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Relationship Between Patient-Health Coach Interactions and Changes in Markers of Glucose Homeostasis

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A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy at Virginia Commonwealth University

by

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

1,5-AG	1,5- Anhydroglucitol
AACC	American Association of Clinical Chemistry
ADA	American Diabetes Association
AMR	Analytical measurement range
ANCOVA	Analysis of Covariance
AV	Analytical variability
BMI	Body mass index
BV	Biological variability
CAP	College of American Pathologists
CAD	Coronary artery disease
CHC	Clinical Health Consultant
CHWP	Connected Health and Wellness Platform
CLIA88	Clinical Laboratory Improvement Act of 1988
CRR	Clinical reportable range
CV	Covariate
DKA	Diabetic ketoacidosis
DPMP	Diabetes Prevention and Management Panel
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
GLM	General Linear Module
GLUT2	Glucose transporter 2
G-6-P	Glucose-6-phosphate
G6PD	Glucose-6-phosphate dehydrogenase
HbA1c	Hemoglobin A1c
HgbA	Hemoglobin A
HIIE	High intensity intermittent exercise
HK	Hexokinase
HPLC	High pressure liquid chromatography
HLA	Human leukocyte antigen
IAA	Insulin antigen antibody
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IMS	Information management system

IT	Information technology
kDa	kilodalton
LDL	Low density lipoprotein
LIS	Laboratory information system
MD	Mahalanobis distance
MI	Motivational interviewing
MODY	Maturity onset of diabetes of the young
NAD+	Nicotinamide adenine dinucleotide (oxidized)
NADH	nicotinamide adenine dinucleotide (reduced)
NDDG	National Diabetes Data Group
NIDDM	Non-insulin dependent diabetes mellitus
nm	Nanometers
OGTT	Oral glucose tolerance test
PI	Principle investigator
PID	Patient identification number
PI3K	Phosphatidylinositol 3-kinase
PPGi	Post-prandial glycemic index
PROD	Pyranose oxidase
QC	Quality control
rBWL	Reduced intensity behavioral weight loss
SAS	Statistical Analysis Software
SD	Standard deviation
SI	System International
SID	Sample identification number
sdLDL	Small dense lipoprotein
SST	Serum separator tube
T2D	Type II diabetes
T1D	Type 1 diabetes
TTM	Transtheoretical model
US	United States

Abstract

RELATIONSHIP BETWEEN PATINET-HEALTH COACH INTERACTIONS AND CHANGES IN MARKERS OF GLUCOSE HOMEMSTASIS

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A dissertation submitted in partial fulfilment of the requirements for the degree of doctor of Philosophy at Virginia Commonwealth University

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Diabetes and insulin resistance are on the rise in the United States. Early detection and deployment of therapies has allowed for the reversal of pancreatic beta cell damage. Unfortunately, not all providers can offer the support to facilitating the required life style modifications. The introduction of clinical health consultants (CHC) as supplemental care has improved patient health for a variety of chronic diseases. Missing in the literature are studies investigating the correlation between the number of CHC interactions and improvement in biomarkers.

The study utilized a non-experimental, retrospective study design to evaluate the relationship between the use between the use of CHCs and the number of CHC interactions, and

the mean changes in glucose, hemoglobin A1c, insulin, proinsulin, C-peptide, and 1,5-anhydroglucitol, over a one-year period for patients presented with the opportunity to participate in CHC interactions. The subjects' follow-up results were compared to their initial results for each group using the ANCOVA and one-way t-test.

A statistically significant difference was detected between the mean change in BMI and the use of CHCs ($p < 0.001$). In addition, a statistically significant relationship was identified between the number of CHC interactions and the magnitude of change in BMI ($p < 0.001$). No statistically significant differences were detected for the other study biomarkers. Initial biomarker values and random error explained a majority of the differences found between the CHC and non-CHC groups. The use of CHC interactions had a minimum effect on the statistical models used to compare the CHC and non-CHC groups.

Chapter 1: Introduction

Overview

The study investigated the relationship between the number of clinical health consultant (CHC) interactions and changes in biomarkers used to evaluate glucose homeostasis related to diabetes, insulin resistance and beta cell dysfunction. The retrospective study utilized previously collected data, including glucose, hemoglobin A1c (HbA1c), 1,5-anhydroglucitol (1,5-AG), insulin, C-peptide, proinsulin, and BMI results, as well as the number of patient-CHC interactions. The results of this study contributed to the knowledge of the potential efficacy of CHCs in improving the management and outcomes of patients with diabetes, insulin resistance or beta cell dysfunction.

Chapter 1 states the study's purpose, hypotheses, and a summary of health coaching and data sources with background information on the health risks related to diabetes, and some of its potential precursors, insulin resistance and beta cell dysfunction. Chapter 2 provides an overview of diabetes and a literature review of biomarkers related to glucose homeostasis, diabetes, insulin resistance, and beta cell dysfunction, disease treatment, and the CHC profession. Chapter 3 details the methods used in the study, including sample selection, biomarker testing, and statistical analysis. Chapter 4 presents the results of statistical analysis and subject demographics.

Chapter 5 offers a discussion of the results, study limitations, and recommendations for future studies.

Background

Diabetes is a disorder of glucose metabolism that affects 30.3 million people in the United States (US), or 9.4% of the American population. Of those, 7.2 million or 23.1% are reportedly undiagnosed (Centers for Disease Control and Prevention, 2014; Centers for Disease Control and Prevention, 2017). Diabetes increases the risk of heart disease and stroke, as well as many other microvascular and macrovascular comorbidities. Improperly managed diabetes can result in kidney disease, blindness, and amputations. There are four classifications of diabetes: Type 1 diabetes (T1D) is caused by the autoimmune or other toxic destruction of pancreatic beta cells. Type 2 diabetes (T2D) is due to a progressive loss of beta cell insulin secretion that follows the development of insulin resistance. Gestational diabetes mellitus (GDM) develops during the second or third trimester of pregnancy, with no diagnosis of diabetes prior to pregnancy. Secondary diabetes is caused by monogenic defects resulting in beta cell dysfunction, including neonatal and maturity-onset diabetes (Riddle, 2018). Additional causes of secondary diabetes include disorders of the pituitary, thyroid, or adrenal glands, diseases of the exocrine pancreas, or drug and chemical related diabetes (Riddle, 2018). Estimates show that of all the individuals with diabetes, 90-95% have T2D. (Centers for Disease Control and Prevention, 2017). Type 2 diabetes, unlike T1D, is potentially a preventable disease. The development of T2D is not an acute process, but rather a slowly progressive condition that results in beta cell dysfunction. Several conditions are associated with the risk of developing T2D including obesity, reduced exercise, smoking, high blood pressure, and high cholesterol (Centers for Disease Control and Prevention, 2017).

Peripheral blood glucose levels are determined by exogenous glucose from the breakdown of consumed food, and endogenous glucose produced by the liver. Hepatic conversion of glycogen to glucose, known as glycogenolysis, and glucose production from amino acids known as gluconeogenesis, occur during times of decreased glucose ingestion such as sleep and between meals. To compensate for increases in plasma glucose concentrations, in insulin resistant states, the pancreatic beta cells secrete more insulin, in an effort to enable peripheral tissues to take up glucose. Over time, the beta cells become exhausted and lose their ability to produce sufficient insulin. Early detection of this process allows for lifestyle modifications that can potentially delay or reverse the progression to T2D. Diabetes was ranked as the seventh leading cause of death in 2015, due to complications such as cardiovascular disease, stroke, end-stage renal disease, and diabetic ketoacidosis. Early detection and intervention has the potential to reduce diabetes related morbidity and mortality (Centers for Disease Control and Prevention, 2017).

The lifestyle modifications required of patients to prevent and/or treat T2D can be controversial, challenging, and sometimes overwhelming to the patient. The American Diabetes Association (ADA) recommends that individuals with diabetes should participate in diabetes self-management education and support (DSMES), medical nutrition therapy (MNT), physical activity, smoking cessation counseling, and psychosocial care (American Diabetes Association, 2018). The introduction of self-management programs has shown to have a positive effect on weight loss and lifestyle modification.

CHCs can help facilitate the process of lifestyle modification and treatment compliance that is required for positive patient outcomes. The development of treatment goals through discussion between the patient and a CHC is an initial step of therapy. CHC involvement

provides support for patients secondary to the care received from their providers. The CHC-patient interaction can increase motivation to comply with recommended therapies through behavior change therapies, identifying underlying factors that may influence an individual's motivation, as well as plan nutritional and exercise options. The main objective of a CHC program is not to treat but to educate the patient and help create the changes needed to overcome their condition.

Research Problem

Many patients find it difficult to change their lifestyle based on health care provider recommendations, despite the understanding that it is needed to improve their overall health. Perceived treatment efficacy, physician trust, worsening of diabetes symptoms, medication cost, complexity of medication dosage and the side effects of medications are factors that lead to patient noncompliance (Polonsky & Henry, 2016). These barriers to patient compliance suggest that doctor-patient interaction is not sufficient for implementing lifestyle changes. Ambivalence related to motivation and lifestyle changes may also present as barriers (Kehler et al., 2008). Studies demonstrate that CHC interactions may facilitate and improve adherence to these lifestyle changes and correlate CHC interactions with changes in markers such as BMI and HbA1c (Leahey & Wing, 2013; Pettitt, 2013; Wayne & Ritvo, 2014; Wolever et al., 2010). The mentioned studies only compare a control group to a treatment group with a set number of CHC interactions. Missing are studies relating the number of CHC interactions to the magnitude of change in specific biomarkers are lacking. Furthermore, there is no literature on the effect of CHC interactions on markers of insulin resistance and beta cell dysfunction such as insulin, C-peptide, and proinsulin, or the more recent glycemic marker, 1,5-AG.

Purpose of the Study and Research Question

The purpose of this study was to retrospectively examine the relationship between patients engaging in CHC interactions and changes in glucose, %HbA1c, 1,5-AG, insulin, C-peptide, proinsulin, and BMI to determine the effectiveness of CHC interactions on potential change in patient health. The study examined patient results and CHC utilization to determine if a correlation between the number of CHC-patient interactions and changes in biomarkers over time exists. The study attempted to answer the research question, is there a relationship between the changes in patients' biomarkers of glucose homeostasis and beta cell health and their interactions with CHCs?

Specific Aims

The study had three Specific Aims:

> Specific Aim 1: Determine if there are statistically significant differences between patients who do or do not participate in CHC interactions in their changes in 1) blood glucose concentration, 2) %HbA1c, 3) blood 1,5-anhydroglucitol concentration (1,5-AG), 4) blood insulin concentration, 5) blood C-peptide concentration, 6) blood proinsulin concentration, and 7) body mass index (BMI).

This was addressed by comparing the difference between initial biomarker results and follow-up results 10-14 months from initial testing, for two groups, 1) those who participated in CHC interactions, and 2) those who did not. Changes in biomarker values were calculated for each marker in both the CHC and non-CHC groups and compared statistically.

> Specific Aim 2: Determine if statistically significant differences exist in the change in diabetes and BMI health scores between subjects who did and those who did not interact with CHCs for glucose and HabA1c.

Arbitrary health scores were assigned to initial and follow-up testing results. Health scores were based upon ADA recommended cutoffs for normal, prediabetic, and diabetic values for glucose and HbA1c, and BMI guidelines for normal, overweight, and obese individuals. The change in health score was calculated between initial and follow-up testing 10-14 months from initial testing for two groups, 1) those who participated in CHC interactions, and 2) those who did not. Group health score differences were then statistically compared.

> Specific Aim 3: Determine the relationship between the number of CHC interactions and magnitude of the change in 1) blood glucose concentrations, 2) % HbA1c, 3) blood 1,5-anhydroglucitol (1,5-AG) concentrations, 4) blood insulin concentrations, 5) blood C-peptide concentrations, 6) blood proinsulin concentrations and 7) body mass index (BMI).

This was determined by the use of a general linear model to compare the magnitude of change in each biomarker between subjects with different numbers of CHC interactions. Change in biomarker values were compared to varying numbers of CHC interactions. Post-hoc Bonferroni tests of multiple comparisons evaluated the mean change between groups.

Significance of the Study

CHC interactions have shown to be effective in the management and treatment of diabetes and other related conditions. Studies investigating the effect of CHC interactions have only utilized the markers of weight loss and HbA1c (Leahey & Wing, 2013; Pettitt, 2013; Wayne & Ritvo, 2014; Wolever et al., 2010). There is no literature on CHC interactions and potential change in insulin, C-peptide, proinsulin, and 1,5-AG, nor on the connection between the number of CHC interactions and the magnitude of the biomarker change. The results of this study increase the knowledge on the relationship between the number of CHC-patient interactions and

improvements in biomarkers for diabetes and insulin resistance. This knowledge could help guide the creation and planning of CHC-mediated therapies to maximize their effectiveness and efficiency for attaining patient treatment goals. Study findings could also serve as a foundation for future studies on how to reduce health care costs and improve patient care utilizing CHCs.

Summary of Data Sources and Analysis

Data for this study was gathered from two databases. The laboratory information system (LIS) at a laboratory in Richmond, VA contained archived patient results for the biomarkers of interest, starting in April 2012. A query of the LIS provided biomarker results, along with subject demographics such as age, gender, and body mass index.

The CHC information management system (IMS) database at the same laboratory housed the dates of patient-CHC interactions. An initial query of the LIS identified patients that met the study inclusion criteria. Then, a query of the IMS accessed information on CHC interactions for the subjects identified from the LIS query.

Data from both the LIS and the IMS were merged into a single Microsoft Excel file. The Principle Investigator imported the data set into Statistical Package for the Social Sciences (SPSS) for data analysis. The study utilized a one-way analysis of covariance (ANCOVA) and linear regression. This approach allowed for the determination of within subject differences in biomarkers, as well as the relationship between CHC-patient interactions, the study independent variable (IV) and the changes in the markers, the study dependent variables (DVs), while adjusting for the patient demographics sex, age, and BMI, the study covariables (CV).

Chapter Summary

Chapter 1 provided background on diabetes, the challenges of lifestyle modification, and how CHCs can help facilitate the change required to improve patient health. Multiple studies

demonstrate the ability of CHCs to change BMI and %HgbA1c but none address the effects of CHCs on other makers used to monitor glucose homeostasis and beta cell health. Chapter 2 offers an overview of the pathophysiology of diabetes, biomarkers of glucose homeostasis, and the potential impact of CHCs on health care outcomes, along with the gaps in literature that this study hopes to fill. Chapter 3 provides the proposed study's methodology, including the target population, sampling strategy, data collection methods and analysis, and potential limitations. Chapter 4 presents the study findings, while Chapter 5 discusses the study results and describes the study limitations, and recommendation for future studies.

Chapter 2: Literature Review

Introduction

Chapter 1 introduced the proposed study including the study purpose, aims, and research questions. Chapter 2 provides an overview of the pathophysiology of diabetes, biomarkers of glucose homeostasis, treatments for diabetes, and the clinical health coach professions. First, an overview of glucose homeostasis and diabetes is presented. Next, information detailing the markers used in the study is discussed. Finally, information describing CHCs and relevant studies demonstrating their effect on improving patient health is presented.

Overview

A key to controlling diabetes, and potentially reversing the progression towards T2D, is the identification of biomarkers of the prediabetic state. Monitoring biomarkers closely linked to diabetes, insulin resistance, and beta cell function allows for the evaluation of diabetes risk and control. Measuring glucose, 1,5- anhydroglucitol (1,5-AG), and hemoglobin A1c (%HbA1c) provide a snapshot of glucose concentration in the peripheral blood at the time of venipuncture, as well as an estimate of the average glucose concentration over the past two weeks to 3 months. Abnormal levels of C-peptide, insulin, and proinsulin in the blood alerts providers to the presence of beta cell dysfunction or death. If detected early, deployment of therapies can potentially reverse the damage sustained to pancreatic beta cells.

