongst participants in a state of IGT at baseline when followed over time for five years.

differences in plasma lipid and amino acid levels. As a result, the purpose of this study is to determine which factors (if any) are associated with the regression from prediabetes to a state of normal glucose tolerance (NGT) in participants when compared to those who either remained prediabetic or else progressed entirely to a state of diabetes when evaluated over a five-year time frame.

While extant research has evaluated the impact of plasma lipid and amino acid levels along with glucoregulatory, adiposity, and demographic measures separately, this paper is novelty in that no study has fully explored the impact that these variables may have upon the reversal of impaired glucose tolerance (IGT) to normal glucose tolerance (NGT) when all of these factors are evaluated simultaneously. By accounting for as many variables as possible, this study attempts to investigate which ones might be associated with a regression from prediabetes to normal glucose regulation by using the Insulin Resistance Atherosclerosis Study (IRAS), which was the first epidemiologic study of its kind that was used and designed with the intention of analyzing relationships between insulin resistance, glycemia, insulinemia, diabetes, and ultimately of cardiovascular disease (CVD) within the context of a large multiethnic cohort [10]. In turn, the IRAS dataset provides a wealth of information through which to examine changes in glucose tolerance status over time due to its prospective cohort design.

1.3 Research questions and hypotheses

1). What is the impact of sex, ethnicity, menopausal status, and age in years on the reversal from a state of Impaired Glucose Tolerance back to one of Normal Glucose Tolerance?

2). What is the impact of weight, BMI, waist circumference, and waist/hip ratio on the reversal from a state of IGT back to one of NGT?

3.) What is the impact of Fasting Insulin, 2-Hr Insulin, Insulin Sensitivity, AIR, Fasting Glucose, 2-Hr Glucose, and Glucose Effectiveness on the potential for reversal from a state of IGT to one of NGT?

4.) What is the impact of Total Cholesterol, LDL, HDL, and Triglyceride levels, along with each specific F_2-Isoprostane/BMI ratio on the potential for reversal from a state of IGT to one of NGT?

5.) What is the impact of Hypertension, the Ankle/Brachial Index, Systolic BP, Diastolic BP, Mean CCA, and Mean ICA on the potential for reversal from a state of IGT to one of NGT?

6.) What is the impact of Smoking Status, Total Energy Expended, High Energy Expended, and Moderate Energy Expended on the potential for reversal from a state of IGT to one of NGT?

7.) What is the impact of urine creatinine and urine albumin on the potential for reversal from a state of IGT to one of NGT?

1.4 Thesis Organization

This thesis is organized into five chapters. Chapter I consists of an introduction of the issue accompanied by relevant background information, the purpose of the study, and subsequently includes the research questions and their respective hypotheses. The literature review is presented in Chapter II. The methods utilized, a description of the sample used, and the measures of interest are all described in Chapter III. Chapter IV is composed of the findings and outcomes associated with the study. Lastly, Chapter V consists of the discussion, a description of the strengths and of any potential limitations, and it in turn concludes with recommendations.

Chapter II: Literature Review

2.1 The Relationship between Glucose Homeostasis and the Progression of Diabetes

Diabetes is one of the leading causes of both mortality and morbidity worldwide, accompanied by cancer, heart disease and stroke. In the United States alone, 34.2 million Americans live with diabetes with another 88 million who are prediabetic, which attests to the enormity of this ever-increasing problem [11]. Diabetes also is at the forefront with regard to expenses in that by 2017, the costs associated with diabetes had steadily increased to an estimated $327 billion. While this figure is primarily composed of $237 billion in direct medical costs alone, the remaining $90 billion arose as a function of absenteeism, a lessened ability to work, along with a general reduction in overall productivity [12]. Diabetes is also a pressing concern worldwide; the International Diabetes Federation (IDF) illustrated that approximately 463 million adults currently have diabetes, with an additional 374 million individuals living in a prediabetic state as of 2019 alone [2].

These figures are on the rise; projections from the International Diabetes Federation anticipate that the global diabetes prevalence is expected to increase to approximately 700 million by 2045 [2]. Prospective studies indicate that risk factors associated with the onset of type 2 diabetes include a propensity towards being overweight/obese accompanied by a lack of physical activity [13, 14, 15]. These findings illustrate the extent to which a low level of physical activity—when in conjunction with obesity-- is associated with impaired glucose tolerance and the eventual onset of type 2 diabetes [15]. Additional research by Hu et. al in 2004 further underscores this link in that researchers concluded that exercise and type 2 diabetes are inversely associated [16]. More specifically, subjects with impaired glucose regulation experienced a nearly five-fold increase in the acquisition of diabetes when compared to participants with

normal glucose levels [16]. To combat the dangers associated with the onset of diabetes mellitus, researchers encourage lifestyle changes (such as alterations made with respect to diet and physical activity) as one approach through which to mitigate potential risk factors associated with disease progression [17].

While much attention is paid to the role of insulin, the onset of type 2 diabetes arises in part due to IGT, which is a disturbance in normal glucose homeostasis that subsequently leads to abnormal glucose regulation. This abnormality is a metabolic disruption that may enable the inception of intermediates-- IGT and impaired fasting glucose (IFG)--which may in turn lead to the eventual development of diabetes [18]. NGT is comprised of both fasting and 2-hour glucose concentrations (<140 mg/dl) [13]. IGT is defined as the presence of an elevated 2-hour plasma glucose concentration (>=140 and <200mg/dl) measured after the ingestion of a 75-gram glucose load during an oral glucose tolerance test (OGTT) when accompanied by a fasting plasma glucose concentration of <126mg/dl [13].

2.2 The Relationship between Glucose Effectiveness, Normal Glucose Tolerance (NGT), Impaired Glucose Tolerance (IGT), and Diabetes

Researchers studying glucose tolerance amongst 827 subjects (out of the initial 1625) within Insulin Resistance Atherosclerosis Study (IRAS) found a notable trend in that they discovered that glucose effectiveness, basal insulin effect (BIE), and glucose effectiveness at zero insulin (GEZI) all deteriorated synchronously with worsening glucose tolerance [19]. This suggests that subjects with NGT at baseline experienced a decline with respect to BIE, GEZI, and overall glucose effectiveness (S_G) when their status transitioned to either a state of IGT or else to one of full-blown diabetes [19]. Amongst those with NGT at baseline, the respective transition to either IGT or else to diabetes was in turn related to declines in S_G (P<0.001), BIE (P=0.009) and GEZI (P<0.001) [19]. This differed starkly in comparison to the findings obtained

for subjects with IGT at baseline in that longitudinal changes in terms of glucose tolerance status led to only mildly significant changes with respect to S_G (P=0.049) and BIE (P=0.042), whereas there was no statistically significant change in GEZI (P=0.332). These findings show that despite subjects reaping the benefits associated with being in a state of NGT, they may nonetheless experience declines in S_G and in GEZI, which accompany a deterioration in glycemic status. However, this is diametrically opposed to that which occurs amongst those who are prediabetic (IGT) given that they encounter only modest changes in S_G and in GEZI. The only exception to this occurs with respect to BIE, which worsens at all stages of glucose tolerance.

Consequently, these findings illustrate that while glucose effectiveness (S_G) is an important determinant of glucose metabolism [20], it also serves as a predictor of the development towards (and the eventual onset) of diabetes [21, 22]. Findings emerging from other studies indicate that S_G is reduced amongst those found to be in either a state of IGT or else who are categorized as being diabetic [22, 23]. This suggests that unlike those who are in a state of NGT, glucose effectiveness may be hard to differentiate in some cases in that it appears to be similar amongst those with IGT and diabetes [23].

2.3 Biomarkers that underlie the Regression from a Prediabetic State to One of Normal Glucose Regulation

While the transition from prediabetes to type 2 diabetes (T2D) has been intensively and abundantly studied, there is a lack of existing research pertaining to the regression from a state of IGT to one of normal glucose regulation. A review published in 2020 indicated that an increased focus should be placed upon the restoration of normal glucose regulation by reversing prediabetes through long-term follow-up and care [1]. Doing so may in turn mitigate future mortality and morbidity rates by reducing not only the complications emerging from diabetes but also by minimizing the risk of dying prematurely [1]. This newly published review from 2020

delves into the biomarkers that underlie the transition from NGT (or normoglycemia) to IGT and that potentially result from changes in factors such as (but not limited to) inflammation, increased adiposity, metabolomics, and glucoregulatory mechanisms [1]. Due to a wealth of extant research investigating the transition from a state of normal glucose regulation to either prediabetes and/or the onset of type 2 diabetes, results indicate that weight gain, deficits in insulin sensitivity, and alterations in pancreatic beta cell function are largely responsible for the rapid progression occurring between states of glucose tolerance and of their eventual deterioration over time.[2] Moreover, when accompanied by biological changes to incretin response that occur alongside changes in inflammatory cytokines and fluctuations in hepatic glucose production, these events happening in unison shed even more light upon the mechanisms that may be responsible for declines in health over time [24, 25].

Oxidative stress arises as a result of Reactive Oxygen Species (ROS), which are particles comprised of highly reactive groups of oxygen-containing molecules. More specifically, these molecules are produced during normal metabolic processes taking place amongst aerobic organisms, although the most notable occurrences in which they are generated happen in the mitochondria through reduction-oxidation reactions [27]. ROS induce damage through their ability to alter both the structural and functional properties of target molecules due to the manner in which they react with nucleic acids, proteins, and lipids [26]. ROS-induced oxidative damage in cells is avoidable when antioxidant defense mechanisms are working properly; a state of redox homeostasis is upheld when both the ROS levels and the antioxidant defenses are in balance [31]. However, this state of redox homeostasis is dismantled when there is an insufficient antioxidant defense system, which subsequently generates oxidative stress within the body. One

[2] Along with their eventual deterioration over time.

method used to measure an individual's oxidative status is through the utilization and measurement of biomarkers such as F_2-isoprostanes [27]. Polyunsaturated fatty acids easily react with reactive oxygen species, which in turn produce oxidation products (such as F_2-isoprostanes) that can be easily measured. F_2-isoprostanes can serve as a powerful tool with respect to quantification [27, 28]. The stability of these molecules enables researchers to both capture and in turn accurately reflect the full extent of the damage —the oxidative injury—occurring *in vivo* [30]. F_2-Isoprostanes play a critical role in that their elevated levels commonly serve as an indication of the occurrence of oxidative stress [27, 30]. Despite this, another interpretation is that these heightened levels may instead reflect the level of functioning with respect to the mitochondrial metabolism instead; some prospective studies have demonstrated that certain individuals with higher F_2-isoprostane levels nonetheless have a lower risk of diabetes and of weight gain [29, 31]. In the latter scenario, this relationship can potentially serve as a protective factor for individuals who are at risk of either developing IGT and/or of acquiring diabetes. More specifically, the isomer with the highest inverse association for those at risk of IGT and/or of type 2 diabetes is the 2,3-dinor-iPF2 α -III(F_2-isoP) in that its protective effects also appear to be amplified even amongst those who are obese [29]. Due to the importance of F_2-isoprostanes, their inclusion into this study can help to define the role that they may have (if any) with respect to IGT, along with the extent to which they may be involved in the reversal from a state of IGT back to one of NGT.