Adherence to the lifestyle changes required to manage or prevent diabetes is not easy. It is estimated that a majority of adult diabetics fail to follow physician prescribed treatments for their disease (Funnell, 2006; Gonzalez, Shreck, Psaros, & Safren, 2014; Willard-Grace et al., 2013). CHC interactions, when coupled with provider therapies, have shown to have a positive effect on health outcomes (Appel et al., 2011; Battista et al., 2012; Eakin, Lawler, Vandelanotte, & Owen, 2007). A common belief in health coaching is that the patient has the ability to adopt healthy behaviors if given the proper guidance. The professional health coach is an educator, one whose goal is to provide information and support that elicit change in patient behavior and physical health.

Glucose Homeostasis

Plasma glucose homeostasis is the result of the dynamic balance between glucose intake and hepatic synthesis, and the demand for and uptake of glucose by organs and cells including the brain, gut, liver, kidneys, pancreas, adipocytes and myocytes. The liver and brain take up glucose directly, not requiring an insulin dependent glucose transporter. The kidneys help regulate glucose by allowing glucose to be excreted into the urine if the glucose renal threshold of 180 mg/dL is exceeded. Beta cells, the site of insulin production, are found in a region of the pancreas called the Islets of Langerhans. Two major types of cells in the Islets of Langerhans, the alpha and the beta cells, are specific to glucose homeostasis. Alpha cells produce glucagon, a hormone that stimulates the production of glucose from glycogen, amino acids, glycerol, and lactate in the liver. Beta cells produce insulin, the hormone responsible for the regulation of glucose transportation from the peripheral blood into the cell.

Insulin production begins with the synthesis of a parent peptide, preproinsulin. (Chan, Keim, & Steiner, 1976). Preproinsulin, a protein comprised of approximately 100 amino acids,

has a short half-life of approximately one minute before it is enzymatically cleaved (Patzelt et al., 1978). Within the rough endoplasmic reticulum (ER) of the beta cells, the 23 amino acids at the N-terminal of preproinsulin are removed and two disulfide bonds are formed, resulting in the formation of proinsulin (Steiner, Cunningham, Spigelman, & Aten, 1967). Proinsulin is then transported to the Golgi apparatus where it is packaged into storage granules along with prohormone convertases 1 and 2, and carboxypeptidase H. This conversion is proportionate with glucose concentration and dependent on the availability of convertase enzymes PC2 and PC1/PC3 (Nagamatsu, Bolaffi, & Grodsky, 1987). Within the Golgi apparatus, the cleavage of the 31 amino acid C-peptide from proinsulin forms the hormone insulin. C-peptide and insulin remain within secretory granules in the beta cells until an increase in blood glucose levels triggers the release of insulin into the blood.

Glucose stimulation of insulin secretion by the beta cells requires a cascade of events. First, the glucose transporter 2 (GLUT2) found on the membrane of beta cells transports glucose from peripheral blood into the beta cell. Glucose then undergoes glycolysis within the beta cell, generating an increase in ATP. The increase in ATP causes the ligand-gate potassium channel to close, resulting in an increase in intracellular potassium level and membrane depolarization. Membrane depolarization allows extracellular calcium to enter the beta cell via voltage-gated Ca2+ channels, with increasing calcium concentrations signaling the insulin-containing vesicles to release insulin and C-peptide. Insulin secretion is biphasic, with the first phase of insulin secretion occurring 2-3 minutes after glucose levels rise and lasting for around 10 minutes. The second phase of insulin release occurs after the initial, with glucose levels still elevated, and continues until glucose homeostasis is achieved. Individuals with T2D are shown to have impaired insulin secretion in the first phase (Cerasi, 1992). Secreted insulin circulates in the

blood and binds to insulin receptors on the surface of cells such as adipocytes and muscle cells, stimulating the translocation of the glucose transporter GLUT4 from intracellular storage vesicles to the cell membrane (Saltiel & Kahn, 2001).

The alpha and beta cells of the pancreas work in tandem to maintain glucose homeostasis. In addition to facilitating the glucose uptake by peripheral cells, a high ratio of insulin/glucagon also promotes the storage of glucose as glycogen in the liver and muscle cells. Conversely, when plasma glucose concentrations fall as in a fasting state, the alpha cells release glucagon, signaling the liver to convert glycogen to glucose and to promote gluconeogenesis. The inability of the body to regulate glucose levels in the peripheral blood, caused by lack of insulin, or insulin resistance, is characteristic of diabetes.

Diabetes

Diabetes is a disorder of glucose metabolism that affects 30.3 million people in the United States (US), or 9.4% of the American population. Of those, 7.2 million or 23.1% are reportedly undiagnosed (Centers for Disease Control and Prevention, 2014; Centers for Disease Control and Prevention, 2017). There are four classifications of diabetes: Type 1 diabetes (T1D) is caused by the autoimmune destruction of pancreatic beta cells. Type 2 diabetes (T2D) is a progressive loss of beta cell insulin secretion that is highly correlated with insulin resistance. Gestational diabetes mellitus (GDM) is a diagnosis of diabetes during the second or third trimester of pregnancy with no diagnosis of diabetes prior to pregnancy. Secondary diabetes is due to causes including neonatal and maturity-onset diabetes, disease of exocrine pancreas, disorders of the pituitary, thyroid, or adrenal glands, pancreatic insufficiency, and drug and chemical related diabetes (Riddle, 2018) . One of the diagnostic clinical signs of diabetes is the inability to regulate plasma glucose concentration. Over time, the metabolic abnormalities of

diabetes can lead to numerous potential microvascular complications such as diabetic nephropathy and failure, neuropathy, retinopathy, macrovascular complications, and atherosclerotic vascular disease (coronary, peripheral, cerebrovascular). Uncontrolled diabetes can also result in a sudden onset of ketoacidosis and hyperosmolar coma, as well as eventual kidney failure, blindness, and amputations (Forbes & Cooper, 2013).

Type 1 Diabetes

Type I diabetes is characterized by an autoimmune destruction of pancreatic beta cells, the cells responsible for secreting the hormone insulin. Genetic mutations, specific to class II human leukocyte antigen (HLA) alleles encoding HLA DRB1*03:01-*DQA1**05:01-*DQB1**02:01 abbreviated DR3 and HLA *DRB1**04:01/02/04/05/08-*DQA1**03:01-*DQB1**03:02/04 (or *DQB1**02; abbreviated DR4 on chromosome 6p21.31, are highly correlated with T1D. The HLA regions I and II are responsible for the production of antigens that bind antigenic peptides involved with T-helper cell presentation. Specifically to T1D, T- cell presentation of autoantigens typically leads to the production of autoantibodies to proteins found in the beta cells. It was noted that mutations resulting in HLA DR3/DR4 heterozygotes are more closely linked to T1D than homozygotes of either haplotype (Nobel & Valdes, 2011). The antibodies typically found in the plasma of Type 1 diabetics are insulin antigen antibody (IAA), and anti-glutamic acid decarboxylase (anti-GAD) (Balakhadze, Giorgadze, & Lomidze, 2016). Type 1 diabetes usually occurs before the age of 15, but may not be diagnosed until later in life. In addition to their age at the time of diagnosis, patients with adult onset of T1D can be distinguished from adolescent T1D by higher body mass index (BMI) and C-peptide values (Törn et al., 2000).

There are several stages of T1D (Table 1), with clinical symptoms usually seen at the later stages (Skylar et al., 2017), (Insel et al., 2015). In stage 1, autoantibodies are present, with the absence of any clinical signs of diabetes and normal glucose metabolism. During stage 2, patients begin to have impaired fasting glucose. Fasting plasma glucose (FPG) levels may rise above the normal cutoff of 99 mg/dL but still below the 126 mg/dL clinical cutoff for diabetes; or the 2-hour post-prandial sample in an oral glucose tolerance test (OGTT) may be between 140-199 mg/dL. Percent HbA1c values may rise above the normal cutoff of 5.6%, but below the diabetic cutoff of 6.5%. Patients in Stage 3 display clinical signs of diabetes, polyuria/polydipsia, with one-third having diabetic-ketoacidosis. Stage 3 is also confirmed with FPG and % HbA1c values consistent with a diagnosis of diabetes: FPG ≥ 126 mg/dL, a two-hour OGTT ≥ 200 mg/dL, or %HbA1c ≥ 6.5.

Table 1: Type 1 Diabetes Diagnosis by Stage

Stage of Type 1 Diabetes	Diagnostic Criteria
1	• Positive for multiple autoantibodies • No impaired glucose tolerance test • No impaired fasting glucose

2	• Positive for multiple autoantibodies
	• Dysglycemia:
	  ○ FPG 100–125 mg/dL (5.6–6.9 mmol/L)
	  ○ 2-h PG 140–199 mg/dL (7.8–11.0 mmol/L)
	  ○ A1C 5.7–6.4% (39–47 mmol/mol) or ≥ 10% increase in A1C

3	• Clinical symptoms ○ polyuria/polydipsia, and one-third with diabetic ketoacidosis (DKA) • Hyperglycemia • Diabetes by standard criteria

Adopted from (Skylar et al., 2017), (Insel et al., 2015), (Cefalu, 2017), (Dabelea et al., 2014)

Controlling T1D involves coordinated management of diet, exercise, and insulin injections (Riddle, 2018). Therapies designed to normalize glucose metabolism in type 1

diabetics have secondary benefits of reducing cardiovascular events. The link between glycemic control and macrovascular disorders is well-established (Klein, 1995; Shamoon et al., 1993).

Type 2 Diabetes

Unlike T1D, T2D is not due to a cellular immune reaction to the beta cells in the pancreas. Rather, the development to T2D begins with accumulation of fat on muscle, liver, and pancreatic tissue, resulting in inflammation, insulin resistance, and then eventual beta cell dysfunction (Riddle, 2018; Skyler et al., 2017). Inflammation can lead to a disruption of the ability of insulin to activate receptors on the cells in insulin dependent tissues such as muscles. This phenomenon, termed "insulin resistance," leads to diminished activity in insulin-mediated pathways, such as the uptake of glucose (Sinaiko & Caprio, 2012). Cusi et al. concluded that the ability of insulin to stimulate the phosphatidylinositol 3-kinase (PI 3-kinase) pathway, the enzyme responsible for the transduction of insulin binding to its receptor and the recruitment of glucose transport proteins to the surface of cells, was reduced in obese patients and almost undetectable in patients with T2D (Cusi et al., 2000). The reduction of glucose transport into the cells results in increased plasma glucose concentrations.

Individuals with insulin resistance can have glucose concentrations and % HbA1c that are still below the diabetic diagnostic threshold. Protracted hyperglycemia due to insulin resistance signals pancreatic beta cells to secrete more insulin, leading to increases in the beta cell products proinsulin, insulin, and C-peptide in the blood. The state of insulin resistance with mild hyperglycemia may be present for many years before beta cell damage is clinically apparent. When the beta cells can no longer produce enough insulin to maintain FPG and %HbA1c within normal levels, the diagnostic threshold of prediabetes are crossed. The American Diabetes Association (ADA) and the International Expert Committee on the Diagnosis and Classification

of Diabetes Mellitus have recognized this group of individuals whose glucose levels do not meet criteria for diabetes as "prediabetic" (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). Prediabetes is not considered its own disease state; patients with prediabetes are at an increased risk for developing T2D and cardiovascular complications (Armstrong, 2017).

At this point, beta cell function continues to deteriorate without lifestyle adjustments. When patients in a prediabetic state are identified, interventions such as lifestyle changes can delay or even prevent progression to T2D. When 80% of beta cell function is lost, circulating levels of insulin, proinsulin, and C-peptide start to decrease, eventually leading to levels of hyperglycemia and %HgbA1c that are consistent with T2D (DeFronzo & Abdul-Ghani, 2011). If interventions fail to preserve the remaining beta cells, the patient will require exogenous sources of insulin, similar to Type 1 diabetics (Weir & Bonner-Weir, 2004).

Figure 1 illustrates the chronological relationships between various parameters of the derangement of glucose homeostasis along the progression to T2D.

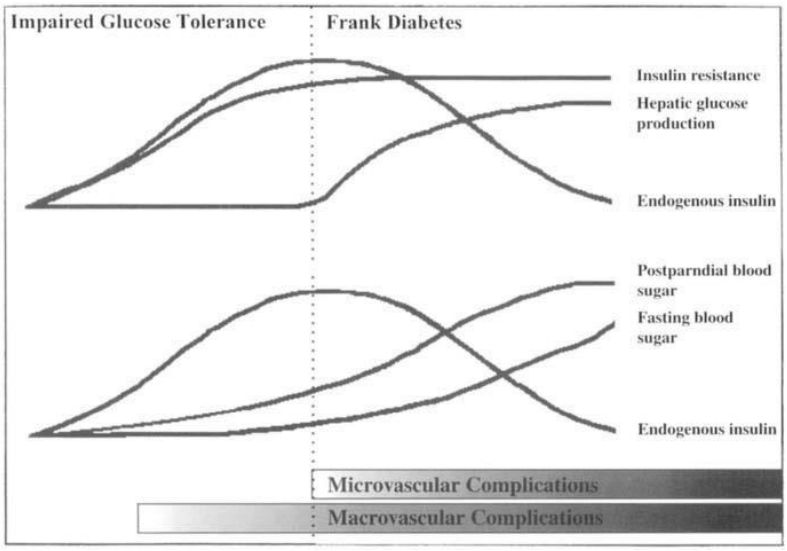


Figure 1: Diabetes Progression Timeline
Duplicated with permission, (Ramlo-Halsted & Edelman, 1999)

Weir, et al have proposed 5 stages in the progression to T2D:

- Stage 1, Compensation. The first stage is characterized by the ability to maintain normal blood glucose concentrations but only because of an increase of insulin production. Compensation is thought to entail an increase in beta cell mass to accommodate the increase in insulin. Monitoring blood glucose concentrations or %HgbA1c would not indicate the presence of insulin resistance or potential progression towards T2D.
- Stage 2, Stable adaptation. Beta cells can no longer maintain normal glucose levels in the peripheral blood, and patients demonstrate sustained elevated post-prandial glucose, and fasting blood glucose levels up to 130 mg/dL, along with impaired glucose stimulation of insulin secretion. Patients may avoid progression to Stage 3 for many years, with lifestyle changes such as diet and exercise (Knowler et.al., 2002).
- Stage 3, Unstable Early Decompensation. Functional beta cells have declined to a level where there is inadequate response to elevated glucose concentrations, and glucose levels rise to as high as 350 mg/dL in a short period of time.
- Stage 4, Stable Decompensation. Beta cell size and mass that is half of that in normal individuals. Most patients with T2D can remain in Stage 4 for the rest of their lives, with a sufficient amount of insulin production to prevent diabetic ketoacidosis.
- Stage 5, Severe Decompensation. The loss of beta cells is so severe that patients become susceptible to ketoacidosis and are totally dependent on insulin for survival. Glucose levels are typically >350 mg/dL (Weir & Bonner-Weir, 2004).