2.4 The Reversal of Prediabetes and Subsequent Restoration of Normal Glucose Regulation

While the bulk of extant research predominately focuses upon the transition from IGT to the onset of diabetes, the regression from prediabetes to a state of normal glucose regulation is of critical importance and should no longer be overlooked. It is preferable to reverse an individual's

condition from a state of prediabetes back to one of normal glucose regulation (NGR) since doing so can substantially lower the risk of acquiring T2D. A shift from IGT to NGT is accompanied by the minimization of future diabetes-related complications [1], which attests to the importance of addressing these health concerns early prior to the commencement and the eventual manifestation of symptoms. Nonetheless, several landmark RCTs have illustrated the importance of lifestyle modification interventions[3] with regard to health outcomes; researchers involved in the Malmö study found that after six years of follow up, 52% of subjects with IGT in the intervention group had successfully reverted back to a state of NGT [32]. This reversion to a state of normal glucose tolerance was complemented by modifications made in terms of blood pressure and hyperinsulinemia. This transition similarly led to improvements in the lipid profiles of the participants and was also accompanied by an amelioration in their overall acute insulin secretory responses to glucose [32].

A second study known as the Diabetes Prevention Program (DPP) similarly demonstrated a successful reversal from a state of IGT back to one of NGT amongst participants who were exposed to a lifestyle-based intervention; over 40% of subjects randomized to the lifestyle intervention group successfully regressed back to a state of NGT—a feat that is double the rate of reversal amongst those who were either exposed to the Metformin arm (20%) [9, 33] or else to the placebo [9, 33] instead. The two aforementioned studies indicated that subjects with a lower baseline fasting plasma glucose (FPG) and a reduced two-hour plasma glucose (2hrPG) when measured during an oral glucose tolerance test (OGTT) were more likely to successfully regress from a state of prediabetes back to one of NGT. Additionally, participants who engaged in sustained weight loss, who incurred greater insulin secretion, and who were younger in age were

[3] When compared to subjects in the placebo group.

significantly more likely to experience a reversal from prediabetes to a state of NGR in approximately 40% of participants, which is an important finding [9, 33]. Overall, both the DPP and the Malmö study demonstrate the value and the benefits associated with the lifestyle-based intervention due to the manner in which a regression from prediabetes to a state of NGR was achieved amongst 40% to 50% of participants [9, 33]. Additional RCTs attempted to evaluate the influence of pharmacological agents in order to determine their effectiveness in terms of initiating the regression from a state of IGT to one of NGT amongst participants when measured either independently or else in conjunction with a lifestyle intervention. One trial involving randomization to either Acarbose (or else to a placebo) yielded promising initial results in that over 35% of participants with IGT had successfully reverted to a state of NGT after a three-year follow-up period.

Despite this, the initially successful regression back to NGT was not sustained in that many participants unfortunately experienced a glycemic rebound after a mere three months [34]. Two additional RCTs investigated the influence of pharmacological agents within participants by testing the effects of Orlistat and Rosiglitazone in order to determine their effectiveness with respect to the initiation of a reversal from a state of IGT to one of NGT. Despite encountering initial success with around 48% of subjects having converted from a state of IGT to one of NGT, this gradually dwindled and is likely a testament to a waning in drug efficacy over time [35, 36, 37]. However, the sustained reversal from a state of IGT to one of NGT amongst the participants who did change their routine health habits and exercise behaviors serves as a testimony to the importance of lifestyle modifications [37, 38, 39, 40]. This finding contrasts against the results found for subjects placed on medication alone due to the manner in which their regression and subsequent ability to remain in a state of NGT was relatively fleeting [37, 38, 39, 40]. A similar

result from the Malmö study that builds upon this outcome is that after twelve years of follow-up, the participants who were successfully engaged in some form of a lifestyle modification experienced a mortality benefit as a result of the sustained intervention [32, 39].

While the substantial weight loss associated with bariatric surgery has led to a greater likelihood of reversing [4] diabetes along with concomitant improvements made for conditions such as hypertension and dyslipidemia, this procedure (if undertaken on its own) is by no means a permanent solution [42]. Even though bariatric surgery can effectively mitigate the incidence of type 2 diabetes, [41] researchers in one study found that a reassessment occurring within a ten-year time frame (after the bariatric surgery took place) nonetheless indicated a reappearance of diabetes in nearly 50 percent of the patients who had previously been in remission [43]. As such, this finding attests to the slowly declining efficacy associated with bariatric surgery; this is likely due to the fact that weight loss alone may not be fully sustained unless the patients have fully incorporated permanent lifestyle modifications into their post-surgery routines.

2.5 The Impact and Benefits of Regression from Prediabetes to Normal Glucose Regulation

Attempts made to incite a reversal from a state of IGT to one of NGT is beneficial and cost-effective in terms of prevention since it is achievable by non-surgical means. Efforts made to change habits early are essential in that doing so can minimize both the risks of morbidity and mortality in a manner that is far less feasible to accomplish upon the onset of type 2 diabetes. More specifically, prediabetes (i.e., IGT) is a condition that encompasses a wide variety of vascular complications such as retinopathy, neuropathy, nephropathy, stroke, myocardial infarction, and even cardiovascular death, all of which may be mitigated with early preventative efforts [25]. The benefits associated with a clinical regression from a state of prediabetes to NGT

[4] or else an amelioration of this.

cannot be overstated due to the manner in which those who have successfully achieved a state of normal glucose regulation (NGR) at least one or more times during a longitudinal study were able to attain a 56% lower risk of incident type 2 diabetes. However, this was not the case amongst subjects who were consistently prediabetic throughout the entirety of the follow-up period [43]. These findings subsequently illustrate how the risk reduction for the incidence of diabetes appear to increase concurrently with the number of times in which a participant was able to successfully achieve a state of NGR; even transitory states of NGR helped to diminish an individual's future risk of developing diabetes [43]. In addition to reducing the onset of type 2 diabetes, successfully regressing back to a state of NGT similarly led to a decrease in the prevalence of microvascular disease-- an outcome that endured over time (which is after a median follow-up period of 15 years) [46]. The regression of prediabetes along with the restoration of normal glucose regulation (NGR) in the DPP/DPPOS cohort similarly produced a decreased macrovascular risk amongst participants [44]. Researchers evaluating the Whitehall study II cohort over time found that while the majority of subjects with HbA1c-defined prediabetes either remained in a state of IGT or else progressed to type 2 diabetes over a five-year period, there was nonetheless a small subset (of the participant population) that experienced a successful reversal from prediabetes to a state of normoglycemia [47]. While these alterations may be due to random fluctuations occurring amongst certain participants, the reversion from 2h PG-defined prediabetes back to a state of NGT was nonetheless beneficial in that it led to a reduction in the risks that are associated with both the onset of heart disease and of CVD-related mortality [47].

Addressing the onset of impaired glucose tolerance is necessary due to the limited success associated with lifestyle interventions alone. Despite the emergence of certain benefits,

these interventions (when undertaken alone) may be insufficient in terms of either reversing prediabetes or else in delaying the onset of type 2 diabetes. By targeting important biomarkers, addressing related glucoregulatory mechanisms, and evaluating alterations in adiposity, metabolites, and in the lipid profile, the ensuing research findings can hopefully help to shed light upon a host of variables responsible for inciting a reversal from a state of IGT back to one of NGT [1]. With a greater understanding of the predictors involved in this regression, further studies can hopefully aid in the creation of targeted and tailored interventions that can mitigate dysglycemia by inducing a reversal backwards to a state of NGT.

Chapter III

Methodology

3.1 Data Source and the Study Population

The data source utilized for the purpose of this secondary data analysis is the Insulin Resistance Atherosclerosis Study (IRAS), which was a prospective cohort epidemiological study that was a novelty due to the manner in which it examined a wealth of risk factors and their subsequent associations with diabetes and insulin resistance syndrome [5][10]. The study began in 1992 with the approval of the Institutional Review Board (IRB) of Wake Forest University School of Medicine [46]. The final sample consisted of a diverse population comprised of non-Hispanic whites (n=614), African Americans (n=464), and Hispanics (n=548) to ensure generalizable and all-encompassing results.

A total of 1625 participants aged between 40 to 69 years at baseline were recruited into this longitudinal study in the time frame spanning from October of 1992 to April 1994. The cohort was comprised of 719 male and 905 female participants, respectively. In order to ensure metabolic diversity, researchers tried to recruit an equivalent number of individuals deemed to be in a state of IGT, one of NGT, or else who were classified as being diabetic. Investigators accomplished this by oversampling nondiabetics who either exhibited an elevated fasting plasma glucose level or who were previously identified as being in a state of IGT. The baseline measurements taken indicated that the majority of subjects had a normal glucose tolerance status (44%), followed by a sizable portion of subjects who were either already diabetic (33%) or else were categorized as being in a state of IGT (23%). Subjects were enlisted from four specific clinical centers located in San Antonio, TX; Los Angeles, CA; San Luis Valley, CO; [and]

[5] to merely name a few of the ensuing conditions.

Oakland, CA. From 1997 to 1998, a follow-up examination was administered to the participants approximately five years after the initial baseline examination (mean 5.2 years [range 4.6-6.6]), which was accompanied by a high response rate of 81% [10]. This indicates that the majority of the initial cohort remained, with only 19% of subjects being lost to follow-up. Each participant gave his/her written informed consent, and the protocols were approved by local IRB committees for both the baseline and follow-up examinations [10].

3.2 Screening and Eligibility, the Sample Selection Process, and Variable Measurement

The oral glucose tolerance test (OGTT) was utilized to measure baseline glucose tolerance amongst participants, which is in direct accordance with the standards established by the World Health Organization [47]. Participants underwent two separate visits to properly obtain all of the required baseline information. With each visit lasting for approximately four hours, the time interval between the two consecutive appointments spanned from one to thirty days. Prior to the morning of the two visits, researchers asked that participants refrain from alcohol consumption, avoid high intensity exercise, and that they also fast for 12 hours beforehand [10]. The OGTT procedure is performed by taking an initial blood sample prior to imbibing a liquid containing 75 grams of glucose.[6] Blood was drawn again two hours after consuming a solution needed to measure the remaining amount of glucose found in the system of each respective subject. Insulin resistance was assessed by using the frequently sampled intravenous glucose tolerance test (FSIGT) during the first and second visits [10]. In order to ensure the accuracy of the insulin levels obtained amongst diabetics, subjects received an injection of insulin as opposed to tolbutamide. Researchers did so this to acquire adequate plasma insulin levels with greater ease given that some diabetics experienced either a blunted or an

[6] This procedure is used to measure a subject's fasting blood glucose level.

absent insulin response to glucose [48]. Researchers also utilized a reduced sampling protocol by requiring that only 12 plasma samples be collected as opposed to the 30 that are more frequently used in this procedure [48]. Measuring insulin resistance was accomplished through the administration of both regular human insulin (0.03 U/kg) and glucose in the form of a 50 % solution (0.3 g/kg). These were injected every 20 minutes amongst subjects through an intravenous line, with researchers inoculating participants with the glucose solution first [10]. 4 mL samples of blood were collected 12 separate times during a period of three hours through a second intravenous line. These twelve respective samples were subsequently assayed in order to measure the respective levels of plasma glucose and insulin concentrations. Participants fasted for a period of twelve hours prior to their blood being drawn in order to measure their lipids, fasting blood glucose levels, and the components of the biochemical profile more effectively.

Subjects filled out questionnaires regarding their self-reported conditions whereas information pertaining to prevalent CVD and peripheral vascular disease was acquired through the use of electrocardiographic and ultrasonographical methods. In addition to measuring blood pressure, trained medical personnel evaluated subjects noninvasively in order to acquire their respective anthropometric measurements. Body Mass Index (BMI) was calculated based on weight measured in kilograms (kg) divided by height (in meters squared) for each of the participants at baseline. During the first visit, F_2-Isoprostanes were measured in urine samples that were acquired and then stored at -70 degrees Celsius before being analyzed using liquid chromatography [49]. Although a total of 901 urine samples were initially collected, researchers only included 857 samples when attempting to measure the F_2 – Isoprostanes concentration. The concentration of F_2 – Isoprostanes was evaluated through the use of liquid chromatography/tandem mass spectrometry and the results were calibrated according to the

urinary concentration of creatinine. More specifically, creatinine was assayed using a fast electrospray ionization-tandem mass spectrometry method [49]. The inclusion of four different F_2 – Isoprostanes isomers was deliberate in order to measure them within the context of potential fluctuations in BMI over time in an attempt to evaluate the influence that they may have (if any) upon the potential for regression from a state of IGT back to one of NGT. The F_2 – Isoprostanes included were the following: iPF(2a)-III, 2,3-dinor-iPF(2a)-III, iPF(2a)-lV, along with 8,12-iso-iPF(2a)-lV. The F_2 -Isoprostanes index—the mean of the four aforementioned standardized F_2-Isoprostanes--was created based on the result for the four F_2 – Isoprostanes isomers. This index allowed for the ranking of participants to occur based upon a specific calculation, which is as follows: $[(X_{1i} – M_1)/SD_1 +(X_{2i} -M_2)/SD_2 +(X_{3i} -M_3)/SD_3 +(X_{4i} -M_4)/SD_4)/4]$. In the context of this formula, "i" is a code for a participant, X_{1-4} represent values indicative of the four F_2 – Isoprostanes isomers, M_{1-4} illustrates the mean of these four isomers, and SD_{1-4} stands for the standard deviation values.

In order to ensure the accuracy of the results, participants were interviewed in their preferred language [10]. The interview component of the two visits was comprised of questions regarding whether or not each subject had any existing history related to previous instances of myocardial infarction, stroke, coronary and/or carotid artery surgery, along with whether or not he/she had endured peripheral vascular surgery [10]. Race/ethnicity was acquired through a self-report manner, and nutrient intake data was both collected and assessed using a 114-item food frequency interview instrument adapted by the National Cancer Institute (NCI) and subsequently modified in order to account for both regional and ethnic food choices [50]. Information related to alcohol consumption—both recent and average lifetime use—was similarly acquired with questionnaires. Participants filled out surveys in order to obtain information regarding physical

activity; responses were based on a one-year activity recall to improve feasibility and to ultimately aid in their recollection of events [51]. In order to properly assess engagement in physical activity, modifications were made to questions in an attempt to enhance the responsiveness of subjects. This was accomplished by accounting for wide variety of activities undertaken either at home, while at work, or even for leisurely purposes given that there was a differential occurring between those living in urban environments relative to subjects residing in rural areas instead [10].

3.3 The Definitions of "IGT" and "NGT" along with Their Respective Classifications

Since the purpose of this study was to evaluate those who were identified as being in a state of IGT at baseline, this reduced the overall sample size from 1625 to only 303 subjects. When measured amongst participants at baseline, IGT was defined as having a fasting glucose < 140 mg/dL and a 2-hr glucose ≥ 140 and <200 mg/dL when using the oral glucose tolerance test (OGTT). In order to differentiate between the two states, those categorized as having NGT had both a fasting and a 2-hr glucose level < 140 mg/dL, whereas those who were diagnosed as diabetic instead were exhibiting fasting glucose levels of ≥ 140 mg/dL or 2-h glucose ≥ 200mg/dL. By evaluating participants with IGT at baseline, there were only three possible combinations (IGT to NGT, IGT to IGT, and IGT to T2D) from which to transition upon visit 2.[7]

However, this group of 303 participants was bifurcated into only two groups (as opposed to the initial three combinations) upon which to make comparisons for simplicity. The first group contains those who successfully reversed from a state of IGT back to one of NGT (n=81), whereas the second group is comprised of subjects who did not successfully transition backwards

[7] This led to a smaller cohort of only 303 individuals.

to a state of NGT. By combining those who remained in a state of IGT along with participants who transitioned from IGT to T2D, this led to the second group being composed of 222 subjects.

3.4 Analytical Cohort

The analytical cohort is comprised solely of participants categorized as being in a state of IGT at baseline. Despite 370 participants having been initially classified as having IGT during visit 1, only 303 individuals were ultimately included given that 65 participants were lost to follow-up. Amongst the remaining 303 subjects, 81 individuals successfully reversed from a state of IGT to one of NGT. The majority of participants (n=222) either remained in a state of IGT during both visits or else had developed type 2 diabetes.

3.5 Statistical Analysis

This study utilizes exploratory data analysis (EDA) in the examination of the Insulin Resistance Atherosclerosis (IRAS) cohort—a dataset initially comprised of 1624 participants. The requisite analyses were performed using SAS software (version 9.4). Descriptive statistics were computed for the entire baseline sample of the IRAS (n=1624) prior to analyzing those who were classified as being in a state of IGT.[8] While there were 370 individuals with IGT at baseline, the final sample consisted of only 303 participants due to 65 subjects being classified as "missing" upon follow-up.[9] Evaluating statistical significance for the categorical variables across both groups[10] was acquired using the Wald Chi-square test. However, given the small sample size (n=189) associated with measuring menopausal status during visits, Fisher's exact test was utilized instead to account for this stipulation.

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[8] This serves as a baseline against which to compare for those who had IGT at baseline relative to the entire cohort.
[9] after the requisite five-year time span had passed.
[10] those who "reversed from IGT back to NGT" vs. subjects who had "no transition to NGT."

In order to properly evaluate the continuous variables, a univariate analysis was utilized in SAS. Basic descriptive statistics such as the mean, median, the number of missing subjects, and the interquartile range are provided. Tests for normality were run beforehand to ensure that the continuous variables of interest could be properly analyzed with the Wilcoxon rank-sum test along with the Kruskal-Wallis test prior to their inclusion. After passing an inspection to ensure normality, every continuous variable was analyzed with a Wilcoxon rank-sum in order to determine the presence of a significant difference (if any) that may have emerged between the two separate groups. Analyzing differences between these two groups both at baseline and again five years later (during the follow-up appointment) was accomplished. This was done in order to determine if there were any significant differences emerging with respect to each variable (across both groups) through the utilization of p-values generated in SAS. The results were found to be of a statistically significant nature if the resulting p-value for a variable was less than 0.05. If the opposite held true, then the results generated were not considered to be significantly different across both groups of interest.

After making comparisons in which both the continuous and categorical variables were evaluated independently across both groups for visits 1 and 2, we then tabulated the difference occurring over time (or "delta"). The difference is equivalent to the results for visit 2 subtracted from the outcomes found for visit 1. By measuring the delta and then calculating the p-value for each variable of interest, this can help to determine whether or not the comparison of change was in fact significantly different between those who transitioned from a state of IGT back to NGT when compared to the subjects who did not. Analyzing the difference over time for each variable was necessary in order to determine whether or not a statistically significant increase or decrease was occurring when measured between these two groups. By looking at a wide array of

variables, this study is a novelty due to its inclusion of as many factors as possible[11] in order to glean insight into a sector of diabetes research that so far has received limited attention.

For the purpose of this study, the continuous and categorical variables were organized into seven respective tables and grouped according to key commonalities. For instance, Table I is comprised of the demographic characteristics of participants with IGT at baseline and it encompasses age, gender, race/ethnicity, and menopausal status. Table 2 consists of the baseline anthropometric measures amongst participants with IGT at baseline and contains variables such as weight, BMI, average waist circumference, and concludes with a waist/hip ratio measurement. Table 3 encompasses the glucoregulatory characteristics of those with IGT at baseline, which ultimately consists of variables such as FPG, 2-hr glucose, glucose effectiveness, insulin sensitivity, AIR, fasting insulin, and 2-hr insulin. Table 4 is comprised of the baseline lipid profile of participants in a state of IGT at baseline; the variables consist of total cholesterol, triglycerides, HDL, and LDL levels, respectively. Table 5 contains the cardiovascular characteristics of the participants with IGT at baseline, which involve blood pressure measurements, variables pertaining to the carotid arteries, and the ankle/brachial index. Table 6 accounts for the baseline lifestyle characteristics of participants with IGT during visit one, and it is comprised of several variables connected to variant levels of energy expenditure along information related to with the self-reported smoking patterns of subjects. Lastly, table 7 encapsulates the remaining characteristics[12] which consist of urinary biomarkers (such as creatinine, albumin, and the four aforementioned F_2-Isoprostanes). Through the incorporation of as many variables as possible, this study attempts to motivate future work and further analyses

[11] All of which are taken from the IRAS data set.
[12] that did not fall into any of the aforementioned categories.

within this area.[13] By emphasizing the importance of prevention, expounding upon the characteristics involved in the reversal from IGT back to a state of NGT is essential before it is too late to effect change.

[13] within the confines of the IRAS data set.

Chapter IV

Results

4.1 Description of the Entire Study Sample

The study population was initially comprised of the entirety of the IRAS cohort—a total of 1625 participants—when measured at baseline. 55.73% of subjects were female with 44.27% of participants identifying as male. Non-Hispanic Whites accounted for the largest subgroup (37.68%), followed by Hispanics (33.74%) and Non-Hispanic Blacks (28.57%) upon further stratification. The cohort was primarily composed of subjects in the age range from 60 to 69 (37.65%), followed by those aged from 50 to 59 years old (34.20%) with only 28.16% of subjects between 40 to 49 years of age. In terms of lifestyle habits and behaviors, most participants had never smoked (43.67%), with a slightly smaller percentage indicating that they had a former history of smoking but had since quit (39.65%). Current smokers (of which there were 270) accounted for the smallest group (16.68%) when assessed by self-report. With respect to hypertension, the majority had normal blood pressure readings (61.01%), with hypertensive participants being in the minority (38.99%) at baseline. In terms of overall diabetes status, 718 participants exhibited normal glucose tolerance (NGT) levels when measured at baseline (44.21%). In addition to those with NGT, 537 subjects were identified as having diabetes (33.07%) with a subset of only 369 individuals categorized as being in a state of IGT (22.72%). Moreover, with 41.62% of subjects having been classified as overweight and another 37.27% found to be obese, only 21.09% of participants were classed as being within the normal weight range.