According to the ADA, a diagnosis of diabetes is made if one of any of the following conditions is demonstrated on more than one occasion: [1] HbA1c is ≥ 6.5%, [2] fasting glucose is ≥ 126 mg/dL, [3] the two-hour specimen from an oral glucose tolerance test (OGTT) is ≥ 200 mg/dL or [4] the patient has a random blood glucose concentration > 200 mg/dL along with the clinical symptoms of diabetes (Cefalu, 2017). Patients without clinical signs of diabetes and whose lab results do not meet the criteria for diabetes may be classified as prediabetic, according to the ADA, if they demonstrate fasting blood glucose concentrations between 100 mg/dL and 125 mg/dL, %HbA1c concentrations between 5.7 and 6.4%, or a two-hour post-prandial blood specimen from an OGTT between 140 mg/dL and 199 mg/dL (Cefalu, 2017). In 2015, it was estimated that in the US, 37% of adults over 18 and 48.3% of all adults aged 65 and above are prediabetic (Centers for Disease Control and Prevention, 2017). It is recommended that individuals who are asymptomatic have their blood glucose and %HgbA1c measured if they have a BMI greater than 25 kg/m^2 or have other risk factors for diabetes such as reduced exercise, a family history of diabetes, or a less than healthy lifestyle (American Diabetes Association, 2013). Increases in age and BMI are highly correlated with the occurrence of T2D (American Diabetes Association, 2013);(Centers for Disease Control and Prevention, 2017).

Gestational Diabetes

Gestational diabetes is the diagnosis of diabetes in the second or third trimester of pregnancy, in the absence of T1D or T2D prior to conception. Gestational diabetes is associated with an increased risk of perinatal complications and maternal T2D after delivery (Riddle, 2018). The exact pathophysiology of GDM is unknown; however, obesity and increased BMI are highly correlated with the development of GDM (Ehrenberg, Dierker, Milluzzi, & Mercer, 2002; Hedderson, Williams, Holt, Weiss, & Ferrara, 2008). Testing for GDM usually occurs at 24-28

weeks gestation in women with no prior diagnosis of diabetes. If a woman is diagnosed with GDM, she is tested again at 4-12 weeks postpartum for persistent diabetes. Testing can be done two ways, a one-time dose of 75g glucose OGTT or a two-step 50g OGTT screen followed by a 100g OGTT for positive screen results. The one-time 75g OGTT cutoffs for diagnosis of GDM are a fasting glucose ≥ 92 mg/dL, 1 hour glucose ≥ 180 mg/dL, or a 2 hour glucose ≥153 mg/dL. According to the ADA, glucose measurements of ≥ 130 mg/dL, 135 mg/dL, or 140 mg/dL at 1 hour following a 50g load are all acceptable positive cutoffs, with the 140 mg/dL cutoff being the most specific (70-88%) and the 130 mg/dL cutoff being the most sensitive (69-89%) (Riddle, 2018). The cutoff used is dependent on the physician's preference. Patients would then receive a 100 g OGTT at their next visit. There are two criteria for 100g OGTT cutoffs, the Carpenter-Coustan and the National Diabetes Data Group (NDDG). According to Carpenter-Coustan, the diagnosis of diabetes is made if at least two of the following are met: Fasting glucose ≥ 95 mg/dL, a 1 h glucose ≥180 mg/dL, a 2 h glucose ≥ 155 mg/dL, a 3 h glucose ≥ 140 mg/dL. According to the NDDG, a diagnosis of diabetes is made if fasting glucose is ≥ 105 mg/dL, 1 hour glucose ≥190 mg/dL, 2 hour glucose ≥ 165 mg/dL, or 3 hour glucose ≥ 145 mg/dL following the 100 g load. Table 2 illustrates the two different methods for diagnosis GDM.

Table 2: Criteria for GDM Diagnosis

One Step strategy 75-g OGTT		**Two-Step Testing 50-GOGTT ≥ 130 mg/dL (7.5 mmol/L) positive 100- OGTT Cut-off**	
		Carpenter-Coustan	**NDDG**
Fasting	92 mg/dL (5.1 mmol/L)	95 mg/dL (5.3 mmol/L)	105 mg/dL (5.8 mmol/L)
1 h	180 mg/dL (10 mmol/L)	180 mg/dL (10.0 mmol/L)	190 mg/dL (10.6 mmol/L)
2 h	153 mg/dL (8.5 mmol/L)	155 mg/dL (8.6 mmol/L)	165 mg/dL (9.2 mmol/L)
3 h	N/A	140 mg/dL (7.8 mmol/L)	145 mg/dL (8.0 mmol/L)

Classification and Diagnosis of Diabetes (Riddle, 2018)

Treatment for gestational diabetes could include a lifestyle changes such as a modified diet and exercise schedule, along with glucose monitoring and/or insulin injections to assist reaching glycemic targets of fasting glucose < 95 mg/dL (Riddle, 2018).

Diabetes Due to Other Causes

Causes for secondary diabetes include single gene mutations causing neonatal diabetes, a diagnosis of diabetes within the first six months of life, and maturity-onset diabetes of the young (MODY), a condition typically characterized by impaired insulin secretion and hyperglycemia before the age of 25. Specifically, MODY is the result of defective insulin production in response to increases in plasma glucose concentration (Fajans & Bell, 2011). In both neonatal and MODY, the cause of genetic mutation can be spontaneous, or the result of autosomal or recessive gene inheritance. Due to their unusual circumstance, these types of diabetes usually require the involvement of a diabetes specialist to determine the best treatment routine. Disease of exocrine pancreas such as cystic fibrosis and pancreatitis are also causes of secondary diabetes. Additionally, disorders in the pituitary, thyroid, or adrenal glands, pancreatic insufficiency, and drug or chemical interactions can cause secondary diabetes (Riddle, 2018).

Treatments for Diabetes

Lifestyle choices such as smoking, lack of exercise, diets high in carbohydrates and low in fiber can contribute to the development towards T2D. If detected early, lifestyle modifications such as regular exercise, lower energy intake, better food choices, smoking cessation, and medication can diminish or halt disease progression and improve cells' sensitivity to insulin (American Diabetes Association, 2018; National Diabetes Information Clearinghouse, 2014). Furthermore, Godsland et al. concluded that loss of beta cell function is highly correlated with increases in age and BMI (Godsland, Jeffs, & Johnston, 2004). Unfortunately, reversal of overt

T2D is much more difficult. By the time diagnostic glycemic thresholds of hyperglycemia have been crossed, severe beta cell damage has already occurred. It is recommended to develop and maintain a healthy lifestyle early, before beta cell damage becomes irreversible. A 2005 study reported that individuals who underwent intensive lifestyle modifications significantly improved insulin sensitivity and beta cell preservation over the course of one year (The Diabetes Prevention Program Research Group, 2005). Diabetes self-management, such as self-monitoring of blood glucose levels and diet selection, combined with education, and support, are correlated with increases in diabetes knowledge and self-care (Haas et al., 2012).

Once a patient is identified as being at risk for progression to T2D, education and support are important components, along with lifestyle changes, of a plan to reverse the process. However, patients often lack the ability to stick to a treatment plan. Gonzales pointed out that less than half of adult diabetics maintain an HbA1c level below recommended glycemic goal, mostly due to non-adherence to medication (Gonzalez, Shreck, Psaros, & Safren, 2014). Furthermore, less than 10% of individuals follow physician guidelines to stop smoking or lose weight (Haynes, 2001). Specifically, less than 17% of patients with T2D reportedly follow providers' prescribed regimens for diet, exercise, medication taking, glucose testing, and appointment keeping (Funnell, 2006; Skovlund & Peyrot, 2005). Data suggests that dependence solely on physicians' directives is not sufficient to successfully manage T2D. Barriers to compliance include: patient's perceived treatment efficacy, worsening of diabetes symptoms and side effects of medication, treatment complexity and convenience, cost of treatment, concerns related to negative effects of the medication being prescribed, and /or physician trust (Polonsky & Henry, 2016). In their 2001 study, Claxton et al. concluded that adherence to diabetes medication is inversely related to the number of daily doses. They found that the percent

adherence dropped from 79% with a single dose per day to 51% for medications requiring four doses per day (Claxton, Cremer, & Pierce, 2001). In 2009, Mann et al investigated the correlation of disease and medication beliefs and drug regimen adherence. Their survey of poorly adherent subjects concluded that some barriers to adherences include beliefs that [1] medication is needed only at times of hyperglycemia (56%, p=0.006), [2] is not needed when their glucose is normal (53%, p=0.02), [3] when the side effects would likely be severe (42% of poorly adherent subjects, p=0.001), and [4] that the medications instructions made them too difficult to take (74% of poorly adherence subjects, p=0.001) (Mann, Ponieman, Leventhal, & Halm, 2009).

Examples of some currently available medications include those to help control glucose levels by promoting insulin secretion (sulfonylureas and meglitinides), by reducing hepatic glucose production (biguanids), by reducing the reabsorption of glucose by kidney (SGLT2 inhibitors), or by reducing the absorption of glucose in the intestines (a-Glucosidase Inhibitors). Medications may not be needed for all patients. Lifestyle modifications, such as a low-calorie, low-fat recommended diets, and moderate intensity exercise for at least 150 minutes per week, have the potential to improve glucose control in patients with T2D (American Diabetes Association, 2018). Nondiabetic patients who adhere to these recommendations saw greater than 50% reduction in the occurrence of T2D, compared to only 31% of those only taking metformin, an oral drug used to help control plasma glucose levels by reducing hepatic glucose production, for 2.8 years (National Institute of Health, 2002). In addition to diet, a structured and monitored moderate exercise program alone may lead to improvement of glucose metabolism (Liao et al., 2015). Torjensen et al. investigated the effect of three interventions, diet alone, diet and exercise, and exercise alone on the reduction of insulin resistance. At the end of one year, they found that the all interventions group showed a decrease in fasting glucose; but the diet and exercise

intervention group showed the greatest (0.3 mmol/L reduction compared to the control group (0.0 mmol/l reduction) ($p < 0.0010$) (Torjesen et al., 1997).

CHC interventions, especially the promotion of increased physical activity, may have an impact on the improvement of 1,5-AG levels in patients. Honda et al. demonstrated that a post prandial exercise routine consisting of stair climbs (two sets) for three minutes, increased peripheral blood 1,5-AG concentrations (Honda et al., 2017). Measuring plasma 1,5-AG concentrations may serve as a marker for the success of CHC intervention to change lifestyle behaviors that lead to improved glycemic control.

Lifestyle modifications used to improve glucose homeostasis may also reduce insulin resistance, as evidenced by lower insulin levels. In their inpatient study, Boden et al. concluded a low carbohydrate diet reduced insulin as well as glucose concentrations in the plasma of patients with T2D (Boden, 2008). Trap et al. demonstrated that high intensity intermittent exercise (HIIE) significantly reduced fasting plasma insulin concentrations in women compared to the control and steady state exercise groups ($p < 0.05$) (Trapp, Chisholm, , Freund, & Boutcher, 2008). Rice also demonstrated that a combination of reduced caloric intake and exercise has a greater effect on reducing insulin levels as opposed to diet alone ($p< 0.05$) (Rice, Janssen, Hudson, & Ross, 1999).

Treatment of patients with diet, exercise, and medication has been shown to reduce proinsulin levels. Medication, such as pioglitazone, a thiazolidinedione class drug, increases insulin sensitivity by increasing glucose transporters 1 and 4, improving glucose uptake by cells, and reducing circulating glucose, thus lowering the demand of insulin secretion (Smith, 2001). Kubo found that treatment of patients with T2D with pioglitazone led to a significant decrease in proinsulin levels from a mean of 24.7 pmol/L to a mean of 14.0 pmol/L ($p <0.01$) (Kubo, 2002).

Torjesen et al. compared the effects of three interventions on insulin sensitivity. Subjects were randomly assigned to a control group, or one of three treatment groups: diet only, exercise only, or diet and exercise combined intervention. After one year, subjects in all three different treatment groups showed significantly lower proinsulin levels compared to subjects in the control group (Torjesen et al., 1997). In addition, the subjects in all three treatment groups showed significantly lower plasma C-peptide concentrations compared to those in the control group (p value of < 0.0011 for all three intervention groups) (Torjesen et al., 1997).

Glucose

Glucose is a monosaccharide that is utilized by every cell in the body to drive the phosphorylation of ADP to ATP. Plasma glucose may originate from the digestion of complex carbohydrates in food, the breakdown of glycogen in the liver, and gluconeogenesis, the production of glucose from non-carbohydrate substances by the liver and kidney (Krebs, 1964). Additionally, in times of prolonged starvation, liver and kidney glucose production is equal in proportion (Owen et al., 1969).

The body normally maintains circulating blood glucose levels between 70 - 99 mg/dL when in a fasting state. In patients with diabetes or prediabetes, prolonged exposure to higher than normal levels of glucose in the peripheral blood have been linked to both microvascular and macrovascular complications. These can include nephropathy, neuropathy, retinopathy, cardiovascular disease and peripheral arterial disease. In their 2005 study of normal male patients, Tirsho et al. concluded higher fasting plasma glucose levels within the normal glycemic range are at an increased risk for T2D (Tirosh et al., 2005).

Hemoglobin A1c

Hemoglobin, the principal cytoplasmic protein of red blood cells (RBC), is a tetramer consisting of four globin chains, each one having a heme group bound to the polypeptide structure. Normal adult hemoglobin A (HgbA) consists of two alpha chains and two beta chains. Within each heme group, one ferrous iron binds to four nitrogen atoms within the heme structure. The ferrous iron binds reversibly with oxygen. In a non-enzymatic reaction, glucose can form a Schiff base with the N-terminal valine of the beta chains at a rate directly proportional to the concentration of glucose in the blood (Brownlee, 1995). The product of this glycation reaction can undergo an Amadori rearrangement to form a stable covalent 1-amino-1-deoxy-2-ketose derivative of hemoglobin A, also known as HbA1c.

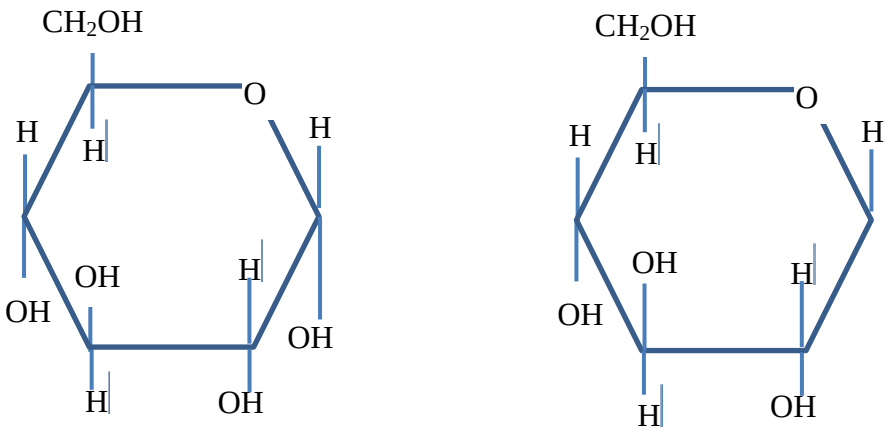
Once hemoglobin is glycated, it remains glycated for the life of the erythrocyte in which it is contained. Since the rate of HbA1c production is directly proportional to the peripheral blood glucose concentration, the higher the average concentration of glucose in the blood, the higher the percent of HgbA that is glycated at any point in time. The average lifespan of normal RBCs is 120 days. Therefore, the percent of HbA1c in a blood specimen reflects the integrated average of blood glucose concentrations over the previous 3 months. As with FPG, the risk of diabetes related microvascular and macrovascular complications in patients with T2D is positively associated with %HbA1c levels >6.0% (Stratton et al., 2000). Furthermore, the risk of myocardial damage increases as patients move from normal to a prediabetic state, to a diagnosis of diabetes (Selvin et al. 2014). Other hemoglobins, such as hemoglobin A2, hemoglobin S, and hemoglobin E can also be glycated; but since their half-life is not the same as HbA1c, the reference range for % HbA1c cannot currently be used to assess glycemic control in patients with these variant hemoglobins. The %HbA1c at any point in time has been shown to be a better

indicator of average blood glucose concentrations over time than periodically measured blood glucose concentrations; therefore, a better indicator of the risk for the long-term complications of persistently elevated plasma glucose concentrations (International expert committee report on the role of the A1C assay in the diagnosis of diabetes, 2009). Treatments aimed at lowering glucose in turn affect %HbA1c over time. As average blood glucose concentrations decrease, the %HbA1c decreases proportionately.

There are limitations if only %HbA1c is used to monitor patients for the risks of complications of diabetes and/or response to diabetes therapy. Variations in RBC lifespan can influence the cumulative glycation of HgbA, and can alter the %HbA1c (R.M. Cohen et al., 2008). This could give %HbA1c values that do not quantitatively reflect average blood glucose concentration. The %HbA1c could be falsely elevated in patients with iron deficiency anemia and asplenia due to the longer lifespan of these RBCs (Christy, Manjrekar, Babu, Hegde, & Rukmini, 2014);(Radin, 2014). Falsely decreased levels may be seen with conditions resulting from increased RBC turnover such as acute or chronic blood loss, splenomegaly, and red cell transfusion (Radin, 2014).

1,5-anhydroglucitol

Another method of monitoring elevated or postprandial blood glucose concentration is to measure 1,5-anhydroglucitol (1,5-AG) concentrations in blood. 1,5-AG (Figure 2) is a dietary monosaccharide that closely resembles the molecular structure of glucose



Glucose 1,5-anhydroglucitol

Figure 2: Glucose and 1,5- anhydroglucitol molecules

Concentrations of 1,5-AG in blood can provide an indication of hyperglycemia and postprandial glycemia. Normally, 1,5-AG is removed from the blood by glomerular filtration, and reabsorbed by the renal tubules in the kidney. Most 1,5-AG is returned to the blood via renal tubular reabsorption, with very little excreted in the urine, thus maintaining a constant level in the blood. In patients with normal glucose homeostasis, the concentration of 1,5-AG in blood remains stable over a 24-hour period, with minimal variation due to short-term dietary changes (Yamanouchi et al., 1987). In states of hyperglycemia, glucose competes with 1,5-AG for renal tubular reabsorption. Due to the similarity in structure, once plasma glucose concentrations exceed the renal threshold for reabsorption of approximately 180 mg/dL, 1,5-AG is also excreted in the urine with a concomitant decrease in plasma 1,5-AG concentrations.

Plasma 1,5-AG concentration is inversely correlated with both FPG concentration and short-term average blood glucose concentrations. In their 1996 study of 56 subjects with newly diagnosed non-insulin dependent diabetes mellitus (NIDDM), Yamanouchi et al. reported slight

changes in glycaemia can be detected within two weeks using serum 1,5-AG as a marker, sooner than with %HbA1c or serum fructosamine concentration. Subject's 1,5-AG levels dropped from 7.4 µg/mL to 4.8 µg/mL (p=0.673), while A1c values showed no significant change (P= 0.001) (Yamanouchi et al., 1996). Dungan et al (2006). were able to demonstrate, using a continuous glucose monitoring system, that plasma 1,5-AG concentrations can be used as indicators of glucose change after a meal in patients in either a prediabetic state or with overt diabetes (Dungan et al., 2006). Akanuma et al. reported plasma 1,5-AG concentrations in newly diagnosed diabetic patients to be 1.9 ± 1.8 µg/mL, compared to 13.4 ± 28.3 µg/mL in healthy subjects (Akanuma, Morita, Fukuzawa, Yamanouchi, & Akanuma, 1988). In addition, decreasing plasma 1,5-AG concentrations have been correlated with the risk of developing T2D. In their 2012 study, Juraschek et al, evaluated serum 1,5-AG and the incidence of diabetes over a three-year period. They concluded that higher baseline quartiles of 1,5-AG were associated with a lower incidence of diabetes (Juraschek, Steffes, Miller, & Selvin, 2012).

Insulin

Insulin, a 6 kilodalton (kDa) peptide hormone consisting of 51 amino acids, is the primary regulator of glucose uptake by peripheral cells. It is composed of one α-chain and one β-chain connected by two disulfide bonds. Insulin is secreted by the beta cells, located in the Islets of Langerhans of the pancreas, in response to elevated plasma glucose concentrations. The binding of insulin to insulin receptors on the surface of insulin-dependent cells stimulates the translocation of the glucose transporter GLUT4 from intracellular storage vesicles to the cell membrane, facilitating the uptake of glucose. Increases in plasma insulin concentration, in concert with decreases in plasma glucagon concentrations, also inhibit hepatic gluconeogenesis. (Saltiel & Kahn, 2001).

Normal fasting serum insulin concentrations range from 3-9 µU/mL or 20.8 – 62.5 pmol/L. In cases of frequent hyperglycemia, prolonged exposure of the body's cells to insulin can lead to a desensitization of the cellular receptors to insulin, leading to a state of "insulin resistance." The decreased ability of cells to take up glucose from peripheral blood exacerbates the hyperglycemia. Beta cells initially compensate by producing additional insulin, with hyperinsulinemia an indication of insulin resistance. Over time, insulin levels continue to rise in response to extended elevated plasma glucose concentrations. The increased demand for insulin results in beta cell stress, with prolonged beta cell stress eventually lead to beta cell death. As beta cell death occurs, the pancreas loses the ability to produce insulin, indicated by hyperglycemia with hypoinsulinemia.

C-Peptide

C-peptide is the 31- amino acid peptide released from proinsulin by prohormone convertases 1 and 2 during the formation of insulin in the storage vesicles of the pancreatic beta cells. Insulin and C-peptide are secreted into the portal vein in equimolar concentrations, but the concentration of C-peptide in peripheral blood is greater than that of insulin. This is due to C-peptide not undergoing first-pass metabolism in the liver as insulin does. As a result, the half-life of C-peptide is around 30 minutes, six times that of insulin (Polonsky et al., 1986). The majority of C-peptide is metabolized and excreted by the kidney (Zavaroni et al., 1987).

C-peptide may have a physiological effect on cell recognition of glucose and may facilitate some transmembrane movement of glucose into cells. Renal tubule cells possess numerous C-peptide binding sites. C-Peptide is involved in numerous cell signaling pathways, as well as protecting kidney cells from tumor necrosis alpha, a contributing factor in diabetic nephropathy (Hills & Brunskill, 2009). In addition, C-peptide has several downstream effects

upon binding to cell surface components, including raising intracellular calcium levels, increasing PI-3-kinase activity, and stimulation of the Na+/K+ ATPase, the protein complex responsible for using energy generated from the hydrolysis of ATP to facilitate the counter transport of NA^+ and K^+ across plasma membranes (Hills & Brunskill, 2008). In their 2012 study, Patel et al. correlated fasting serum C-peptide levels with cardiovascular risk. They concluded that nondiabetic patients in the highest quartile (72%) had a significantly higher incidence of cardiovascular death, compared to those in the lowest quartile (60 %) (Patel, Taveira, Choudhary, Whitlatch, & Wu, 2012). In addition, Heding and Rasmussen concluded in their study that mean C-peptide levels of 0.37 nmol/L or 1.11 ng/dL were indicative of normal subjects, and mean C-peptide of 0.86 nmol/L or 2.6 ng/mL were correlated with maturity onset of diabetes (Heding & Rasmussen, 1975).

Plasma C-peptide concentrations may be used to evaluate beta cell function. In their systematic review, Jones concluded that C-peptide is useful in evaluating insulin secretion and diabetes management (Jones & Hattersley, 2013). C-peptide allows for the evaluation of beta cell function in patients taking exogenous insulin. Serum insulin concentrations include both endogenous and exogenous insulin, whereas C-peptide concentrations reflect only the endogenous activity of the beta cells (Clark, 1999). Plasma C-peptide concentrations also aid in the differentiation between T1D and T2D, with higher values associated with insulin secretion and the progression to T2D (Service, Rizza, Zimmerman, & Dyck, 1997).

Proinsulin

Proinsulin is formed from the polypeptide preproinsulin. Within the endoplasmic reticulum of the beta cells, the 23 amino acid-residue signal peptide of preproinsulin is removed to form proinsulin. The primary structure of proinsulin consists of the sequence of amino acids

that eventually become the alpha and beta chains of insulin, along with the C-peptide fragment. Most of the proinsulin is converted to insulin and C-peptide in the storage vesicles of the beta cells, and only a small amount of proinsulin is released in to the peripheral blood. However, plasma concentrations of proinsulin are usually higher than insulin. Proinsulin has a much longer half-life, around 17 minutes, compared to insulin, with a half-life of around 5 minutes (Starr & Rubenstein, 1974). In addition, proinsulin has a much lower hepatic extraction than insulin (Horwitz, Starr, Mako, Blackard, & Rubenstein, 1975).

In states of insulin resistance, there is an increased demand for insulin. Proinsulin levels may rise in the peripheral blood due to impaired formation of C-peptide and insulin. This is due to the increased compensatory synthesis of preproinsulin and the decreased availability of beta cell carboxypeptidase H, the enzyme needed to cleave the amino acids from the C-terminal of proinsulin to form insulin and C-peptide (Pfützner, Pfützner, Larbig, & Forst, 2004). Research has shown that fasting plasma proinsulin concentrations can predict the conversion from impaired glucose tolerance (IGT) to T2D along with the severity of hyperglycemia. (Nijpels, Popp-Snijders, Kostense, Bouter, & Heine, 1996). Elevated plasma proinsulin concentrations can be found in states of glucose intolerance, even in the absence of elevated plasma C-peptide or insulin concentrations (Krentz, Clark, Cox, & Nattrass, 1993). Vangipurapu et al. reported a correlation between increased plasma proinsulin concentrations and the worsening of hyperglycemia and conversion to T2D (Vangipurapu et al., 2015). Saad et al. reported that the degree of proinsulin elevation was directly related to the severity of hyperglycemia (Saad et al., 1990). This was subsequently confirmed by Røder et al (Røder, Porte, Schwartz, & Kahn, 1998). Furthermore, elevated proinsulin levels have been shown to be a risk factor for premature coronary artery disease (CAD) (Katz, Ratner, Cohen, Eisenhower, & Verme, 1996). In addition,

Pfutzner et al. concluded that fasting proinsulin levels above 10 pmol/L were associated with insulin resistance (Pfützner et al., 2004).

The previous sections described the various markers of the progression to diabetes. Although there is evidence that patients can achieve improvements in biomarkers of glucose homeostasis through lifestyle modification, these changes are not easily accomplished. The next section describes the various treatments for diabetes and prediabetes, the CHC profession, and the potential impact of CHCs on biomarker results.

Health Coaches

Health or wellness coaches have become popular as part of the movement towards preventative health. Three types of health coaches exist: peer, mentor, and professional (Leahey & Wing, 2013). Peer coaches are individuals with the same condition as the patient, while mentors have overcome the affliction of interest. Both peers and mentors can help others achieve similar success by sharing experiences of what worked for them at different times of their disease/condition. The third type is a professional health or wellness coach. Professional health coaches, described as clinical health consultants (CHC) in this study, receive formal training by means of procedure review, training checklist completion, and attendance of required training seminars. CHCs are also often certified in facilitating support by organizations such as the National Society of Health Coaches, the American Council on Exercise, or the Wellness School of Coaching. The role of a CHC is not to treat the patient, but rather to supplement the work of physicians with information and support (Leahey & Wing, 2013). The CHC is an educator whose overall goal is eliciting change in a patient's behavior and physical health.

A search of the available literature demonstrates that patient-health/wellness coach interactions reduce weight and %HbA1c concentration in diabetic patients, and have a major

impact on improving health behaviors (Liddy, Johnston, Nash, Ward, & Irving, 2014). Various programs involving repetitive education and support outlets influence positive health outcomes (Haas et al., 2012; Liddy et al., 2014), (Leahey & Wing, 2013), (Wayne & Ritvo, 2014). Professional education, especially when delivered frequently and over a long period, may yield more of the desired patient outcomes for those with T2D (Loveman, Frampton, & Clegg, 2008). In a 2007 study, Ko monitored the %HbA1c of subjects who participated in 30 hours of diabetes education for five days, followed by a three-hour reinforcement educational session during annual follow-up sessions over a course of four years. They found that the study group had lower mean %HbA1c values (7.9%), compared to their control group, those who only received an initial education of four hours with no annual reinforcement during follow-up visits, (8.7%), ($P< 0.05$) (Ko et al., 2007).

A common belief among all coaching approaches is that the patient has the ability to adopt healthy behaviors if given the proper guidance. The CHC interactions are geared toward changing the behavior of the patient to foster changes in their health. Achievement of this goal for T2D, or patients in a prediabetes state, is dependent on the patient changing their lifestyle to include getting regular exercise, eating healthier foods, smoking cessation, or changing other negative behaviors that are unique to a specific patient.

Grounded in coaching psychology, CHCs foster and promote the behavior change processes that help produce desired health outcomes (Pettitt, 2013; Wayne & Ritvo, 2014). One key element in reaching the desired change is the patient's self-efficacy, or belief in their capability to reach their goal. Promoting self-efficacy in patients is a major strategy in health coaching to help patients develop the confidence needed to initiate change (Bandura, 1977). In addition, it is important that health coaches develop positive experiences with their patients, as

negative attitudes can have a major effect on a patient's self-efficacy (Gonzalez et al., 2014). A therapy designed to enhance self-efficacy increases patient involvement and activities that can improve both their overall well-being and health outcomes (Wu et al., 2011).

Clinical health coaches often utilize the framework of the Transtheoretical Model (TTM), a model that identifies stages of behavior change. Use of the TTM can help determine the patient's current position on change, as well as their readiness to make the behavior change. Transtheoretical Model based interventions focused on physical activity, nutrition and behavior change have shown reductions in weight, percent calories from fat and overall calorie intake, and increases in exercise (Johnson et al., 2008; Riebe et al., 2003). The TTM consists of five stages of change: precontemplation, contemplation, preparation, action, and maintenance. In the precontemplation stage, persons are not intending to change in the near future. These individuals may not be aware of their poor diet choice or the harmful effects of smoking and lack of exercise. In the contemplation stage, persons are more aware of the pros and cons of change, and have intentions of changing within 6 months. Persons in the preparation phase are ready to change, usually within the next month. They have a plan of action and made accommodations to move to the next phase. They may have researched exercise routines and/or gym options, or researched healthy eating options. The action phase marks the first significant effort to change their behavior. In the action phase, lifestyle modifications, such as quitting smoking, refraining from buying high sugar/high fat foods, and beginning an exercise regimen, are started. Those in the maintenance phase are trying to prevent a relapse back to the undesired behavior. These individuals continue to eat healthy, exercise regularly, and avoid poor health choices. Movement from phase-to-phase is different for each individual; however, those participating in a TTM are

more likely to move to action and maintenance stages for self-monitoring of blood glucose and healthy eating (H. Jones et al., 2003).

Adopting the TTM, CHCs often use motivational interviewing (MI) in coaching sessions. Motivational interviewing is a coaching approach centered on the patient's readiness to change, and is defined as "client centered, directive method for enhancing intrinsic motivation to change by exploring and resolving ambivalence" (Miller, 2002). Motivational interviewing assists the CHC to determine how the CHC interaction session should be carried out (Marley, Carbonneau, Lockner, Kibbe, & Trowbridge, 2011). Motivational interviewing has the potential to improve patient self-efficacy, patient activation (the patient's empowerment to work with their provider to manage their health), and perceived health status (Linden, Butterworth, & Prochaska, 2010). In addition to MI, CHCs are trained to provide empathy during their interaction with the patient. Empathy is cognitive understanding of what the patient is feeling or going through and being able to project this in a way that can foster assistance for the other individual (Hojat, Louis, Maio, & Gonnella, 2016; Hojat, 2007). Empathy can create a bond and strengthen trust between the patient and CHC (Hojat, Louis, Maio, & Gonnella, 2013). The use of MI during CHC interactions can further foster empathy from the CHCs. When used during health coaching sessions, a combination of MI and patient education session has shown to reduce BMI and waist circumference, and increase physical activity compared to those receiving education alone ($p < 0.001$) (Barrett, Begg, O'Halloran, & Kingsley, 2018). Furthermore, in their 2007 study, Brug et al. demonstrated that the use of MI to elicit empathy in patient centered coaching sessions results in lower saturated fat intake compared to patients seen by non-MI based dietitians (Brug et al., 2007).