Table 1. Categorical Variables that are Baseline Characteristics for the Entire IRAS Cohort

Categorical Variables	**N (missing values)**	**Percent**
Gender	1624 (0)	100
Males	719	44.27
Females	905	55.73
Race/Ethnicity	1624 (0)	100
Non-Hispanic White	612	37.68
Non-Hispanic Black	464	28.57
Hispanic	548	33.74
Diabetes Status	1624 (0)	100
Normal Glucose Tolerance (NGT)	718	44.21
Impaired Glucose Tolerance (IGT)	369	22.72
Diabetic	537	33.07
Hypertension	1616 (8)	100
Yes (High)	630	38.99
No (Normal)	986	61.01
Smoking Status	1619 (5)	100
Never Smoked	707	43.67
Former Smoker	642	39.65
Current Smoker	270	16.68
Weight Categorization	1617 (7)	100
Normal	341	21.09
Overweight	673	41.62
Obese	603	37.29

Table 2. Continuous Variables that are Baseline Characteristics for the Entire IRAS Cohort

Continuous Variables	N (missing values)	Mean (SD)
Age (measured in years)	1624 (0)	55.647 (8.469)
Fasting Glucose (mg/dl)	1623 (1)	123.269 (49.53)
BMI (kg/m^2)	1617 (7)	29.47 (5.978)
2-hour Glucose (mg/dl)	1612 (12)	186.52 (104.567)
Triglycerides (mg/dl)	1615 (9)	152.17 (120.33)
Fasting Insulin (uU/ml)	1620 (4)	18.37 (15.52)
HDL (mg/dl)	1615 (9)	45.040 (14.609)
F_2-IsoP Index[1]	627 (997)	0.030 (0.845)
8,12-iso-iPF(2a)-lV (ng/mg CN)	627 (997)	4.25 (2.938)
iPF(2a)-lV (ng/mg CN)	627 (997)	6.539 (4.1265)
2,3-dinor-iPF(2a)-III (ng/mg CN)	627 (997)	4.55 (3.223)
iPF(2a)-III (ng/mg CN)	627 (997)	0.251 (0.197)
Insulin sensitivity (SI, $x10^{-4}minutes^{-1}/\mu Uml$)	1482 (142)	1.645 (1.892)
Acute Insulin Response (microU/ml)	1560 (64)	345.358 (455.081)
Urine Creatinine (mg/dL)	1587 (37)	133.743 (80.81)
Urine Albumin (mg/L)	1592 (32)	22.955 (65.35)
LDL (mg/dl)	1560 (64)	141.224 (35.216)
AIR (mean of 2,4 min ins)	1535 (89)	53.152 (51.308)
Total Energy Expended (kcal/kg/yr) for Visit I	1596 (28)	14573.62 (2641.46)
High Energy Expended (kcal/kg/yr) for Visit I	1603 (21)	1173.07 (2381.38)
Moderate Energy Expended (kcal/kg/yr) for Visit I	1600 (24)	2642.40 (2454.00)

Note: The Index is the mean of four standardized F_2-Isoprostanes

4.2 Prevalence of participants with IGT when measured at Baseline

As shown in Table 1, 369 individuals were identified as being in a state of IGT at baseline (22.72%). Due to attrition occurring over the five-year time frame, only 303 of those with IGT at baseline remained.[14] At follow-up, the participants were bifurcated into two respective categories, the first of which consists of those who reversed from a state of IGT to one

[14] given that 66 subjects were counted as missing during visit 2.

of NGT (n=81). The second group contains subjects who remained prediabetic (i.e., IGT) during both visits (n=121) along with those who fully transitioned from a state of IGT to overt diabetes (n=101) which total to 222 when combined.

4.3 The Characteristics of Participants with IGT at Baseline and the Potential for Reversal

In table 3, sex (p=0.0438) and menopausal status (p=0.0350) were statistically significant, whereas this was not the case for ethnicity and age during visit 1. Amongst those who successfully regressed from a state of IGT back to NGT (n=81), 46.91% were male participants accompanied by a slightly higher composition of female subjects (53.09%). In table 4, subjects with a lower average waist circumference (mean of 91.70) were more likely to reverse from a state of IGT backwards to one of NGT; participants who failed to transition were more likely to have a higher average waist circumference (mean=96.07) (p=0.0061). Additionally, those with lower BMIs (mean=28.84) experienced a higher rate of regressing from a prediabetic state back to NGT relative to their peers with higher average BMIs (mean=31.10) (p=0.0056). Lastly, no statistically significant differences emerged across groups with respect to the waist/hip ratio (p=0.1324), weight (p=0.0853) and height (p=0.1003) in table 4.

As illustrated in table 5, those with a lower fasting insulin level (mean=15.43) at baseline were significantly more likely to reverse from a state of IGT back to NGT when compared to subjects who did not (mean=20.85) (p=0.0029). Moreover, individuals with greater levels of insulin sensitivity (mean=1.79) were significantly more likely to regress from IGT to NGT when compared to those with lower levels of insulin sensitivity (mean=1.10) (p=0.0035). Participants with a lower amount of fasting glucose (mean=100.73) were also more likely to experience a successful reversal of prediabetes relative to subjects who had higher fasting glucose levels (mean=106.11) (p=0.0003). Those with a greater acute insulin response (AIR) (mean=478.89)

were more likely to reverse from IGT to NGT when compared to those with a lessened AIR (mean=313.79) when calculated with the mathematical minimal model (p=0.0026). Participants exhibiting lower levels of 2-hr glucose (mean=155.38) were also more likely to experience a successful reversal back to a state of NGT relative to those who had higher levels of 2-hr glucose (mean=106.11) (p<.0001).

In table 6, participants exhibiting a greater HDL level (mean=48.67) were significantly more likely to transition backwards from a state of IGT to one of NGT when compared to those with a lower HDL level (mean=43.82) (p=0.0061). Subjects with higher levels of triglycerides (mean=163.08) were less likely to successfully return to NGT from a prediabetic state relative to participants with lower triglyceride levels (mean=131.52) (p<0.0001). However, other variables from which the baseline lipid profile is comprised did not generate significant findings between the two groups, such as total cholesterol (p=0.3559) and LDL levels (p=0.5428). As shown in table 7, there were no statistically significant links between cardiovascular characteristics and a subsequent transition from a state of IGT to NGT.

As showcased in table 8, there were no statistically significant outcomes occurring between baseline lifestyle characteristics--the types of energy expenditure and smoking status--and an eventual transition from a state of IGT to NGT. As delineated in table 9, the four F_2-Isoprostanes along with urine creatinine and urine albumin did not produce any statistically significant differences between groups.

Table 3: Demographic Characteristics of Participants with IGT at Baseline

Participant Characteristics*	**Reversal from IGT to NGT** upon Follow-Up **(N=81)**	**No Transition to NGT** at Follow-Up **(N= 222)**	**P-Value**
Sex			
Male **N (%)**	38 (46.91%)	76 (34.23%)	***0.0438**
Female **N (%)**	43 (53.09%)	146 (65.77%)	
Missing **N (%)**	0	0	
Ethnicity			
Non-Hispanic White **N (%)**	27 (33.33%)	90 (40.54%)	0.3575
Non-Hispanic Black **N (%)**	27 (33.33%)	57 (25.68%)	
Hispanic **N (%)**	27 (33.33%)	75 (33.78%)	
Missing **N (%)**	0	0	
Menopausal Status (At visit 1)			
Pre- **N (%)**	11 (25.58%)	15 (10.27%)	***0.0350**
Peri- **N (%)**	2 (4.65%)	11 (7.53%)	
Post- **N (%)**	30 (69.77%)	120 (82.19%)	
Age (in years) **(**At visit 1)			
Mean (SD)	56.75 (8.75)	56.47 (7.69)	0.6357
Median (IQR)	58.00 (15.00)	57.00 (13.00)	
Missing	0	0	

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test; Fisher's Exact Test was used to analyze Menopausal Status due to its small sample size (n=189) whereas the Wald chi-square test was utilized for the remaining categorical variables. These are stratified above with the respective N and % provided for each of the levels.

Table 4: Baseline Anthropometric Measures in Participants with IGT (at Visit 1)

Participant Characteristics*	**Reversal from IGT to NGT** upon Follow-Up **(N=81)**	**No Transition to NGT** at Follow-Up **(N= 222)**	**P-Value**
Average Waist Circumference (At visit 1)			
Mean (SD)	91.70 (15.72)	96.07 (12.84)	***0.0061**
Median (IQR)	89.00 (81.50, 98.20)	95.15 (87.00, 102.35)	
Missing	0	1	
BMI (kg/m^2) (At visit 1)			
Mean (SD)	28.84 (5.96)	31.10 (6.60)	***0.0056**
Median (IQR)	27.68 (25.39, 31.05)	29.53 (26.41, 34.72)	

Missing	0	0	
Waist/Hip Ratio (At visit 1) Mean (SD) Median (IQR) Missing	 0.86 (0.10) 0.85 (0.76, 0.94) 0	 0.88 (0.08) 0.87 (0.81, 0.94) 1	 0.1324
Weight (in Kg.) (At visit 1) Mean (SD) Median (IQR) Missing	 81.39 (18.96) 78.09 (67.00, 93.50) 0	 85.13 (18.55) 82.50 (73.55, 93.80) 0	 0.0853
Height (in cm.) (At visit 1) Mean (SD) Median (IQR) Missing	 167.77 (9.36) 166.30 (161.40, 175.00) 0	 165.57 (9.36) 164.50 (158.80, 173.00) 0	 0.1003

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 5: Glucoregulatory Characteristics of Participants with IGT at Visit 1

Participant Characteristics*	**Reversal from IGT to NGT** upon Follow-Up **(N=81)**	**No Transition to NGT** at Follow-Up **(N= 222)**	**P-Value**
Fasting Insulin (uU/mL) (At visit 1) Mean (SD) Median (IQR) Missing	 15.43 (10.41) 12.00 (8.00, 21.00) 0	 20.85 (23.84) 16.00 (12.00, 23.00) 0	 ***0.0029**
2 Hr Insulin (uU/mL) (At visit 1) Mean (SD) Median (IQR) Missing	 130.81 (126.47) 98.00 (69.00, 155.00) 0	 137.51 (102.56) 110.50 (77.00, 165.00) 0	 0.1712
Si value (At visit 1) Mean (SD) Median (IQR) Missing	 1.79 (1.70) 1.33 (0.79, 2.22) 4	 1.10 (0.84) 1.00 (0.48, 1.56) 19	 ***0.0035**

Sg value (At visit 1) Mean (SD) Median (IQR) Missing	 0.02 (0.01) 0.02 (0.01, 0.02) 4	 0.02 (0.01) 0.02 (0.01, 0.02) 19	 *0.0617
AIR from MINMOD (At visit 1) Mean (SD) Median (IQR) Missing	 478.89 (465.01) 344.73 (149.45, 625.45) 2	 313.79 (332.70) 215.00 (77.96, 444.68) 6	 ***0.0026**
2 Hr Glucose (mg/dL) (At visit 1) Mean (SD) Median (IQR) Missing	 155.38 (14.08) 151.00 (145.00, 162.00) 0	 166.23 (17.40) 165.00 (151.00, 181.00) 0	 ***<.0001**
Fasting Glucose (mg/dL) (At visit 1) Mean (SD) Median (IQR) Missing	 100.73 (10.63) 100.00 (94.00, 107.00) 0	 106.11 (10.78) 106.00 (98.00, 113.00) 0	 ***0.0003**

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 6: Baseline Lipid Profile of Participants with IGT (at Visit 1)

Participant Characteristics*	**Reversal from IGT to NGT** upon Follow-Up **(N=81)**	**No Transition to NGT** at Follow-Up **(N= 222)**	**P-Value**
Tot Chol (mg/dL) (At visit 1) Mean (SD) Median (IQR) Missing	 211.53 (37.73) 206.00 (183.00, 237.00) 2	 216.08 (37.77) 211.50 (185.50, 239.00) 2	 0.3559
HDL (mg/dL) (At visit 1) Mean (SD) Median (IQR) Missing	 48.67 (15.31) 45.00 (39.00, 59.00) 2	 43.82 (14.08) 41.00 (35.00, 50.00) 1	 ***0.0061**
LDL (mg/dL) (At visit 1) Mean (SD) Median (IQR) Missing	 141.85 (37.08) 140.00 (115.50, 166.00) 5	 144.35 (34.73) 144.00 (120.00, 164.00) 11	 0.5428

Trigs (mg/dL) (At visit 1) Mean (SD) Median (IQR) Missing	 131.52 (99.80) 104.00 (75.00, 159.00) 2	 163.08 (88.19) 140.00 (99.00, 197.00) 1	 ***<.0001**

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 7: Cardiovascular Characteristics of Participants with IGT (at Visit 1)

Participant Characteristics**	**Reversal from IGT to NGT** upon Follow-Up **(N=81)**	**No Transition to NGT** at Follow-Up **(N= 222)**	**P-Value***
Hypertension (At visit 1) High **N (%)** Normal **N (%)** Missing **N (%)**	 30 (37.04%) 51 (62.96%) 0	 93 (41.89%) 129 (58.11%) 0	 0.4463
Ankle/Brachial Index (at visit 1) Mean (SD) Median (IQR) Missing	 1.21 (0.09) 1.21 (1.14, 1.28) 0	 1.19 (0.12) 1.20 (1.12, 1.26) 0	 0.3355
Mean Internal Carotid Artery Far Wall Max. (At visit 1) Mean (SD) Median (IQR) Missing	 0.91 (0.32) 0.81 (0.75, 0.94) 8	 0.93 (0.42) 0.79 (0.71, 1.01) 29	 0.3480
Mean Common Carotid Artery Far Wall Max. (At visit 1) Mean (SD) Median (IQR) Missing	 0.85 (0.23) 0.81 (0.70, 0.93) 6	 0.82 (0.17) 0.80 (0.70, 0.89) 23	 0.6044
SYS1V1 Systolic (Baseline) Visit 1 (Ave 2&3) Mean (SD) Median (IQR) Missing	 124.00 (18.23) 123.00 (111.00, 135.00) 0	 125.58 (17.33) 124.00 (114.00, 135.00) 0	 0.4623
DIAS1V1 Diastolic (Baseline) Visit 1 (Ave 2&3) Mean (SD) Median (IQR)	 78.26 (8.87) 79.00 (70.00, 85.00)	 77.69 (9.07) 78.00 (71.00, 83.00)	 0.6591

Missing	0	0	

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test; the Wald chi square test was utilized for the remaining categorical variables.

Table 8: Baseline Lifestyle Characteristics of Participants with IGT (at Visit 1)

Participant Characteristics*	**Reversal from IGT to NGT** upon Follow-Up **(N=81)**	**No Transition to NGT** at Follow-Up **(N= 222)**	**P-Value**
Smoking Status (At visit 1)			
Never- **N (%)**	38 (46.91 %)	107 (48.20 %)	0.6693
Ex- **N (%)**	30 (37.04 %)	88 (39.64 %)	
Current- **N (%)**	13 (16.05 %)	27 (12.16 %)	
Missing **N (%)**	0	0	
Total Energy Expended (kcal/kg/yr) (At visit 1)			
Mean (SD)	14226.56 (2030.44)	14367.56 (2517.73)	0.9598
Median (IQR)	13626.00 (12967.25, 14717.25)	13765.94 (12756.00, 15232.00)	
Missing	0	4	
High Energy Expended (kcal/kg/yr) (At visit 1)			
Mean (SD)	902.07 (1449.33)	1011.03 (2145.40)	0.4003
Median (IQR)	288.00 (0.00, 1152.00)	234.00 (0.00, 1152.00)	
Missing	0	4	
Mod. Energy Expended (kcal/kg/yr) (At visit 1)			
Mean (SD)	2452.00 (2110.69)	2514.57 (2158.55)	0.8023
Median (IQR)	1772.00 (984.0, 3162.0)	1816.00 (878.0, 3648.0)	
Missing	0	3	

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test; the Wald chi-square test was utilized for the remaining categorical variables.

Table 9: Urinary Biomarkers amongst Participants at Baseline

Participant Characteristics*	**Reversal from IGT to NGT** upon Follow-Up **(N=81)**	**No Transition to NGT** at Follow-Up **(N= 222)**	**P-Value**
Urine Creatinine (mg/dL) (At visit 1)			
Mean (SD)	149.94 (97.67)	139.21 (77.02)	0.7564
Median (IQR)	127.05 (90.30, 186.90)	131.25 (79.80,180.60)	
Missing	1	4	
Urine Albumin (mg/L) (At visit 1)			
Mean (SD)	13.00 (21.80)	17.21 (52.39)	0.7151
Median (IQR)	6.20 (6.20, 8.15)	6.20 (6.20, 10.60)	
Missing	1	5	
mean of 4 standardized F2-isoPs.			
Mean (SD)	0.0 (0.63)	0.08 (0.85)	0.9730
Median (IQR)	-0.18 (-0.53, 0.40)	-0.15 (-0.50, 0.48)	
Missing	23	41	
iPF_2a			
Mean (SD)	0.24 (0.15)	0.24 (0.16)	0.9843
Median (IQR)	0.20 (0.13, 0.29)	0.21 (0.13, 0.29)	
Missing	23	41	
dinor_ng_mg_cn			
Mean (SD)	4.55 (2.29)	4.95 (3.20)	0.8162
Median (IQR)	3.99 (2.93, 5.37)	4.17 (2.83, 6.22)	
Missing	23	41	
ipf2_ng_mg_cn			
Mean (SD)	6.37 (3.56)	6.87 (4.79)	0.9695
Median (IQR)	5.56 (3.71, 8.32)	5.28 (3.69, 8.61)	
Missing	23	41	
iso_iPF_2a			
Mean (SD)	4.31 (2.64)	4.34 (2.92)	0.9010
Median (IQR)	3.78 (2.37, 5.35)	3.59 (2.50, 5.43)	
Missing	23	41	
(Mean of 4 standardized F2-isoPs/BMI) *(1000)			
Mean (SD)	7.77(4.60)	7.64 (4.92)	0.7417
Median (IQR)	6.96 (3.95, 10.19)	6.45 (3.99, 9.78)	
Missing	23	41	
(IPF_2a/BMI) *(1000)			
Mean (SD)	210.21 (120.64)	216.52 (147.26)	0.7368
Median (IQR)	178.00 (124.23, 259.38)	168.69 (118.93, 275.80)	
Missing	23	41	

(Dinor_ng_mg_cn/BMI) *(1000)			
Mean (SD)	139.24 (76.68)	136.59 (87.67)	0.5311
Median (IQR)	118.91 (88.29, 179.55)	116.63 (75.98, 172.65)	
Missing	23	41	
(ipf2_ng_mg_cn/BMI) *(1000)			
Mean (SD)	147.28 (66.87)	155.08 (97.83)	0.8820
Median (IQR)	136.25 (103.86, 181.44)	137.94 (84.66, 196.73)	
Missing	23	41	
(Iso_iPF_2a/BMI) *(1000)			
Mean (SD)	-1.00 (19.65)	1.61 (24.89)	0.8623
Median (IQR)	-5.74 (-17.52, 14.86)	-4.26 (-15.73, 12.48)	
Missing	23	41	

*All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.
**Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

4.4 Results found for Participants with IGT at Baseline when Examined during Follow-up

In table 10, menopausal status produced a statistically significant result in which female subjects who either pre-menopausal (n=7, 16.28%) or else peri-menopausal (n=3, 6.98%) were more likely to successfully reverse from IGT back to a state of NGT (p=0.0089). Amongst those who did not transition backwards, 93.15% of the women in that group (n=136) identified as being post-menopausal upon follow up; this percentage is far higher than the 76.74% of post-menopausal women who did successfully revert from a state of IGT (n=33) back to one of NGT. While this reinforces previous results found with respect to the statistically significant influence of gender, age did not elicit a statistically significant result (p=0.6080).

Subjects with a lower average waist circumference (mean= 93.61) as shown in table 11 were more likely to reverse from prediabetes to a state of NGT; those who did not had higher average waist circumferences (mean=98.39) overall (p=0.0031). Subjects with lower BMIs at follow-up (mean=29.22) were more successful in reversing from a state of IGT to one of NGT. In contrast, subjects with higher BMIs (mean=31.79) were less likely to successfully transition

backwards (p=0.0011). No statistically significant differences emerged for the waist/hip ratio (p=0.1081) or else for the weight variable (p=0.0562) when measured at follow-up.

In table 12, subjects with a lower fasting insulin (mean=18.23) were significantly more likely to reverse from a state of IGT back to one of NGT in comparison to those with higher fasting insulin measurements (mean=23.83) at follow-up (p=0.0006). Similarly, participants with lower 2-hr insulin levels (mean=117.45) at follow-up had more success in regressing from a prediabetic state back to one of NGT relative to those with higher 2-hr insulin levels (mean=167.62) given that the latter had far more difficulty achieving this reversal (p<0.0001). Additionally, individuals with greater levels of insulin sensitivity (mean=1.47) were also significantly more likely to reverse from a state of IGT to one of NGT in comparison to those with decreased insulin sensitivity (mean=0.80) overall (p<0.0001). Subjects with a lower amount of fasting glucose (mean=98.39) were also more likely to experience a successful reversal of prediabetes relative to their peers who exhibited higher fasting glucose levels (mean=121.53) at follow-up (p<0.0001). Participants with a greater acute insulin response (AIR) (mean=648.09) were more likely to reverse from a state of IGT to one of NGT in comparison to those with a lesser AIR (mean=263.46) during visit 2 (p<0.0001).[15] In terms of 2-hr glucose, subjects with higher levels (mean=212.15) were less likely to transition backwards from a state of IGT to NGT when compared to participants who exhibited lower levels of 2-hr glucose (mean=119.76) at follow-up (p<0.0001).

Table 13 indicates that the subjects with greater HDL levels (mean=50.58) were significantly more likely to transition from a state of IGT to one of NGT relative to those with lower HDL levels (mean=45.19) at follow-up (p=0.0153). Participants with higher levels of

[15] when calculated utilizing the mathematical minimal model.

triglycerides (mean=155.64) were less likely to successfully regress to NGT from a prediabetic state when compared to those with lower triglyceride levels (mean=116.93) ($p<0.0001$). The inclusion and analyses of the ratios of the four F_2-Isoprostanes[16] did not produce any significant results. Moreover, no significant findings emerged for total cholesterol ($p=0.0706$) or for LDL levels ($p=0.1339$) when both variables were measured during the follow-up visit.

In table 14, no statistically significant findings emerged with regard to cardiovascular characteristics and the potential for a reversal from prediabetes to a state of NGT. The same held true for lifestyle characteristics in that no statistically significant findings arose for the three types of energy expenditure or for smoking status (which is displayed in table 15). As shown in table 16, both urine creatinine ($p=0.3162$) and urine albumin ($p=0.7782$) failed to generate statistically significant findings with respect to regressing from a state of prediabetes back to one of NGT.

Table 10. Demographic and Baseline Characteristics by Glucose Tolerance Status (at Visit 2)

Participant Characteristics	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value**
Menopausal Status* (At visit 2) Pre- **N (%)** Peri- **N (%)** Post- **N (%)** Missing **N (%)**	 7 (16.28%) 3 (6.98%) 33 (76.74%) 0	 7 (4.79%) 3 (2.05%) 136 (93.15%) 0	***0.0089**
Age (in years) **(**At visit 2) Mean (SD) Median (IQR) Missing	 62.09 (8.72) 64.00 (15.00) 0	 61.71 (7.81) 62.00 (13.00) 0	0.6080

*****As a categorical variable, Menopausal Status required the utilization of Fisher's Exact test in order for the p-value to appropriately account for the small sample size (n=189). This variable is stratified above with the N and % provided for each level.

[16]when each of the F_2-Isoprostanes are divided by the BMI acquired for visit 2.