Health coaches have been used to improve the general health of patients as well as in the treatment of patients with chronic conditions such as hypertension and diabetes. In a recent study, Leahey and Wing investigated the impact of three different types of health coaches on subjects in a weight loss program over six months. Subjects were all enrolled in a reduced intensity behavioral weight loss treatment, with caloric intakes limited to 1200-1500 kcal daily. They were expected to increase activity to 40 minutes each day for 5 days per week. This program consisted of weekly meetings for the first six weeks, biweekly meetings (once every two weeks) for the next six weeks, and then monthly meetings for the last three months of the six-month program. Eligible participants were randomized into one of the three types of health coaches: professional (n=14), peer (n=16), or mentor (n= 12). The study results demonstrated that participants across all of the groups showed reduction in body weight; however, those who received the professional health coach intervention had the greatest percentage of weight loss (Leahey & Wing, 2013).

Apple et al. investigated the effects of different types of health coach interactions on weight loss and the ability of participants to meet the goal of either 5% and/or 10% weight reduction. Four hundred and fifteen obese patients were randomly assigned to one of three groups: 1) a control group with self-directed weight loss management, 2) a remote intervention group receiving weight loss support over the telephone, a study website, and email, and 3) an in-person intervention group receiving both group and individual weight loss management support. After 24 months, both intervention groups showed significant weight loss vs. the control group ($p<0.001$). There was no significant difference in weight loss or participant ability to meet weight loss goals between the remote or in-person intervention groups (Appel et al., 2011). The

study also provided evidence that CHC interactions can be impactful using technology, and that face-to-face communication may not be necessary to influence patient behavior change.

Battista et al. investigated the effects of a 12-month dietitian-coached program on T1D and T2D patients. In their study, participants were assigned to either a control group with only endocrinologist guidance (n=50), or a treatment group receiving quarterly on-site diabetes self-management education with annual endocrinologist follow-up (n=51). They reported that repetitive dietitian guidance significantly reduced %HbA1c levels (0.6% reduction, p=0.04) vs the control group (Battista et al., 2012).

Mobile technologies could help facilitate coaching by introducing a new method of communication to aid in client/health coach interactions. The use of smartphones would allow faster responses to client questions and encourage a consistent stream of communication between the health coach and the client. In 2014, Wayne and Ritvo demonstrated that health coach interaction conducted via smartphone technology could aid in the management of clients with T2D. The goal of their study was to test a newly developed smartphone-assisted intervention to improve behavioral management of T2D in an ethnically diverse, lower socioeconomic population within an urban community health setting. A new smartphone application, the Connected Health and Wellness Platform (CHWP) Health Coach App, was tested in a 24-week intervention with reduction of %HbA1c levels as the primary outcome of interest. For the 19 individuals entered into their study, the overall mean reduction in HbA1c level was 0.28%. In 12 subjects who started the program with %HgbA1c values > 7%, the mean reduction was 0.43% ($p < 0.05$) (Wayne & Ritvo, 2014).

Research into CHC interaction and improvement of health outcomes shows patient benefit with CHC utilization. However, there is no published research on the effect of CHC

interactions on early markers of insulin resistance and beta cell dysfunction, or research concerning the number of interactions required to achieve these benefits. In a systematic review of literature on health/wellness coaching and effects on various aspects of health, Wolever found that of the 284 health/wellness coach studies identified, 148 did not specify the total number of coaching sessions used (Wolever et al., 2013). Of the remaining 136, the authors did not investigate the effects of varying numbers of sessions. In addition, only 185 of the 284 total studies were empirical with systematic data collection. In the relevant articles reviewed, study participants in the intervention group underwent structured initial coaching sessions, with several studies investigating the effects of long-term coaching over several years. Variations in the timeframe of coaching interventions were noticed in several studies in their review; however, research specifically the effects of the number of coaching sessions on change in health outcomes was not reported.

In their 2008 study, Bray et al. investigated the effects of a diabetes life coach on recently diagnosed T1D and T2D patient health. Study participants were assigned a life coach for guidance on exercise and diet lifestyle modification. Participants were deemed engaged, participating in face-to-face and telephone interventions, or non-engaged. Life coach-patient interactions were at least biannually, with the opportunity for unlimited access if necessary. They found that individuals who were engaged were 50% more likely to meet ADA guidelines of %HbA1c levels < 7.0%, with interventions improving adherence to diet and exercise routines, and medication regimens ($p < 0.001$). The number of visits was not recorded, but could have been a potential co-variate in their analysis (Bray, Turpin, Jungkind, & Heuser, 2008).

In their systematic review of the effect of telephone interventions on exercise and dietary behavior change, Eakin et al. noted that factors associated with a positive outcome included

programs that lasted between 6-12 months and those with 12 or more interactions during their study (Eakin et al., 2007). Research describing the effects of differences in the number of CHC interactions and changes in biomarkers of glucose homeostasis could provide evidence that scheduling additional CHC interactions could improve the health outcomes of patients with or at risk of developing diabetes. Therefore, additional research on the effectiveness of CHCs has the potential to influence patients to pursue treatment routes that include CHC engagement. Furthermore, the lack of research on the effects of CHC interactions on other markers of glucose homeostasis such as 1,5-AG and markers of beta cell health such as proinsulin, insulin, and C-peptide represents a gap in the knowledge of CHC capabilities. This study investigated if there is a relationship between CHC interactions and changes in biomarkers of glucose homeostasis. Finally, the study explored if the number of CHC interactions is related to those changes.

Chapter Summary

Chapter 2 reviewed disorders of glucose homeostasis, biomarkers of this process and of diabetes and prediabetes, and reviewed the CHC profession. Chapter 2 summarized the impact of CHC interactions on changes in personal behavior and lifestyle modifications. The review of available literature demonstrates the ability of CHC interaction to improve patient health and its importance to health care. Literature on CHC interactions and changes in diabetes-related markers is not limited. However, research on the effect of the number of CHC interactions on the levels of specific biomarkers is lacking. Furthermore, knowledge of CHC interactions and their effect on additional markers of glucose homeostasis and beta cell health is absent.

Chapter 3: Methodology

Overview

Chapter 3 discusses details of the research design, subject database creation, subject selection, the research setting, and study variables. The databases used to gather patient results and CHC interaction are described, including data extraction, assessment of validity, and database management. Statistical analyses used to evaluate each Specific Aim are discussed.

Problem Statement

Many patients find it difficult to change their lifestyle based on health care provider recommendations, despite the understanding that it is needed to improve their overall health. Less than 20 % of adult diabetics comply with provider prescribed medications and lifestyle modifications (Funnell, 2006; Gonzalez et al., 2014; Willard-Grace et al., 2013). This suggests that doctor-patient interaction is not sufficient in implementing lifestyle changes. Some studies demonstrate that CHC interactions may help facilitate and improve adherence to these lifestyle changes, improving BMI and % HbA1c, a marker of glucose homeostasis (Haas et al., 2012; Leahey & Wing, 2013; Liddy et al., 2014; Wayne & Ritvo, 2014) . However, there are no published studies relating the number of CHC interactions to the magnitude of change in specific markers. There is also no published research on how CHC interactions affect changes in other

markers of glucose hemostasis such as 1,5-AG, or markers of beta cell function such as proinsulin, insulin, and C-peptide.

Research Question, Specific Aims, and Hypotheses

The research question for this study is: is there a relationship between the changes in patients' biomarkers of glucose homeostasis and beta cell function and utilization of CHCs?

Specific Aim 1: Determine if there are statistically significant differences between patients who do or do not participate in CHC interactions in their changes in 1) blood glucose concentration, 2) %HbA1c, 3) blood 1,5-anhydroglucitol concentration (1,5-AG), 4) blood insulin concentration, 5) blood C-peptide concentration, 6) blood proinsulin concentration, and 7) body mass index (BMI).

To accomplish this aim, the following hypothesis was tested:

There is no significant difference in the changes in blood glucose, %HbA1c, 1,5-AG, insulin, C-peptide, proinsulin or BMI between patients who interacted with CHCs and those who did not.

This was tested by comparing the difference between initial and follow-up biomarker results, 10-14 months from initial testing, for two groups, 1) those who participated in CHC interactions, and 2) those who did not. Changes in biomarker values were calculated for each marker in both the CHC and non-CHC groups and compared statistically.

Specific Aim 2: Determine if statistically significant differences exist in the change in glucose, HabA1c, and BMI health scores between subjects who did and those who did not interact with CHCs.

To accomplish this aim, the following hypotheses will be tested:

There is no significant difference in the change in health scores for glucose, %HabA1c, and BMI for patients who had CHC interactions and those who did not.

Aim 2 was accomplished by first assigning a "health score" to the initial and follow-up biomarker results. Health score were based upon ADA recommended cutoffs for normal, prediabetic, and diabetic values for glucose and HbA1c, and BMI guidelines for normal, overweight, and obese individuals. Normal values for glucose and %HbA1c and a normal BMI were given a score of 0. Individuals in a pre-diabetic state based on glucose and %HbA1c results, and those with BMIs in the overweight category were assigned a score of 1. Those in the diabetic and obese classifications were assigned a value of 2. Changes in health score were evaluated by subtracting the follow-up score from the initial score and compared between the treatment and control group.

Specific Aim 3 Determine the relationship between the number of CHC interactions and the magnitude of the change in 1) blood glucose concentrations, 2) % HbA1c, 3) blood 1,5-anhydroglucitol (1,5-AG) concentrations, 4)blood insulin concentrations, 5) blood C-peptide concentrations, 6) blood proinsulin concentrations and 7) body mass index (BMI).

To accomplish this aim, the following hypotheses will be tested:

There is no relationship between the number of CHC interactions and the change in blood glucose concentration, %HbA1c, 1,5-AG, insulin, C-peptide, proinsulin, or BMI.

Specific Aim 3 will be accomplished by linear regression of the magnitude of change in patient's marker results versus the number of CHC interactions. Additionally, a one-way t-test was used to compare the mean change of those subjects who utilized CHC interactions.

Research Design

The current study utilized a retrospective non-experimental study design to investigate the relationship between changes in patients' biomarker results and CHC interactions (Polit, 2008). The retrospective design allows for the collection of data across varying ages, sex, and patient BMI over a span of four years, which would not be practical if a true experimental design were utilized. Repeated measures were used to identify the changes in marker results from the subjects' initial blood draw to their follow-up blood draw 12±2 months after their initial draw.

Sampling Strategy

Preliminary research identified over 200,000 patients that had initial test results and at least one set of results 10 to14 months later. The study sample included all of the patients who did not meet the exclusion criteria:

- Patients under the age of 18 years old.
- Patients over the age of 89 years old
- Patients missing age, sex, and BMI demographics

Population and Sample

The target population was all persons seen by a health care provider that had laboratory testing performed at the Richmond, VA based laboratories, Health Diagnostics Laboratory and True Health. This population included patients from across the continental United States. The sample for the study consisted of patients with follow-up testing 12±2 months after initial testing between April 2, 2012 and July 15, 2016. A data use agreement between Virginia Commonwealth University (VCU) and True Health was signed, allowing research collaboration. In addition, an approved VCU IRB (IRB HM20013795) qualified for exemption according to 45 CFR 46.101(b), Category 4 prior to data collection. In 2012, the laboratory began offering

providers a menu of tests specific to diabetes, glucose homeostasis, and pancreatic health. This offered providers a tool to screen patients for their risk of developing diabetes and to help monitor patients already diagnosed with diabetes or insulin resistance.

Handling of Protected Health Information

For this study, only deidentified patient information was provided to the principle investigator (PI). The information technology (IT) team removed patient identifiers such as name, date of service, medical record number, and sample ID, and assigned a study number to each subject, prior to allowing the PI access to the data. Only the subjects' biomarker test results, age, gender, and BMI were included in the final Excel worksheet used for the data analysis. A data use agreement between True Health and VCU along with VCU IRB approval (IRB HM20013795) ensure patients are protected and permission granted to use deidentified patient data.

Variables

Disease marker results were from previously tested subject samples. The same dependent variables were utilized for both Specific Aim 1 and 3: changes in the results for glucose, %HbA1c, 1,5-AG, proinsulin, insulin, and C-peptide, and BMI over a 10-14-month period. The units of measurement for all dependent variables are given in Table 3.

Table 3: Units of Measure

Test	Units of Measurement of Change
Glucose	mg/dL
Hemoglobin A1c	%
1,5-Anhydroglucitol	µg/mL
Insulin	µU/mL
C-Peptide	ng/mL
Proinsulin	pmol/L
BMI	Weight (Kg)/(height (m))^2

Specific Aim 1 has one independent variable, CHC interactions or no CHC interactions. The independent variable for Specific Aim 3 was the number of CHC interactions over the 10-14 month interval for those subject in the study. Specific Aim 2 had the same subjects as Specific Aim 1 for glucose, HbA1c, and BMI. The IV was also the same, but the DVs were the changes in health score for glucose, HbA1c, and BMI. Specific Aim 2 health scores are described in Table 4.

Table 4: Health Score Variable Table

Test	Initial Testing Health score	Follow-up Testing Health score	Mean Change in Health score
Glucose	Health score (Category)	Health score (Category)	Absolute health score change
Hemoglobin A1c	Health score (Category)	Health score (Category)	Absolute health score change
BMI	Health score (Category)	Health score (Category)	Absolute health score change

Patient demographics of age, sex, initial BMI, and initial marker values were used as covariates (CV) in the statistical analysis for all three Aims. The CHCs used in this study were registered dietitians, exercise specialist, or registered nurses. These CHCs may have prior certifications in health or wellness coaching; however, no certifications in were required for employment. All CHCs did complete an initial training, including the completion several online based training specific to behavior change, as well as annual competency assessments. The study variables described above are listed in Table 5.

Table 5: Study Independent and Dependent Variables

Variable	Level of Measurement	Definition of Variable	By Aim, IV, DV, or CV
CHC (Y/N)	Dichotomous	Exposure to CHC. Transferred to 0 and 1 for statistical analysis. 1 defines CHC visits, 0 defines no CHC visits	Aim 1-IV Aim 2-IV
Number of CHC interactions	Interval	If utilized CHC, the number of interactions during study timeframe	Aim 3-IV
Change in glucose	Ratio	Difference between initial and follow-up glucose	Aim 1-DV Aim 3-DV
Change in % hemoglobin A1c	Ratio	Difference between initial and follow-up %HgbA1c	Aim 1-DV Aim 3-DV
Change in 1,5-Anhydroglucitol	Ratio	Difference between initial and follow-up 1,5-anhydroglucitol	Aim 1-DV Aim 3-DV
Change in insulin	Ratio	Difference between initial and follow-up	Aim 1-DV Aim 3-DV
Change in C-peptide	Ratio	Difference between initial and follow-up proinsulin	Aim 1-DV Aim 3-DV
Change in proinsulin	Ratio	Difference between initial and follow-up C-peptide	Aim 1-DV Aim 3-DV
Change in BMI	Ratio	Difference between initial and follow-up BMI	Aim 1-DV Aim 3-DV
Change in glucose health score	Ratio	Difference between initial and follow-up Glucose health score	Aim 2-DV
Change in HbA1c health score	Ratio	Difference between initial and follow-up HbA1c health score	Aim 2-DV
Change in in BMI health score	Ratio	Difference between initial and follow-up BMI health score	Aim 2-DV

CV= covariate, DV = dependent variable, IV = independent variable

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As previously mentioned, the patient demographics of age, sex, and initial BMI were collected and used as covariables in the statistical analysis of the data. Increases of age as well as BMI have been highly correlated with incidences of diabetes. (American Diabetes Association, 2013); (Centers for Disease Control and Prevention, 2017). Using these as

covariates helped identify the effects of age, sex, and BMI on potential change in biomarker values. For evaluating change in BMI, only the subject's age and sex were included as covariates since changes in BMI is the dependent variable. To evaluate the possible propensity for a subject to participate in CHC interactions, the relationship between initial biomarker value and changes in biomarker values was investigated using ANCOVA. The study CVs are listed in Table 6.