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

Table 11: Anthropometric Measures in Participants with IGT (at Follow-Up)

Participant Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value**
Average Waist Circumference (At visit 2) Mean (SD) Median (IQR) Missing	 93.61 (15.63) 91.35 (17.35) 3	 98.39 (13.70) 96.80 (14.50) 0	 ***0.0031**
BMI (kg/m^2) (At visit 2) Mean (SD) Median (IQR) Missing	 29.22 (6.83) 27.97 (5.93) 4	 31.79 (7.08) 29.86 (8.62) 1	 ***0.0011**
Waist/Hip Ratio (At visit 2) Mean (SD) Median (IQR) Missing	 0.87 (0.13) 0.85 (0.79, 0.94) 3	 0.88 (0.08) 0.88 (0.82, 0.94) 0	 0.1081
Weight (in Kg.) (At visit 2) Mean (SD) Median (IQR) Missing	 82.78 (21.60) 79.09 (68.20, 91.36) 4	 86.52 (19.43) 83.30 (73.50, 97.32) 1	 0.0562
Height (in cm.) (At visit 2) Mean (SD) Median (IQR) Missing	 168.05 (9.16) 166.40 (160.70, 175.10) 4	 165.20 (9.58) 164.35 (158.20, 172.70) 0	 ***0.0323**

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 12: Glucoregulatory Characteristics of Participants with IGT at Follow-Up

Participant Characteristics*	Reversal from IGT to NGT (N=81)	No Transition to NGT (N= 222)	P-Value
Fasting Insulin (uU/mL) (At visit 2) Mean (SD) Median (IQR) Missing	 18.23 (9.82) 15.00 (12.00, 21.00) 0	 23.83 (14.85) 20.50 (14.00, 30.00) 2	 *** 0.0006**
2 Hr Insulin (uU/mL) (At visit 2) Mean (SD) Median (IQR) Missing	 117.45 (123.42) 14.00 (62.00, 129.00) 3	 167.62 (148.76) 126.00 (89.50, 182.00) 6	 ***<.0001**
Si value (At visit 2) Mean (SD) Median (IQR) Missing	 1.47 (1.56) 1.13 (0.55, 1.67) 13	 0.80 (1.58) 0.52 (0.00, 1.02) 33	 ***<.0001**
Sg value (At visit 2) Mean (SD) Median (IQR) Missing	 0.02 (0.01) 0.02 (0.01, 0.02) 13	 0.02 (0.01) 0.02 (0.01, 0.02) 34	 ***0.0020**
AIR from MINMOD (At visit 2) Mean (SD) Median (IQR) Missing	 648.09 (749.28) 376.14 (186.14, 718.36) 13	 263.46 (316.56) 196.91 (38.55, 383.18) 36	 ***<.0001**
Fasting Glucose (mg/dL) (At visit 2) Mean (SD) Median (IQR) Missing	 98.39 (11.75) 98.00 (91.50, 107.00) 0	 121.53 (37.91) 110.50 (99.50, 131.00) 3	 ***<.0001**
2 Hr Glucose (mg/dL) (At visit 2) Mean (SD) Median (IQR) Missing	 119.76 (15.52) 122.00 (110.50, 132.50) 2	 212.15 (69.23) 192.00 (167.00, 237.00) 4	 ***<.0001**

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 13: Baseline Lipid Profile of Participants with IGT (at Visit 2)

Participant Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value**
Tot Chol (mg/dL) (At visit 2) Mean (SD) Median (IQR) Missing	 203.71 (44.65) 201.00 (174.50, 226.50) 5	 211.45 (36.62) 208.50 (188.00, 232.00) 6	0.0706
HDL (mg/dL) (At visit 2) Mean (SD) Median (IQR) Missing	 50.58 (16.85) 47.00 (39.00, 59.00) 5	 45.19 (13.40) 43.50 (36.00, 52.50) 6	*** 0.0153**
LDL (mg/dL) (At visit 2) Mean (SD) Median (IQR) Missing	 129.76 (42.51) 127.00 (103.50, 147.00) 5	 135.11 (33.87) 131.50 (113.00, 154.00) 6	0.1339
Trigs (mg/dL) (At visit 2) Mean (SD) Median (IQR) Missing	 116.93 (71.39) 93.00 (71.00, 141.00) 5	 155.64 (75.49) 141.00 (99.00, 190.50) 6	***<.0001**
Ratio of (iPF_2a)/BMI_2 (At visit 2) Mean (SD) Median (IQR) Missing	 0.01 (0.00) 0.01 (0.00, 0.02) 25	 0.01 (0.00) 0.01 (0.00, 0.02) 42	0.7367
Ratio of (ipf2_ng_mg_cn)/BMI_2 (At visit 2) Mean (SD) Median (IQR) Missing	 0.21 (0.13) 0.18 (0.13, 0.26) 25	 0.21 (0.15) 0.16 (0.11, 0.28) 42	0.7537
Ratio of (Iso_iPF_2a)/BMI_2 (At visit 2) Mean (SD) Median (IQR) Missing	 0.13 (0.07) 0.12 (0.09, 0.17) 25	 0.13 (0.09) 0.12 (0.07, 0.17) 42	0.6670

Ratio of (Dinor_ng_mg_cn)/ BMI_2 (At visit 2) Mean (SD) Median (IQR) Missing	 0.15 (0.07) 0.13 (0.10, 0.19) 25	 0.15 (0.10) 0.13 (0.09, 0.19) 42	 0.8842

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 14: Cardiovascular Characteristics of Participants with IGT (at Visit 2)

Participant Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value**
Hypertension (At visit 2) High **N (%)** Normal **N (%)** Missing **N (%)**	 35 (43.75%) 45 (56.25%) 1 (0.012%)	 123 (55.56%) 98 (44.34%) 1 (0.004%)	 0.0974
Ankle/Brachial Index (at visit 2) Mean (SD) Median (IQR) Missing	 1.12 (0.13) 1.13 (1.05, 1.20) 3	 1.12 (0.14) 1.14 (1.07, 1.20) 1	 0.9205
Mean Internal Carotid Artery Far Wall Max. (At visit 2) Mean (SD) Median (IQR) Missing	 1.04 (0.48) 0.84 (0.40) 6	 1.03 (0.61) 0.86 (0.36) 8	 0.4065
Mean Common Carotid Artery Far Wall Max. (At visit 2) Mean (SD) Median (IQR) Missing	 0.85 (0.24) 0.80 (0.23) 3	 0.87 (0.21) 0.82 (0.24) 4	 0.1860
SYS2V2 Systolic (Baseline) (Ave 2&3) (At visit 2) Mean (SD)	 126.92 (18.91) 125.00 (116.00,	 129.24 (16.95) 127.00 (118.00,	 0.2064

Median (IQR) Missing	136.00) 6	140.00) 14	
DIAS1V1 Diastolic (Baseline) (Ave 2&3) (At visit 2) Mean (SD) Median (IQR) Missing	77.59 (9.95) 79.00 (70.00, 84.00) 6	78.52 (10.04) 78.00 (71.00, 84.00) 14	0.5965

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test; the Wald chi-square test was utilized for the remaining categorical variables. The categorical variable is stratified above with the respective N and % provided for each level.

Table 15: Lifestyle Characteristics of Participants with IGT at Follow-Up

Participant Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value**
Smoking Status (At visit 2) Never- **N (%)** Ex- **N (%)** Current- **N (%)** Missing **N (%)**	 38 (46.91%) 33 (40.74%) 10 (12.35%) 0	 107 (48.63 %) 96 (43.63 %) 17 (7.70 %) 2 (0.009%)	0.4584
Mod. Energy Expended (kcal/kg/yr) (At visit 2) Mean (SD) Median (IQR) Missing	 2148.63 (2498.00) 1582.00 (1496.00) 2	 2268.92 (2343.88) 1520.00 (2350.00) 3	0.5956
Total Energy Expended (kcal/kg/yr) (At visit 2) Mean (SD) Median (IQR) Missing	 14011.05 (2458.92) 13181.25 (1536.63) 2	 14213.19 (2794.36) 13538.63 (2337.38) 3	0.5283
High Energy Expended (kcal/kg/yr) (At visit 2)	 860.35 (2000.92) 160.00 (864.00)	 1019.36 (2465.68) 168.00 (1072.00)	0.9113

Mean (SD) Median (IQR) Missing	2	3	

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test; the Wald chi-square test was utilized for the remaining categorical variables. "Smoking status" is stratified above with the respective N and % provided for each level.

Table 16: Urinary Biomarkers amongst Participants at Follow-Up

Participant Characteristics**	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value***
Urine Creatinine (mg/dL) (At visit 2) Mean (SD) Median (IQR) Missing	 125.38 (64.85) 116.00 (77.00, 160.00) 2	 134.61 (75.41) 125.00 (86.00, 160.00) 3	 0.3162
Urine Albumin (mg/L) (At visit 2) Mean (SD) Median (IQR) Missing	 14.61 (18.00) 6.10 (5.80, 13.30) 2	 54.79 (391.95) 6.00 (5.80, 13.20) 3	 0.7782
(Mean of 4 standardized F2-isoPs/BMI for visit 2) *(1000) Mean (SD) Median (IQR) Missing	 7.73 (4.72) 7.00 (4.00, 10.50) 25	 7.53 (4.89) 6.17 (3.90, 9.72) 42	 0.7367
(IPF_2a/BMI for visit 2) *(1000) Mean (SD) Median (IQR) Missing	 208.74 (129.78) 175.60 (127.66, 255.43) 25	 213.17 (146.22) 164.42 (112.29, 282.93) 42	 0.7357
(Dinor_ng_mg_cn/BMI for visit 2) *(1000) Mean (SD) Median (IQR) Missing	 133.80 (73.95) 118.48 (88.12, 170.93) 25	 134.57 (87.22) 115.64 (73.83, 165.65) 42	 0.6670

(ipf2_ng_mg_cn/BMI for visit 2) *(1000) Mean (SD) Median (IQR) Missing	 145.77 (71.46) 132.80 (97.81, 185.21) 25	 153.16 (100.28) 127.28 (87.55, 190.33) 42	 0.8842
(Iso_iPF_2a/BMI for visit 2) *(1000) Mean (SD) Median (IQR) Missing	 -1.69 (19.62) -8.51 (-16.98, 14.36) 25	 1.79 (24.66) -3.86 (-15.87, 12.42) 42	 0.6101

*All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.
**Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

4.5 The Comparison of Change in Characteristics (between visits 1 & 2) between the Groups of Participants who had IGT at Baseline.

In table 17, menopausal status (p=0.0365) was statistically significant in terms of the comparison of change across both groups over time. 13.95 percent of women who reversed from IGT to a state of NGT were pre-menopausal both at baseline and again upon follow-up, which is higher relative to those who never transitioned backwards. 82.19% of women who never successfully reversed were post-menopausal during both visits, which is a higher percentage compared to the mere 67.44% of subjects who did successfully regress from IGT to NGT. Changes in age between the two visits bore no statistically significant impact upon the outcome of interest (p=0.1759). In table 18, the comparison of change between groups for BMI was statistically significant (p=0.0348). This indicates that participants who did not successfully reverse to a state of NGT had a larger increase in mean BMI over time (mean=+0.76 kg/m^2) relative to the changes in BMI amongst subjects who did successfully transition from a state of IGT to NGT (mean=+0.34 kg/m^2). However, there were no statistically significant differences occurring with respect to changes in the waist/hip ratio, weight, and average waist circumference.

In table 19, subjects who reversed from a state of IGT to NGT experienced a decrease in 2-hr insulin between visits 1 and 2 (mean= -15.97) whereas those who failed to transition had an increase in 2-hr insulin levels (mean=30.34) over time (p=0.0032). Subjects who successfully reversed from IGT to NGT similarly had a decrease in 2-hr glucose levels (mean= -35.81) whereas those who did not regress encountered higher 2-hr glucose levels (mean=45.94) over time (p<0.0001) A similar pattern arose with fasting glucose in that those who regressed from IGT to NGT had a decrease between visits 1 and 2 (mean=2.34) whereas subjects who did not transition backwards from IGT had an overall increase in their respective levels (mean=15.48) over time (p<0.0001). Participants who encountered an increase in overall acute insulin response (AIR) (mean=192.23) between the two visits were more likely to reverse from a state of IGT to NGT relative to their peers who experienced a decrease in AIR (mean= -59.52) over time[17] (p=0.0007). However, the comparison of change for fasting insulin (p=0.1291), insulin sensitivity (p=0.1723), and glucose effectiveness (p=0.6576) were not significantly different between those who transitioned from a state of IGT to NGT relative to subjects who did not.

As shown in table 20, no statistically significant findings occurred when comparing the change over time for characteristics that comprise the lipid profile. In table 21, subjects who reversed from IGT to NGT had a lower average Common Carotid Artery[18] (CCA) value (mean=0.00) relative to those who did not transition backwards from IGT (mean=0.05). The subjects who failed to regress to NGT experienced an increase in their mean CCA values over time between visits (p=0.0369). There were no significant findings emerging for HDL, LDL, total cholesterol, and triglyceride levels. Similarly, no significant differences arose with regard to the F_2-isoprostane ratios when measured over the five-year time frame. In table 22, no

[17] with the mathematical minimal model.
[18] The Mean CCA is with regard to the far wall max.

statistically significant changes emerged for lifestyle characteristics when measured for both groups between visits 1 and 2. In table 23, no statistically significant changes occurred for urine albumin (p=0.6372) or for urine creatinine (p= 0.3887) when both biomarkers were evaluated over time.

Table 17: The Change in Demographic Characteristics between Visits 1 & 2 amongst Participants with IGT at Baseline

Characteristics	**Reversal from IGT to NGT**	**Did not Reverse to NGT**	**P-value** (For comparison of change between the groups)
Menopausal Status (N=189) *			
Pre – Pre-**N (%)**	6 (13.95%)	6 (4.11%)	***0.0365**
Pre – Peri **N (%)**	3 (6.98 %)	2 (1.37%)	
Pre – Post **N (%)**	2 (4.65%)	7 (4.79%)	
Peri– Peri **N (%)**	0 (0.00%)	1 (0.68%)	
Peri– Post **N (%)**	2 (4.65%)	9 (6.16%)	
Post– Peri **N (%)**	0 (0.00%)	0 (0.00%)	
Post – Post **N (%)**	29 (67.44%)	120 (82.19%)	
Missing **N (%)**	1 (2.33%)	1 (0.68%)	

*Fisher's Exact test was utilized for the categorical variables of interest due to the small sample size affiliated with menopausal status. The categorical variable is stratified above with the respective N and % provided for each level.

Table 18: The Change in Anthropometric Measures between Visits 1 & 2 amongst Participants with IGT at Baseline

Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N=222)**	**P-Value** (For the comparison of change between the groups)
BMI (kg/m^2)			
Mean (SD)	0.34 (2.24)	0.76 (2.31)	***0.0348**
Median (IQR)	0.11 (-1.03, 1.24)	0.72 (-0.55, 1.77)	
Missing	4	1	

Average Waist Circumference Mean (SD) Median (IQR) Missing	 1.72 (5.36) 1.63 (-1.60, 5.50) 3	 2.37 (5.93) 1.90 (-1.00, 5.60) 1	 0.6362
Waist/Hip Ratio Mean (SD) Median (IQR) Missing	 0.01 (0.09) 0.01 (-0.02, 0.03) 3	 0.00 (0.05) 0.00 (-0.02, 0.03) 1	 0.7641
Weight (in. Kg) Mean (SD) Median (IQR) Missing	 0.78 (6.15) 0.36 (-2.30, 3.60) 4	 1.70 (5.95) 1.74 (-2.10, 5.10) 1	 0.1075
Height (in cm.) Mean (SD) Median (IQR) Missing	 -0.15 (1.15) -0.20 (-0.90, 0.5) 4	 -0.36 (1.59) -0.30 (-1.00, 0.3) 0	 0.3996

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR, which is the 75^{th}-25^{th} percentile contrast.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 19: The Change in Glucoregulatory Characteristics between Visits 1 & 2 When Measured amongst Participants with IGT at Baseline

Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N=222)**	**P-Value** (For the comparison of change between the groups)
Fasting Insulin (uU/mL) Mean (SD) Median (IQR) Missing	 2.80 (9.65) 3.00 (-2.0, 7.0) 0	 2.98 (26.33) 5.00 (-2.0, 10.0) 2	 0.1291
2 Hr Insulin (uU/mL) Mean (SD) Median (IQR) Missing	 -15.87 (107.31) -7.50(-52.0, 27.0) 3	 30.34 (113.52) 9.00 (-25.0, 65.0) 6	 ***0.0032**
Si value			

Mean (SD) Median (IQR) Missing	-0.31 (1.80) -0.26(-0.74, 0.25) 14	-0.36 (1.53) -0.33 (-0.95, 0.0) 45	0.1723
Sg value Mean (SD) Median (IQR) Missing	0.0 (0.01) -0.0 (-0.01, 0.01) 14	-0.00 (0.01) -0.0 (-0.01, 0.00) 46	0.6576
AIR from MINMOD Mean (SD) Median (IQR) Missing	192.23 (556.17) 52.82 (-87.73, 374.64) 13	-59.52 (273.94) -32.09 (-145.55, 68.09) 39	***0.0007**
Fasting Glucose (mg/dL) Mean (SD) Median (IQR) Missing	-2.34 (10.45) -1.50 (-7.5, 4.5) 0	15.48 (34.53) 6.50 (-2.0, 17.50) 3	***<.0001**
2 Hr Glucose (mg/dL) Mean (SD) Median (IQR) Missing	-35.81 (20.56) -30.5 (-48.0, -19) 2	45.94 (68.03) 28.25 (2.0, 72.0) 4	***<.0001**

- All continuous variables are reported with the mean, standard deviation, median, and IQR.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 20: The Change in the Lipid Profile Occurring between Visits 1 & 2 When Measured amongst Participants with IGT at Baseline

Participant Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value** (For the comparison of change between the groups)
Tot Chol (mg/dL) Mean (SD) Median (IQR) Missing	-6.38 (32.14) -2.50 (-28.00, 10.00) 7	-6.08 (30.51) -3.50 (-23.0, 11.0) 8	0.7460
HDL (mg/dL) Mean (SD) Median (IQR) Missing	1.96 (9.49) 3.00 (-4.00, 7.00) 7	1.22 (11.31) 1.00 (-3.00, 7.00) 7	0.8737

LDL (mg/dL) Mean (SD) Median (IQR) Missing	 -9.31 (28.86) -8.00 (-27.00, 6.00) 10	 -9.50 (31.05) -9.00 (-27.0, 10.0) 17	0.6706
Trigs (mg/dL) Mean (SD) Median (IQR) Missing	 -16.78 (70.04) -10.00 (-37.00, 12.00) 7	 -7.46 (78.33) -3.00 (-36.0, 31.0) 7	0.1505
Ratio of (iPF_2a)/BMI Mean (SD) Median (IQR) Missing	 -0.00 (0.00) -0.00 (-0.01, 0.00) 25	 -0.00 (0.00) -0.00 (-0.00, 0.00) 42	0.1275
Ratio of (ipf2_ng_mg_cn)/BMI Mean (SD) Median (IQR) Missing	 -0.00 (0.02) -0.00 (-0.01, 0.00) 25	 -0.00 (0.02) -0.00 (-0.01, 0.00) 42	0.0881
Ratio of (Iso_iPF_2a)/BMI Mean (SD) Median (IQR) Missing	 -0.00 (0.01) -0.00 (-0.00, 0.00) 25	 -0.00 (0.01) -0.00 (-0.01, 0.00) 42	0.0755
Ratio of (Dinor_ng_mg_cn) /BMI Mean (SD) Median (IQR) Missing	 -0.00 (0.01) -0.00 (-0.00, 0.00) 25	 -0.00 (0.01) -0.00 (-0.01, 0.00) 42	0.0684
Ratio of F_2-IsoP Index/BMI[1] Mean (SD) Median (IQR) Missing	 0.0 (0.00) 0.0 (-0.00, 0.00) 25	 0.0 (0.00) 0.00 (-0.00, 0.00) 42	0.8480

Note: The index is simply the mean of four standardized F2-isoprostanes.[1]

- All continuous variables are reported with the mean, standard deviation, median, and IQR.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

Table 21: The Change in the Cardiovascular Characteristics Occurring between Visits 1 & 2 When Measured amongst Participants with IGT at Baseline

Participant Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value** (For the comparison of change between the groups)
Hypertension High-High **N (%)** High-Normal **N (%)** Normal-High **N (%)** Normal-Normal **N (%)** Missing	 26 (32.50%) 4 (5.00%) 9 (11.25%) 41 (51.25%) 1	 87 (39.37%) 6 (2.71%) 36 (16.29%) 92 (41.63%) 1	 0.2674
Ankle/Brachial Index Mean (SD) Median (IQR) Missing	 -0.09 (0.14) -0.07 (-0.17, -0.0) 3	 -0.07 (0.13) -0.06 (-0.15, 0.01) 1	 0.4199
Mean Internal Carotid Artery Far Wall Max. Mean (SD) Median (IQR) Missing	 0.12 (0.30) 0.03 (-0.06, 0.16) 10	 0.07 (0.44) 0.02 (-0.09, 0.18) 34	 0.4143
Mean Common Carotid Artery Far Wall Max. Mean (SD) Median (IQR) Missing	 0.00 (0.13) -0.0 (-0.06, 0.07) 6	 0.05 (0.16) 0.03 (-0.04, 0.12) 24	 ***0.0369**
Systolic (Ave 2&3) Mean (SD) Median (IQR) Missing	 6.97 (17.27) 7.00 (0.00, 14.0) 6	 5.86 (15.97) 7.00 (-4.00, 15.00) 17	 0.5908
Diastolic (Ave 2&3) Mean (SD) Median (IQR) Missing	 -0.45 (9.71) 0.00 (-6.0, 6.00) 6	 0.75 (9.26) 1.00 (-6.00, 7.00) 14	 0.6406

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test; the Wald chi-square test was utilized for the remaining categorical variables.

Table 22: The Change in Lifestyle Characteristics occurring between Visits 1 & 2 When Measured amongst Participants with IGT at Baseline

Participant Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value** (For the comparison of change between the groups)
Smoking Status Never- Never **N (%)** Never- Ex **N (%)** Ex- Never **N (%)** Ex- Ex. **N (%)** Ex- Current **N (%)** Current- Never **N (%)** Current- Ex. **N (%)** Current-Current **N (%)**	36 (44.44%) 2 (2.47%) 2(2.47%) 26 (32.10%) 2 (2.47%) 0 (0.00%) 5 (6.17%) 8 (9.88%)	102 (46.36%) 3 (1.36%) 5 (2.27%) 80 (36.36%) 3 (1.36%) 0 (0.00%) 13 (5.91%) 14 (6.36%)	0.8254
Mod. Energy Expended (kcal/kg/yr) Mean (SD) Median (IQR) Missing	-276.94 (2617.78) -400.0 (-1568.0, 776.0) 2	-267.52 (2666.31) -228.00 (-1520.00, 978.00) 6	0.6273
Total Energy Expended (kcal/kg/yr) Mean (SD) Median (IQR) Missing	-187.31 (1976.92) -290.63 -1107.38, 558.0) 2	-165.12 (3185.60) -162.63 (-1197.38, 1012.00) 7	0.5323
High Energy Expended (kcal/kg/yr) Mean (SD) Median (IQR) Missing	-37.22 (1587.39) 0.00 (-404.00, 216.00) 2	5.49 (3007.07) 0.00 (-384.0, 260.0) 7	0.9468

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test; the Wald chi-square test was utilized for the remaining categorical variables.