Table 6: Study Covariates

Variable	Level of Measurement	Definition of Variable	By Aim, IV, DV, or CV
Subject ID	Nominal	Assigned study number	
Sex	Dichotomous	Transformed to 0 and 1 for statistical analysis. 1 defines male, 0 defines female	Aim 1-CV Aim 2-CV Aim 3-CV
Age at initial testing	Interval	Time in years	Aim 1-CV Aim 2-CV Aim 3-CV
Initial BMI	Ratio	BMI at initial blood Draw is used as part of the DV, change in BMI. BMI could be a CV for all biomarkers.	Aim 1-CV/DV Aim 2-CV/DV Aim 3-CV/DV
Initial glucose	Ratio	Used as a CV	Aim 1-CV Aim 2-CV Aim 3-CV
Initial HbA1c	Ratio	Used as a CV	Aim 1-CV Aim 2-CV Aim 3-CV
Initial 1,5-AG	Ratio	Used as a CV	Aim 1-CV Aim 3-CV
Initial insulin	Ratio	Used as a CV	Aim 1-CV Aim 3-CV
Initial C-peptide	Ratio	Used as a CV	Aim 1-CV Aim 3-CV
Initial proinsulin	Ratio	Used as a CV	Aim 1-CV Aim 3-CV

Marker Testing Methods

Glucose. Glucose was measured using the Beckman Coulter 5800 automated chemistry analyzer. This analyzer utilizes the hexokinase method for glucose measurement (Stein, 1965). The Beckman Coulter 5800 has an analytical measurable range (AMR) between 10-800 mg/dL and a Clinical reportable range (CRR) between 10-2400 mg/dL with auto dilution. To evaluate assay performance, daily quality control (QC) results were averaged amongst all instruments used for patient testing over the lifetime of a single lot. Based upon quality control data for four instruments from January 1, 2018 and June 20, 2018, the coefficient of variation for this assay was 1.08% at a concentration of 59.57 mg/dL, and 1.74% at a concentration of 362.85 mg/dL. The most recent calibrator lot verification, performed in November 2017, indicated a 0% bias between the previous lot and the new lot.

HbA1c. Prior to March 2015, the Richmond based laboratory utilized the BIO-RAD VARIANT II Turbo for measuring %HgbA1c. This method utilizes ion exchange high-pressure liquid chromatography (HPLC) (Jones 1979). In 2014, the laboratory changed to the Premier Hb9210™ HgbA1c Analyzer, manufactured by Trinity Biotech, for %HgbA1c measurement, which utilizes boronate affinity HPLC (Fairbanks & Zimmerman 1983); (Millia 1981). The method comparison between the two showed a slight positive bias in the Trinity assay (Y), $Y=1.0553x-0.1088$, $R^2 = 0.9873$.

The daily QC results were averaged amongst all instruments over the lifetime of a single lot to evaluate assay performance. The Trinity Premier quality control data for seven instruments used of patient testing from October 2, 2017 to June 20, 2018 the coefficient of variation for this assay was 1.23% at a concentration of 6.04% HgbA1c, and 1.61% at a concentration of 9.70% HgbA1c. The most recent calibrator lot verification, performed in May

2018, indicated a 1 % bias between the previous lot and the new lot. The BioRad Turbo Variant II quality control data for six instruments from December 11, 2013 to March 22, 2014, had a coefficient of variation of 2.36% at a concentration of 5.36% HgbA1c, and 1.61% at a concentration of 9.70% HgbA1c.

1,5-AG. Serum 1,5-AG was measured on the Beckman Coulter AU 5800 platform, with a two-step enzymatic assay and reagents produced by Glycomark (Yamanouchi 1996). Assay AMR is 1.0-100 µg/mL. The daily QC results were averaged amongst all instruments used for patient testing over the lifetime of a single lot. The coefficient of variation for this assay was 3.9% at a concentration of 5.47 µg/mL, and 2.56% at a concentration of 14.51 µg/mL for a single lot of QC used on seven instruments between December 19, 2017 to April 2, 2018. The most recent calibrator lot verification, performed in July 2017, indicated a -2% bias between the previous lot and the new lot.

Insulin. Insulin was measured on the Roche EMOD™ electro-chemiluminescence testing platform. The EMOD™ Elecsys insulin assay utilizes the sandwich immunoassay principle utilizing monoclonal antibodies specific for insulin (Sapin et al., 2001). The AMR is 1-1000 µU/mL. Daily QC results were averaged amongst all instruments over the lifetime of a single lot. Based upon quality control data for six instruments totaling 12 measuring cells between April 25, 2018 and June 20, 2018, the coefficient of variation for this assay was 2.51% at a concentration of 25.39 µU/mL, and 2.29% at a concentration of 77.08 µU/mL. The most recent calibrator lot verification, performed in August 2017, indicated a -2% bias between the previous lot and the new lot.

C-peptide. C-peptide was measured on the Roche EMOD™ electro-chemiluminescence testing platform. The Roche EMOD ™ assay utilizes the sandwich principle, and uses two

monoclonal antibodies specific for C-peptide (Kao, Talyor, & Heser 1992). The AMR is 0.3 – 40 ng/mL. The daily QC results were averaged amongst all instruments over the lifetime of a single lot. The coefficient of variation for this assay was 2.02% at a concentration of 1.94 ng/mL, and 2.46% at a concentration of 9.65 ng/mL for four instruments, totaling eight measuring cells for a single lot of QC between February 20, 2018 and June 20, 2018. The overall mean bias for two calibrator lot verifications, performed in 2018, was 0.0%.

Proinsulin. Proinsulin was measured on the Dynex DSX™ testing platform, utilizing an ELISA kit from Mercodia™. The proinsulin assay is a solid phase assay based on the sandwich immunoassay principle, employing two monoclonal antibodies against separate antigenic determinants on the proinsulin molecule (Kjems et al, 1993). The AMR is 2-150 pmol/mL, with a CRR of 2-1500 with auto dilution. To evaluate assay performance, daily QC results were averaged amongst all plate runs over the lifetime of a single lot. Based upon quality control data between May 1, 2018 and June 20, 2018, the coefficient of variation for this assay was 3.92% at a concentration of 9.11pmoL/L, and 6.13% at a concentration of 31.23 pmoL/L. The most recent lot verification, performed in November 2017, indicated a -2 % bias between the previous lot and the new lot.

From April 2012 to July 2016, all of the assays used to measure the biomarkers included in this study underwent changes in reagent and calibration lots that could have potentially led to differences in biomarker results from the initial measurement to the follow-up measurement. The Richmond based laboratory utilized the following protocol to minimize shifts in patient results due to lot changes:

- For reagent lot changes, 10 patients that ranged from the low-end to the high-end of the assay AMR, along with QC, were run using the current lot of reagent, and

again using the new lot of reagent. A percent bias of less than 10% was required for the new lot to be accepted.

- For calibrator lot changes, 10 patients that ranged from the low-end to the high-end of the assay AMR, along with QC, were run using the current calibration curve , and again using the new calibration curve. A percent bias of less than 10% was required for the new lot to be accepted.

BMI. Body mass index is calculated as the patient's mass divided by the square of their height in meters = $\frac{Mass\ (kg)}{(Height\ (m))^2}$. Height and weight, if available, were collected at the time of blood draw. Body mass index can be categorized as underweight, normal, overweight, and obese, with obesity subdivided into three classes. The BMI classifications for the current study are displayed in Table 7. For the purpose of this study, health scores were assigned solely according to the categories of normal, overweight, and obese.

Table 7: Patient Classification by BMI

BMI category	BMI (kg/m^2)	Study Classification
Underweight	< 18.5	NA
Normal	18.5 – 24.9	Normal
Overweight	25.0 – 29.9	Overweight
Obesity Class 1	30.0 – 34.9	Obese
Obesity class 2	35.0 – 39.9	Obese
Extreme Obesity Class 3	≥ 40	Obese

Adopted from (*Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults : The evidence report* 1998)

Research Setting

Laboratory testing was completed at either Health Diagnostics Laboratory or True Health Diagnostics, in Richmond, Virginia. The patient population was dispersed throughout the continental United States. Most, but not all specimens, were collected at a physician's office or at other draw sites by a phlebotomist. All samples were sent to Richmond, Virginia for testing

and usually arrived within 24 hours of the venipuncture. All samples were shipped with ice packs to ensure the optimal temperatures were maintained during sample transportation, with temperature verification if samples were suspected of being out of optimal refrigeration temperature range of 2-8°C. The laboratory Pre-Analytics Department received and accessioned all serum separator tubes (SST) and whole blood Ethylenediaminetetraacetic acid (EDTA) tubes. Samples received unspun or outside the optimum temperature range were flagged and affected tests were not tested.

Patient-CHC interactions were conducted in in a variety of locations. For face-to-face interactions, the CHC met with the patient either at a physician's office or a wellness location, an office provided to CHCs and other health practitioners. Patient-CHC interactions could have also occurred over the phone. In either setting, the CHCs were instructed to follow the same CHC visit protocol in accordance with their training. In addition, CHC interactions could vary in length of time or topics discussed based on patient conditions. There was no data recorded on the length of the visit or topics discussed during the CHC interaction.

Data collection and analysis took place at True Health Diagnostics. Senior IT analysts wrote the code that allowed a search of the LIS and IMS databases, and assisted with sorting and refining data once extracted.

Data Collection

Data collection was a two-step process. First, a query of the LIS identified potential study subjects. The IT team at the laboratory identified patients who had an initial blood draw after April 1, 2012, and that had a follow-up blood draw 10 to 14 months after their initial blood draw, up to July 15, 2016. Once potential study subjects were selected from the LIS query, a search of the CHC information management system (IMS) for the same subjects provided the number and

date of the subjects' CHC interactions, if any. The format of each of the data elements that were extracted from each of the two databases are listed in Table 8.

Table 8: Subject Data Elements Extracted from Harvest LIS and Health Coach IMS

Data Element	Format
Patient identification number	XXXXXXX
Sample ID at initial draw	YYMMDDXXXXX
Date of initial draw	MM/DD/YYYY
Age at initial draw	Digit-Continuous
BMI at initial draw	Digit-Continuous
Sample ID at follow up draw	YYMMDDXXXXX
Date of follow up draw	MM/DD/YYYY
Age at follow up draw	Digit-Continuous
BMI at follow up draw	Digit-Continuous
CHC interactions During Study	0 for no CHC or 1for CHC
Sex	M/F
Fasting Status at initial draw	0 for non-fasting, 1 for fasting, or 2 for not indicated
Fasting Time at initial draw	Digit-Continuous
Fasting status at follow up draw	0 for non-fasting, 1 for fasting, or 2 for not indicated
Fasting time at follow up draw	Digit-Continuous
Glucose at initial draw	Digit-Continuous
%HgbA1c at initial draw	Digit-Continuous
Insulin at initial draw	Digit-Continuous
Proinsulin at initial draw	Digit-Continuous
C-peptide at initial draw	Digit-Continuous
1,5-anhydroglucitol at Initial Draw	Digit-Continuous
Glucose at follow up draw	Digit-Continuous
%HgbA1c at follow up draw	Digit-Continuous
Insulin at follow up draw	Digit-Continuous
Proinsulin at follow up draw	Digit-Continuous
C-peptide at follow up draw	Digit-Continuous
1,5-anhydroglucitol at follow up draw	Digit-Continuous

The data from the LIS and IMS were merged into a single Microsoft Excel file. After verification of the data, the IT team provided the PI with a deidentified file. The final deidentified study components are listed in Table 9.

Table 9: Deidentified Study Dataset

Data Element	Format
Subject Number	Digit
Age at Initial Draw	Digit
Age at Follow-up Draw	Digit
Sex	M/F
Fasting at Initial	0, 1, or 2
Fasting Time at Initial Draw	Digit
Fasting at Follow-up Draw	0, 1, or 2
Fasting Time at Follow-up Draw	Digit
Number of CHC Visits During the Study Timeframe	0 or 1
Number of CHC interactions During Study	Digit-Continuous
BMI at Initial Draw	Digit-Continuous
BMI at Follow-up Draw	Digit-Continuous
Insulin at Initial Draw	Digit-Continuous
Insulin at follow up draw	Digit-Continuous
Glucose at Initial Draw	Digit-Continuous
Glucose at follow up draw	Digit-Continuous
Hemoglobin A1c at Initial up draw	Digit-Continuous
Hemoglobin A1c at follow up draw	Digit-Continuous
C-peptide at Initial up draw	Digit-Continuous
C-peptide at follow up draw	Digit-Continuous
Proinsulin at Initial up draw	Digit-Continuous
Proinsulin at Follow-up draw	Digit-Continuous
1,5-AG at Initial up draw	Digit-Continuous
1,5-AG at follow up draw	Digit-Continuous

Validity and Reliability

Specimen integrity, accuracy of specimen testing and verification of data collection, were essential to ensure the results of the current study were valid and reportable. Improperly collected, transported, or stored sample could have varying effects on the quality of results generated from laboratory instrumentation. Preanalytical component of laboratory quality control included a check of specimen integrity and proper labeling prior to testing. Blood specimen integrity, i.e. proper tube labeling, shipping temperature of 2-8°C (specimens were acceptable if shipped with a cold pack and/or temperature was verified by infrared thermometer if temperature

was questioned), signs of hemolysis, clotted or specimens partially spun samples, was checked upon arrival to the laboratory that performed the analysis. The Pre-Analytics team reviewed all specimens for proper shipping conditions. The date and time of collection was also recorded by the Pre-Analytics team, but not included in the study dataset.

To ensure accuracy of specimen testing, the laboratory adheres to analytical quality control measures. Daily, weekly, monthly, and annual maintenance was performed and documented for all instruments and equipment used in the preanalytical and analytical phases of testing, according to manufacturer's guidelines and standards set forth by the College of American Pathologists (CAP). Calibration and QC of all testing methods were performed in accordance with manufacturers' guidelines or laboratory operating procedure, whichever was more stringent. New lots of reagent and calibrator were verified according to the laboratory's standard operating procedures, which require that 10 patient specimens that span the AMR along with quality controls for the analyte were measured with both the current and new lot of reagents or calibrator. The % bias slope between the two lots must be less than ±10% to be acceptable.

Quality control measures were also taken in the post analytical phase of data collection. The use of two databases allowed for the collection and merging of data while reducing the potential of human error associated with transcription. Results were automatically transferred from the LIS and MS to the Excel file without human manipulation. The IT team checked 100 random subjects from the combined Excel database file and compared their name, initial and follow-up biomarker testing results, and demographics to those in the LIS and IMS to ensure they match. Once the data set was established, The IT team de-identified the subjects by replacing patient name and sample number with a new unique study number that was unrelated to the laboratory patient identification system.