- All continuous variables are reported with the mean, standard deviation, median, along with the IQR.

Table 23: The Change in the Urinary Biomarkers Occurring between Visits 1 & 2 When Measured amongst Participants with IGT at Baseline

Participant Characteristics*	**Reversal from IGT to NGT (N=81)**	**No Transition to NGT (N= 222)**	**P-Value** (For the comparison of change between the groups)
Urine Creatinine (mg/dL) Mean (SD) Median (IQR) Missing	 -21.58 (90.85) -7.70 (-64.90, 32.50) 3	 -5.26 (90.85) -6.20 (-56.50, 48.70) 7	 0.3887
Urine Albumin (mg/L) Mean (SD) Median (IQR) Missing	 1.56 (17.65) -0.20 (-0.40, 2.50) 3	 38.51 (378.19) -0.20 (-0.40, 5.10) 8	 0.6372

- All continuous variables are reported with the mean, standard deviation, median, and IQR.

*Differences between groups for continuous variables were assessed using the Wilcoxon Rank Sum/Kruskal Wallis Test.

CHAPTER V

DISCUSSION

5.1 Discussion of the Research Question

Due to a dearth of RCTs within this specific area of diabetes research, the purpose of this study is to discover characteristics that may help to induce a reversal from a state of IGT back to one of NGT. This study takes a novel approach by using exploratory data analysis (EDA) in order to evaluate findings obtained from the Insulin Resistance Atherosclerosis Study (IRAS) cohort. In addition to the lack of extant studies examining the reversal from prediabetes to a state of NGT, those that are in existence focus specifically upon lifestyle and/or medication interventions.[19] The findings generated from some of these extant studies are mixed, with efficacity waning over time in some instances—particularly in relation to the use of medications and lifestyle regimens.[20] In order to acquire more information on that which may aid in the reversal from IGT to NGT, this study delves into several previously unexplored areas through the inclusion of the following: measures of adiposity, the lipid profile, cardiovascular characteristics, glucoregulatory mechanisms, along with anthropometric, lifestyle, and demographic characteristics of interest. This study goes one step further by attempting to understand the interplay of the potential factors that may be involved in this reversal when measured in simultaneity, which may help to stimulate future research studies due to the potential for synergistic effects.

The first hypothesis centers upon the notion that demographic characteristics—sex, ethnicity, menopausal status, and age-- may serve as predictors of a reversal from a state of prediabetes (or IGT) back to one of NGT. The results of this study revealed that menopausal

[19] These have ultimately produced varied results in terms of success.
[20] such as exercise routines.

status and gender [21] are statistically significant; men were more likely to reverse from IGT back to NGT relative to their female counterparts—an outcome that runs contrary to previous research.

The second research hypothesis relating to the impact that anthropometric measures have upon a regression from IGT to NGT yielded mixed findings. While adults with higher BMIs were significantly less likely to transition from a state of IGT to NGT relative to those with lower BMIs, there were no significant differences across groups for the average waist circumference, waist/hip ratio, and weight. We conjectured that changes in glucoregulatory characteristics would engender a reversal from a state of IGT to one of NGT. While the study did find statistically significant outcomes for 2-hr insulin, AIR from MINMOD, fasting glucose, and 2-hr glucose, this was not the case for insulin sensitivity (S_I), glucose effectiveness (S_G), or for fasting insulin. Decreases in 2-hr insulin, fasting glucose, 2-hr glucose levels, and a higher AIR did incite a regression from a state of IGT to one of NGT, whereas there were no statistically significant findings emerging across groups for changes in S_I, S_G, and fasting insulin when measured over the five-year time frame.

We also hypothesized that changes in the lipid profile over time, which is comprised of total cholesterol, HDL and LDL levels, triglyceride levels, and the ratio of all four F_2-isoprostanes divided by BMI, would serve as predictors facilitating the transition from IGT back to an improved state of NGT. Since no statistically significant results were produced, this did not lend support to the findings from the current landscape of research regarding the lipid profile. This runs counter to the results found in the current existing literature in which lower levels of

[21] This comes as no surprise given that menopausal status is nested within gender and in turn applies only to female participants.

triglycerides and LDL cholesterol (when accompanied by a higher supply of HDL cholesterol) were found to prompt a transition from a state of prediabetes back to one of NGT.

The fifth research hypothesis posits that cardiovascular risk factors--hypertension, the ankle/brachial index, systolic and diastolic measures of blood pressure, mean CCA, and the mean ICA--aid in the reversal from a state of prediabetes to one of NGT. However, since none of these variables generated statistically significant findings, this suggests that these cardiovascular characteristics may be inadequate predictors of a reversal from a state of IGT to one of NGT.

The sixth research hypothesis seeks to evaluate the impact (if any) that lifestyle characteristics (such as energy expenditure and smoking status) have upon the potential for regression from a state of IGT to one of NGT. In a manner similar to cardiovascular risk factors, no statistically significant differences emerged with regard to lifestyle characteristics. Smoking status along with the variant types of energy expenditures (which were total, moderate, and high) did not successfully predict the onset of a transition from a state of IGT to one of NGT. The final hypothesis seeks to measure the impact of urine creatinine and urine albumin upon the potential for reversal from a prediabetic state back to one of NGT. Since no statistically significant findings arose for urinary biomarkers, this result indicates that urine albumin and urine creatinine were not effective predictors of a regression from a state of IGT to one of NGT.

When testing for statistical significance, the outcomes for demographic and adiposity measures produced mixed results.[22] The findings obtained for BMI in this study mirrors results that were found in the POP-ABC analysis from 2014[23] in that a lower BMI is an effective predictor in the ability to transition from IGT back to a state of NGT [1]. The reverse similarly held true in that in that those with higher BMIs were less likely to regress from a state of IGT to

[22] in terms of regressing from a state of IGT to NGT as measured in the context of this study.
[23] by Dagogo-Jack et. al.

one of NGT and instead were more likely to transition from IGT towards the onset of full-blown T2D.

While the outcome found for BMI corroborated finding from existing research, findings for gender in this study ran contrary to that which is in the current background of prediabetes literature. This study found that men were more likely to achieve a successful regression from a state of IGT back to one of NGT, whereas the reverse held true for their female counterparts. Despite the unexpected nature of the outcome found for gender, this was supported by the statistically significant results found for menopausal status.[24] These findings are notable in that they highlight a predisposition that female subjects may have given that they appeared to transition from a state of prediabetes (or IGT) to overt type 2 diabetes more frequently than their male peers. This outcome suggests that some of the underlying mechanisms relating to gender may not yet be fully understood. As a result, these conflicting findings merit additional research in order to fully address the underpinnings that at this point remain both poorly understood and minimally studied.

The results acquired in this study may vary slightly in comparison to some of the findings from previously existing research due to several considerations. One reason for this could be due to the sample size being comprised of only 303 individuals. Participants met the inclusion criteria only if they were categorized as being in a state of IGT when measured at baseline, which subsequently led to a small sample size. With only 303 participants being classified as having IGT upon visit 1, this small n-size may in turn serve as a limitation. Another potential explanation that may account for the discrepancies in terms of what was deemed to be of

[24] When measured at baseline and again at follow-up in the context of our current study.

statistical significance [25] may be due to the criteria utilized and codified by researchers accountable for the implementation and analysis of the Insulin Resistant Atherosclerosis Study. The specific criteria used may influence the number of participants who were categorized as being in a state of IGT in addition to affecting the the number of subjects who were potentially discounted from being classified as prediabetic as well. The criteria used for the purpose of this study are based on measuring impaired glucose tolerance (IGT) using the oral glucose tolerance test (OGTT) along with the frequently sampled intravenous glucose tolerance test (FSIGT) [10]. While the OGTT and the FSIGT are commonly utilized procedures, one potential concern is that the results from the IRAS data set may differ from those found in other studies due to manner in which both IGT and NGT are operationally defined. More specifically, the operational definitions and criteria used can impact the overall findings by influencing the statistical significance of the variables chosen. Another consideration is that the decision of whether to accept or reject the null hypothesis may differ across studies based upon the significance levels and/or confidence intervals used.[26]

5.2 Study Strengths and Limitations

The prospective cohort study design exemplified by the insulin resistance atherosclerosis study (IRAS) is a powerful tool. It is a rare opportunity through which to evaluate changes occurring over time for a wide assortment of characteristics in order to find potential predictors that may incite a reversal from a state of IGT to one of NGT. A strength of this study is that analyzing the cohort from the IRAS data set allows for the observation of trends occurring over a five-year time frame for an entire host of factors. The extensive number of variables contained within the IRAS data set can ultimately set the stage for a wealth of associations between

[25] in addition to the variables that were deemed to not be statistically significant.
[26] Although this is rather unlikely.

variables to be analyzed over time in future research studies. Another strength of IRAS is that it is the first epidemiologic study of its kind intended to evaluate relationships between factors such as insulin resistance, insulinemia, glycemia, and the prevalence of cardiovascular disease (or CVD) when assessed in the context of a large multiethnic cohort. Moreover, researchers went to great lengths (and even oversampled) in an attempt to ensure as much equal representation as possible across all respective levels of gender, race/ethnicity, geographic region, along with the glucose tolerance status of participants.

Although the criteria and the operational definitions utilized in the IRAS to measure glucose tolerance are widely accepted, the number of individuals who were characterized as having IGT at baseline was limited (n=303). As a result, this rendered a generalizable conclusion difficult to make and is accompanied by the inability to infer causality. Due to the unanticipated results arising with regard to menopausal status, it is quite possible that there may be underlying mechanisms or metabolic processes faced by female participants that have not yet been fully understood or encapsulated and that merit future research. With respect to female subjects, drawing conclusions regarding menopausal status and the potential for a transition in glucose tolerance status is difficult to make on account of the small sample size (n=189) and as a result the findings should be interpreted with caution. Nonetheless, the statistical methodology used in this study to tackle this problem—Fisher's Exact test—does account for this exceedingly small sample size. This test helped to generate p-values that are more reflective of reality (despite the small n-size) which is another strength of this study. Lastly, the use of exploratory data analysis (EDA) sets the stage for future research that can build off of these preliminary results since this study merely serves as a starting point.

5.3 Conclusions and Recommendations

Despite the small sample size (n=303) of the cohort used in this study, the goal is to bring attention to the importance of reversing prediabetes back to a state of NGT before these patients ultimately transition from a state of IGT to T2D and the health-related damage incurred is irreversible. The contradictory nature of some of the abovementioned findings and the lack of statistical significance with regard to certain variables[27] attest to the current paucity of studies and serve as a testament for why further research needs to be conducted in this area in order to bridge existing gaps. Novel research is needed to better understand the issues surrounding this inadequately studied area of prediabetes and can also help in the creation of targeted interventions aimed at mitigating dysglycemia and at restoring NGT.

[27] that one would expect to serve as adequate predictors of a regression from a state of IGT to NGT.

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