Polit and Beck (2008) define internal validity as the ability of the study to measure a true effect rather than another external factor (Polit, 2008). This study was subject to threats of internal validity concerning the laboratory testing and CHC interactions. Threats to internal validity concerning the laboratory instrumentation were reduced by limiting testing platform variation by following the analytical standards described above required by the laboratory and accrediting agencies. Concerning CHC interactions, threats to internal validity were reduced by ensuring CHCs followed a structured protocol for behavior change during interactions. Before CHCs were allowed to interact with clients, all CHCs were required to participate in company mandated training courses. The goal of their training was to standardize the approach of the consultation while adapting the best counseling method dependent on the patient's readiness and ability to change the behavior of interest. However, CHC interactions may vary from one patient to another, as well as from health coach to health coach.

Sample Size and Statistical Power

Sample size requirements were dependent on effect size, power, significance, and the number of predictors. A post-hoc sample size calculation was performed with a confidence of 80% against Type II errors and a confidence of 95% against Type I errors. Effects size, the difference between two groups, aids in the statistical explanation of the effectiveness of a particular intervention.

The study utilized Cohen's d to generate estimates of effect size for all biomarkers and BMI. Cohen's d=$\frac{Group\ 1\ mean - Group\ 2\ mean}{Groups\ common\ SD}$. A Cohen's of 0.2 is considered a small effect size, a Cohen's d of 0.5 is considered a medium effects size, and a Cohen's d of 0.8 is considered a large effect size (J. Cohen, 1988). To assess effect size of CHC interactions and change in the study biomarkers and BMI, the effects size was calculated post hoc using means and SDs

gathered during data collection and calculated from SPSS frequency data analysis. Soper's online calculator for apriori estimates for a multiple regression analysis was used to determine sample size requirements, using a power 0.8, probability level of 0.05, the effects size for each DV, and up to five predictors dependent on individual model CVs where significant (Soper, 2018).

Data Cleaning

Data from both the laboratory LIS and the CHC IMS were merged into a single Microsoft Excel file. The Excel data set was examined for accuracy by the IT team. One hundred random subjects from the unaltered data set were checked against the LIS and IMS for biomarker, demographics, and CHC interaction data. The following were removed from the Excel spreadsheet prior to loading into SPSS:

- Patients that did not have both an initial and follow-up result for the disease marker being analyzed
- Non-fasting initial and final draw results for glucose, proinsulin, insulin, or C-peptide.

After the data set was reviewed in Excel, the data set was imported into SPSS v24 for data analysis (IBM Corp. 2016).

Randomization was required for the selection of the control groups (non-CHC groups) to test the hypotheses for Specific Aims 1and 2. Subjects from the non-CHC group were randomly selected to match the approximate number of subjects in the CHC group for each biomarker, with the exception of 1,5-AG. For 1,5-AG, the number of subjects in the CHC group (n = 142) was > 10% of the non-CHC group (n = 25). Therefore, randomization was not required. The number of subjects in the CHC group was divided into the number of subjects in the non-CHC

group to calculate the percent of subjects needed to for random selection. Once the percentage was calculated, SPSS was used to randomly select the corresponding number of subjects from the non-CHC group. The number of subjects used to test each hypothesis is shown in Table 10.

Table 10: Non-CHC Random Selection

Test	CHC and non-CHC Sample	CHC Subjects	Non-CHC Subjects	% of non-CHC Subjects Selected for Analysis	Calculated non-CHC Sample	Final Sample Size for Analysis
Glucose	15,803	969	14,834	6.5	975	1944
Hemoglobin A1c	37,594	1,357	36,597	3.7	1328	2685
1,5-AG	142	25	117	21.4	NA	NA
Insulin	15,375	1031	14,344	7.1	922	2023
C-Peptide	3739	407	3332	12.2	425	832
Proinsulin	2303	277	2026	13.7	257	534
BMI	88747	4029	84718	4.8	4043	7854

To ensure the randomly selected sample was not statistically different from the total non-CHC population, ANOVA was performed on the age, sex, BMI, the initial value for each marker to answer the question, was whether there was a difference in each of the variables between the group randomly sampled from the non-CHC data set and the complete non-CHC data set? The results from the ANOVA, as shown in Table 11, demonstrate that there was no statistically significant difference in age, sex, initial BMI or initial marker result between the entire non-CHC sample and the subjects randomly selected for inclusion in the statistical analysis.

Table 11: Non-CHC Random Selection ANOVA

Test	Age p value	Sex p value	Initial BMI p value	Initial Marker p value
Glucose	0.306	0.142	0.464	0.084
Hemoglobin A1c	0.378	0.444	0.563	0.160
1,5-AG	NA	NA	NA	NA
Insulin	0.365	0.194	0.956	0.361
C-Peptide	0.651	0.372	0.916	0.982
Proinsulin	0.717	0.858	0.870	0.366
BMI	0.370	0.795	0.613	0.711

Missing data was not a threat to statistical validity as all missing biomarker data were eliminated prior to loading the data set into SPSS. Descriptive statistics such as, means and maximum and minimum values, for age, sex, initial BMI, and mean change in biomarker along with their frequency distributions generated were generated in SPSS prior to data analysis.

Univariate outliers, or outliers within a single variable, were identified by analyzing descriptive statistics and standardized z scores. The standardized score, or z score is the number of standard deviations (SD) a particular value is from the mean of all values (Tabachnick & Fidell, 2007). To reduce the effect of BMI univariate outliers on the statistical analyses, all initial BMI values were converted to a standardized z score to identify potential outliers. Eligible subjects from all six biomarker groups were entered into a single SPSS file. Descriptive analysis performed in SPSS was able to generate a z score of each initial BMI to confirm with 99.9% confidence that the cases in the BMI dataset are part of the population represented by the study samples (Tabachnick & Fidell, 2007). Normalization of all subjects' initial BMI was calculated in SPSS to generate a standardized z score. BMI outliers were defined as a BMI z score of < -3.3 or > 3.3. Subjects with an initial BMI >52.26 were eliminated as having a z score > 3.3. None of the initial BMI values had a BMI z score of < -3.3. However, The Diagnostic and Statistical

Manual of Mental Health Disorders (DSM-5), derived from World Health Organization categories for thinness in adults, classifies BMI between 24 and 17 as mild anorexia, BMI between 16.0-16.99 as moderate anorexia, BMI between 15.0-15.99 as sever anorexia, and BMI < 15 as extreme anorexia (American Psychiatric Association, 2013). Subjects with BMIs <15 were eliminated as extreme values.

To identify univariate outliers, or extreme values for a single variable, the change in each marker, with the exception of HbA1c, and BMI were converted to standardized z score in SPSS. Markers with z scores < -3.3 and > 3.3 were eliminated as univariate outliers. For %HbA1c, Tukey's extreme values were used to eliminate univariate outliers related to difference in initial and final HbA1c values. Tukey's extreme values were chosen as opposed to z score distribution due to the large number of cases that would have been eliminated if z score distributions were used (Tukey, 1977). A change in %HbA1c > 9% was determined to be an outlier if z score distributions were used, this was not such an abnormal value. In a 2015 study of %HbA1c values and the risk of chronic obstructive pulmonary disease in patients with T2D, Li et al. classified patients with HbA1c values > 10% as high, with 9,390 of their 45,753 subjects in that category (Li et al, 2015).

Multivariate outliers, or a combination of extreme values, were identified by Mahalanobis distance test (Mahalanobis, 1936). This test generates a score for each subject based upon the combination of values of all variables and compares the score to the centroid for all other subjects. The cutoff for MD with 6 degrees of freedom is 22.46 and 20.52 for 5 degrees of freedom (Pearson and Hartly, 1958). Cases with MD greater than the cutoff were eliminated.

Biomarker minimum and maximum values were compared to the clinical reportable range (CRR) for their respective methods in the excel file prior to loading into SPSS. Values outside their CRR (Table 12) were identified and deleted.

Table 12: AMR and CRR

Test	AMR	Dilution	CRR
Glucose mg/dL	10-800	X3	10-2400
Hemoglobin A1c %	3.8-18.5	NA	3.8-18.5
1,5-Anhydroglucitol	1.0-110	NA	1.0-110
Insulin uU/mL	1-1000	NA	1-1000
C-Peptide	0.3-40.0	NA	0.3-40.0
Proinsulin	2.0-150	X10	2-1500

Data Analysis

The goal of Specific Aim 1 was to determine if there are differences in the changes in markers between patients who interacted with a CHC and those who did not. The change for each marker for each case was calculated. The mean change for each marker for all subjects in each group was then calculated, along with the variance in marker change within each group. The change for each marker was compared between the two groups. Patient demographics of age, sex, and initial BMI were used as covariates in the analysis of change in marker results and included in the final statistical model if significant. For evaluating the relationship between CHC interactions and changes in BMI, patient demographics of sex and age were used as covariates. To evaluate the possibility that the propensity for a subject to seek CHC interactions might influence the change in marker results, the relationship between initial biomarker values and the change in biomarker values was investigated. A structural model was developed using the general linear model (GLM) procedure in SPSS. For each marker change, CHC interaction and all CVs were proposed as predictors. The GLM entered each variable using stepwise regression and returned one or more models that were statistically significant ($p<0.05$) on their own and

statistically significantly different from the prior (reduced) model. Estimates of the magnitude of the correlation and statistical significance ($P<0.05$) of marker differences and exposure to CHC interactions were provided for each model returned by GLM. For those markers in which a significant relationship was found, initial biomarker values were used as covariates in the final analysis. For each model returned by GLM, an ANOVA table provided sums of squares, an F statistic, degrees of freedom for the model, a p value and an eta squared statistic. The final model for each analysis included CHC interaction and any CVs that were statistically significant ($p<0.05$). Standardized beta weights for each significant predictor in each model, and a t test for each beta and zero-order correlations determined the effect size for each of the biomarkers or how much variance is shared with the independent variable. The final model output stated if the means between the two groups were statistically significantly different, as well as if the use of CHC explained a statistically significant amount of that difference. A p value less than 0.05 indicated a statistically significant difference between groups, as well as a relationship between CHC interaction and change in biomarker and BMI values.

The goal of Specific Aim 2 was to evaluate the change in glucose, %HgbA1c, and BMI health scores. For this specific aim, the general construct of diabetic health was measured by transformation of initial and final glucose, %HgbA1c, and BMI results into health scores. The criteria for the assignment of health scores is listed in Table 13. Interaction with a CHC or no interaction with a CHC was the IV. The change in health score was a separate DV. Interaction or no interaction with a CHC was the IV, and initial marker values, if significant, along with age, sex, initial BMI, and effects of the CHC interaction, were inserted step-wise as covariates into a linear regression to attain estimates of the interactions. A p value less than 0.05 indicated a

statistically significant difference between groups, as well as a relationship between CHC interaction and change in biomarker and BMI health scores.

Table 13: Disease Marker Health scores

	Health Score		
Test	**0**	**1**	**2**
Glucose (mg/dL)	≤ 99	100-125	>125
Hemoglobin A1c (%)	≤ 5.6	5.7-6.4	≥ 6.5
BMI	18.5-24.9	25.0-29.9	> 29.9

For Specific Aim 3, the SPSS GLM procedure was again used to perform an ANCOVA to provide relevant statistical evidence relating the number of CHC interactions to change in mean marker values, using the number of CHC visits as the IV. As for Specific Aim 1, marker differences were used as the DVs, each analyzed separately. Covariates were initial marker values, age, sex, and initial BMI. Patients who did not interact with a CHC were not included in this analysis. Linear regression was used to attain estimates of the relationship between the number of CHC interactions and the changes in biomarker results. As with Aim 1, p values less than 0.05 indicates a significant relationship between the number of CHC visits and marker differences. A one way paired t-test was conducted to investigate the mean marker change between the number of CHC interactions defined as one, two, three, and four or more visits. A Bonferroni post hoc test was used to assess the significance of mean differences between these groups (Dunn, 1961). The resulting statistics provided evidence relevant to a proportional relationship between the number of CHC interactions and the magnitude of the changes in marker results.

Data Interpretation

For all three Specific Aims, a stepwise regression model was created to provide evidence relevant to each research question for each marker difference or health score difference. The

results were presented as a series of numbered models, one for the addition of each predictor, regardless of whether the predictor was considered a CV or the IV. The first model identified the predictor that explained the greatest proportion of the variance in the DV. The next model identified the predictor that explained the greatest proportion of the remaining variance in the DV. This process was repeated until the addition of the next predictor resulted in a model that was no longer statistically significant. This process generated from one to four models that revealed the statistically significant CVs, and the IV. For each marker, a model summary table was constructed with the R, R^2, R^2 change, F-score change, and the significance of the change in F-score. The R^2 represents the proportion of the variance in the DV that is explained by the CV or IV. The change in R^2 represents the change in variance explained with the addition of each CV, if applicable. The change in F-statistic and accompanying p value indicate the significance of each CV when added to the model, as well as the significance of the addition of the IV in the final model.

For Aims 1and 2, an initial t-test determined if there was a statistically significant difference in the unadjusted means between the CHC and non-CHC groups. The ANCOVA generated for Specific Aims 1 and 2 determine if there were statistically significant differences in the mean changes in the DV between those who had CHC interactions and those who did not. The significance of the final model would also determine if the addition of the IV to the model explained a statistically significant amount of that difference.

For Specific Aim 3, the F-score change and accompanying p values only indicate the effect of the addition of each CV on the change in BMI or marker value and the number of CHC interactions. Since the number of CHC interactions is not a categorical level of measurement, an ANCOVA was not utilized to provide a statement of significance concerning the difference

between the IV groups. However, ANCOVA was used to describe statistically significant relationships between marker difference and number of CHC visits. Subjects were grouped based on the number of CHC interactions they had. There was a group with one visit, another with two visits, one with three visits, and if needed, a group for four or more visits. Groups were created for each marker to ensure that each group was as equal in size as possible to preserve homogeneity of variance between the groups. Differences in mean BMI and marker differences between groups was determined by a one-way ANOVA and pairwise comparisons. In these situations, a summary table for the ANOVA and a table of paired comparisons, with a Bonferroni adjustment was presented.

Chapter Summary

Chapter 3 discussed information on the study methodology. Information was provided concerning the population and sampling strategies employed, as well as details concerning how the data was acquired from two data courses and merged. Steps required for data cleaning were discussed, along with how the data was analyzed for each Specific Aim. Finally, Chapter 3 concluded with an explanation of how the data was examined and interpreted for statistical significant findings.

Chapter 4: Results

Introduction

Chapter 4 describes the subject demographics generated for all dependent variables across all three Aims. In addition, sample selection for each biomarker is presented, along with sample exclusion criteria and resulting sample sizes. Next, Chapter 4 provides statistical evidence related to all three aims; diabetes related disease markers related to CHC interaction, diabetes related disease markers related to health scores, and diabetes related disease markers related to CHC frequency.

Specific Aim 1

The purpose of Aim 1 was to determine if there are statistically significant differences between subjects who do or do not participate in CHC interactions in their changes in blood glucose concentration, %HbA1c, blood 1,5-AG, blood insulin concentration, blood C-peptide concentration, blood proinsulin concentration, and BMI. Before statistical analysis was performed, the data set was cleaned and reviewed for univariate and multivariate outliers. The results of this process are presented for each marker, following the summary tables for the changes before and after adjustment for covariates.

The changes in markers were determined by subtracting the subjects' follow-up marker values from their initial marker values. A negative change indicates an improvement in glucose homeostasis, with the exception of 1,5-AG. For 1,5-AG, a positive change in marker value

indicates an improvement. Table 14 displays the mean changes in markers before adjustment for covariates. The group of subjects who interacted with CHCs showed significantly greater improvements in insulin, proinsulin, and BMI than the group with no CHC interactions. Both groups showed worsening of %HgbA1c and 1,5-AG. However, the differences between the groups was not statistically significant.

Table 14: Mean Changes in Markers of Glucose Homeostasis in Subjects with and without CHC Interactions, and their Statistical Significance

Test	CHC[a]	Non-CHC[b]	F	df	p value
Glucose (mg/dL)	-0.57	0.08	1.195	1	0.275
HbA1c (%)	0.10	0.11	0.790	1	0.374
Insulin (μU/mL)	-0.78	-0.11	7.369	1	0.007
C-Peptide (ng/mL)	-0.11	-0.03	1.838	1	0.176
Proinsulin (pmol/L)	-1.17	0.23	4.163	1	0.042
1,5-AG (μg/mL)	-0.37	-1.29	1.300	1	0.256
BMI	-0.49	-0.20	33.878	1	<0.001

a.)subjects who interacted with CHCs. b.)subjects who did not interact with CHCs

To investigate the role of potential covariates on the mean change in the marker results, linear regression was used to evaluate the change in model significance with stepwise additions of the CVs age, sex, initial BMI, and initial marker value, and the IV, the use of CHCs. The results of this analysis, summarized in Table 15, revealed that the initial result for each marker was a significant covariate with CHC-patient interactions for the change in that marker. Other covariates influenced some, but not all markers. After adjusting for the covariates, only the change in BMI was significantly different between subjects with CHC interactions and those without CHC interactions.

Table 15: The Significance of CHC Interactions After Adjusting for Covariates on the Difference Between the Change in Biomarkers

Test	Mean Marker Change CHC	Mean Marker Change Non-CHC	Significant Covariates	Significance of CHC Interactions (p value)
Glucose (mg/dL)	-0.57	0.08	Initial glucose, Age, sex	0.546
HbA1c (%)	0.10	0.11	Initial HbA1c	0.768
1,5-AG (μg/mL)	-0.37	-1.29	Initial 1,5-AG	0.379
Insulin (μU/mL)	-0.78	-0.11	Initial insulin, BMI, sex	0.112
C-Peptide (ng/mL)	-0.11	-0.03	Initial C-peptide, BMI, Age	0.453
Proinsulin (pmol/L)	-1.17	0.23	Initial proinsulin, sex, age, BMI	0.104
BMI	-0.49	-0.20	Initial BMI	< 0.001

Subject selection and results of analysis of the change in blood glucose concentration. The initial number of available subjects was 171,614. Subjects that did not fast for at least 8 hours were excluded from the analysis of the change in glucose concentration. Subjects that did not have both an initial and a follow-up measurement of glucose concentration were also excluded. After these exclusions, 16,150 subjects remained with 992 having CHC interactions and 15,158 without CHC interactions. From this sample of subjects, one was removed for having a BMI < 15, and 100 were removed for having a BMI > 52.26. Z scores were generated to identify univariate outliers, yielding 124 subjects with changes in glucose concentration corresponding to z scores <-3.3, and 122 with a z score > 3.3. These subjects were eliminated prior to random selection of subjects without CHC interaction. Prior to randomization, 15,803 subjects remained with 969 having had CHC interactions and 14,834 without CHC interactions. After randomization, 1944 total subjects remained. To eliminate multivariate outliers, a Mahalanobis Distance (MD) was generated using study number as the

DV, and age, sex, initial BMI, CHC yes or no, initial insulin, and difference in BMI as the IVs. The cutoff for Chi Square table with 6 degrees of freedom is 22.46, indicating that a MD > 22.46 would be considered an outlier (Pearson and Hartly, 1958). Based upon the MD generated, 49 subjects showed multicollinearity with MD > 22.46. These were removed from the dataset for prior to the statistical . Table 16 describes the subject demographics for the glucose biomarker sample.

Table 16: Demographics and Change in Glucose Concentrations for Subjects With and Without CHC Interactions

CHC Interactions		**Initial Age**	**Sex**	**Initial BMI**	**Initial Glucose** (mg/dL)	**Change in Glucose** (mg/dL)
Yes						
n= 962	Mean	56.0	33% M	30.0	99.2	-0.57
	SD	13.4	67% F	6.6	22.8	12.77
No						
n=964	Mean	54.5	45% M	28.8	98.1	0.08
	SD	14.2	55% F	6.0	24.2	13.31
Total						
n =1926	Mean	55.2	39% M	29.4	98.8	-0.24
	SD	13.8	61% F	6.3	23.5	13.04

Statistical analysis using a t-test in SPSS revealed there was no statistically significant difference in the mean change in glucose concentration between subjects that utilized CHC interactions and those who do not with 95% confidence ($p = 0.275$).

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown in Table17. The final model includes initial glucose concentration, age, and sex as significant CVs and the IV. Initial glucose concentration accounted for 11.1 % of the variance in the model, while, age and sex accounted for 0.8 and 0.5 % respectively. After adjusting for initial glucose, age and sex, linear regression confirmed no statistically significant difference in the

mean change in glucose concentration between those who did (- 0.57 mg/dL), and those who did not (0.08 mg/dL), participate in CHC interactions, (p=0.546).

Table 17: Significance of Covariates and CHC Interactions on the Mean Change in Glucose Concentration

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial Glucose	.334	.111	.111	241.063	< 0.001
2	Initial Glucose, Age	.346	.119	.008	17.797	< 0.001
3	Initial Glucose, Age, Sex	.353	.125	.005	11.660	0.001
4	Initial Glucose Age Sex CHC Y/N	.354	.125	.000	.365	0.546

Subject selection and results of analysis of the change in %HbA1c. The initial number of available subjects was 38,320. Fifty-four subjects had results below the AMR for either their initial or follow-up result, and were excluded. After excluding subjects that did not have both initial and follow-up results, 38,266 subjects remained, with 1,368 having had CHC interactions and 36,898 without CHC interactions. There were 290 subjects with BMI > 52.26 and 1 case with BMI < 15. These subjects were also excluded. Next, extreme cases of changes in marker results were identified, using the extreme values in the SPSS explore function. Values that fell outside 3 times the interquartile range (IQR) were deemed extreme values (Tukey, 1977). One subject was excluded due to a change in %HgbA1c > -9, and 13 subjects were excluded due to a change in %HgbA1c > 7.

Prior to randomization, 37, 954 subjects remained, 1,357 with CHC interactions, and 36,597 without CHC interactions. To eliminate multicollinearity outliers, a MD was generated

using study number as the DV and age, sex, initial BMI, CHC yes or no, initial %HbA1c, and difference in BMI as the IVs. The cutoff for chi square table with 6 degrees of freedom is 22.46, indicating that a MD > 22.46 would be considered an outlier (Pearson and Hartly, 1958). Based upon the MD generated, 85 cases were deemed multivariate outliers and were excluded prior to the statistical analysis. Table 18 describes the subject demographics for the HbA1c biomarker sample.

Table 18: Demographics and Change in %HbA1c for Subjects With and Without CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial HbA1c (%)	Change in HbA1c (%)
Yes						
n= 1315	Mean	45.3	45% M	30.4	5.60	0.10
	SD	14.4	55% F	6.8	0.67	0.40
No						
n=1295	Mean	55.9	46% M	29.5	5.56	0.11
	SD	14.0	54% F	6.4	0.69	0.39
Total						
n =2610	Mean	50.6	46% M	29.9	5.58	0.10
	SD	15.1	54% F	6.6	5.68	0.40

Statistical analysis using a t-test in SPSS revealed no statistically significant difference in the mean change in %HbA1c between those that utilized CHCs and those who did not ($p = 0.374$). The mean change in %HbA1c was positive for both the CHC (0.10%) and non-CHC (0.11%) groups, indicating an increase in average blood glucose concentration over time.

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 19. The only significant CV was the initial %HbA1c, which accounted for 15.4% of the variance. After adjusting for initial %HbA1c, linear regression confirmed

no statistically significant difference in the change in %HbA1c between those who did, and those who did not have CHC interactions (p=.768).

Table 19: Significance of Covariates and CHC Interactions on the Mean Change in %HbA1c

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial %HbA1c	.392	.154	.154	473.571	< 0.001
2	Initial %HbA1c CHC Y/N	.392	.154	.000	0.87	0.768

Subject selection and results of analysis of the change in 1,5-AG concentration. The initial number of available subjects for 1,5-AG was 150. Five subjects had results below the AMR and were excluded. Only 145 subjects remained with 25 having CHC interactions and 120 without CHC interactions. One subject had a BMI > 55.26 and was excluded. Z scores were used to identify outliers for the change in 1,5-AG. Two subjects had z scores greater than 3.3, corresponding to a change in 1,5-AG greater than 18 µg/mL. Randomization of the subjects without CHC interactions was not performed since the number of subjects with CHC interactions was > 10% of the number of subjects without CHC interactions. No multivariate outliers were identified. Table 20 describes the subject demographics for the 1,5-AG biomarker sample.

Table 20: Demographics and Change in 1,55-AG Concentrations for Subjects With and Without CHC Interactions

CHC Interactions		Start age	Sex	Initial BMI	Initial 1,5-AG (µg/mL)	Change in 1,5-AG (µg/mL)
Yes						
n= 25	Mean	51.5	36% M	28.4	15.80	-0.37
	SD	15.0	64% F	5.6	9.01	3.67
No						
n=117	Mean	50.3	33% M	28.2	17.05	-1.29
	SD	15.23	67% F	6.9	6.81	3.64
Total						
n =142	Mean	50.6	34% M	28.3	16.83	-1.13
	SD	15.15	66% F	6.7	7.23	3.7

Statistical analysis using a t-test in SPSS revealed no statistically significant difference in the mean change in 1,5-AG between those with CHC interactions and those without CHC interactions (p=0.256).

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 21. The only significant CV was the initial 1,5-AG, which accounted for 19.2% of the variance. After adjusting for initial 1,5-AG, linear regression confirmed no statistically significant difference mean change in 1,5-AG between those who did, and those who did not have CHC interactions (p=0.379).

Table 21: Significance of Covariates and CHC Interactions on the Mean Change in 1,5-AG Concentration

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial 1,5-AG	.438	.192	.192	33.171	< 0.001
2	Initial 1,5-AG CHC Y/N	.443	.196	.005	.779	0.379

Subject selection and results of analysis of the change in insulin concentration. The initial number of available subjects for insulin was 123,401. Subjects that did not fast for at least 8 hours were excluded from the analysis of the change in insulin concentration. Subjects that did not have both an initial and a follow-up measurement of insulin concentration were also excluded. After exclusions, 15,629 subjects remained with, 1,031 having CHC interactions and 14,344 without CHC interactions. From this sample, one was eliminated for having a BMI < 15 and 105 for having a BMI < 52.26. Z scores were generated to identify univariate outliers, yielding 69 subjects with changes in insulin corresponding to z score >3.3 and 79 with a z score <-3.3. These subjects were eliminated prior to random selection of subjects without CHC interactions. Prior to randomization, 15,375 subjects remained, with 1031 having had CHC interactions, and 14,344 without CHC interactions. After randomization, 2023 subjects remained. To evaluate multivariate outliers, a MD was generated using study number as the DV and age, sex, initial BMI, CHC yes or no, initial insulin, and difference in BMI as the IVs. The cutoff for chi square table with 6 degrees of freedom is 22.46, indicating that a MD > 22.46 would be considered an outlier (Pearson and Hartly, 1958). Based upon the MD generated, 40 subjects showed to have multivariate outliers with MD > 22.46. These were removed and analysis ran. Table 22 describes the patient demographics for the insulin biomarker sample.

Table 22: Demographics and Change in Insulin Concentrations for Subjects With and Without CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial Insulin (μU/mL)	Change in Insulin (μU/mL)
Yes						
n= 973	Mean	55.6	34% M	30.0	11.9	-0.78
	SD	13.8	66% F	6.4	7.5	5.65
No						
n=1010	Mean	54.1	40% M	28.7	10.7	-0.11
	SD	13.2	60% F	6.2	7.2	5.31
Total						
n =1983	Mean	54.9	37% M	29.4	11.3	-0.45
	SD	13.5	63% F	6.4	7.4	5.50

Statistical analysis using a t-test in SPSS revealed a statistically significant difference in the mean change in inulin between those with CHC interactions and those without CHC interactions (p=0.007).

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 23.

Table 23: Significance of Covariates and CHC Interactions on the Mean Change in Insulin Concentration

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial Insulin	.401	.161	.161	380.081	< 0.001
2	Initial Insulin Initial BMI	.426	.181	.020	48.825	< 0.001
3	Initial Insulin, Initial BMI Sex	.431	.186	.005	11.369	0.001
4	Initial Insulin Initial BMI Sex CHC Y/N	.432	.187	.001	2.523	0.112

The final model included initial insulin, initial BMI, and sex as CV and the IV, use of CHC. Initial insulin accounted for 16.1% of the variance in the model, while initial BMI and sex accounted for 2% and 0.5% respectively. After adjusting for initial insulin, initial BMI and sex, linear regression confirmed no statistically significant difference was detected for the change in mean insulin between those who did (-0.78 μU/mL), and those who did not (-0.11 μU/mL), participate in CHC interactions (p = 0.112).

Subject selection and results of analysis of the change in C-peptide concentration. The initial number of available subjects for C-peptide was 28,415. Subjects that did not fast for at least 8 hours were excluded from the analysis of chance in C-peptide concentration Subjects that did not have both an initial and a follow-up measurement of C-peptide concentration, and/or that had results outside the AMR, were also excluded. After these exclusions, 3,816 subjects remained with 414 having had CHC interactions and 3,402 that did not. From this sample of subjects, one was eliminated for having a BMI < 15 and 76 were eliminated for having a BMI > 52.26. Z scores were generated to identify univariate outliers, yielding 24 subjects with changes in C-peptide concentration corresponding to z score >3.3 and 26 with a z score <-3.3. Prior to random selection of the non-CHC groups, 3,739 subjects remained. After randomization, 832 subjects remained. The MD was generated using study number as the DV and age, sex, initial BMI, CHC yes or no, initial insulin, and difference in BMI as the IVs. The cutoff for chi square table with 6 degrees of freedom is 22.46, indicating that a MD > 22.46 would be considered an outlier (Pearson and Hartly, 1958). Based upon the MD generated, nine subjects were considered multivariate outliers with MD > 22.46. These were removed and analysis ran. Table 24 describes the patient demographics for the insulin biomarker sample.

Table 24: Demographics and Change in C-peptide Concentrations for Subjects With and Without CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial C-peptide (ng/mL)	Change in C-peptide (ng/mL)
Yes						
n= 401	Mean	56.1	32% M	30.2	3.02	-0.10
	SD	14.0	68% F	6.2	1.34	0.83
No						
n=422	Mean	52.5	41% M	29.2	2.76	-0.03
	SD	14.6	59% F	6.5	1.26	0.73
Total						
n =832	Mean	54.24	37% M	29.7	2.88	-0.07
	SD	14.413	63% F	6.3	1.30	0.78

Statistical analysis a t-test in using SPSS revealed no statistically significant difference in the mean change in C-peptide concentrations between subjects that utilized CHCs and those who did not ($p = 0.176$).

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 25.

Table 25: Significance of Covariates and CHC Interactions on the Mean Change in C-peptide Concentration

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial C-peptide	.351	.123	.123	115.356	< 0.001
2	Initial C-peptide Initial BMI	.374	.140	.016	15.605	< 0.001
3	Initial C-peptide Initial BMI Start Age	.380	.145	.005	4.839	0.028
4	Initial C-peptide Initial BMI Start Age CHC Y/N	.381	.145	.001	.563	0.453

The final model includes initial C-peptide concentration, initial BMI, and age as significant CVs, and the DV change in C-peptide. Initial C-peptide accounted for 12.3% of the variance in the model, while initial BMI and age accounted for 1.6% and 0.5% variance respectively. After adjusting for initial C-peptide, initial BMI, and age, linear regression confirmed no statistically significant difference was detected for the change in mean C-peptide between those who did (- 0.11 ng/mL), and those who did not (0.03 ng/mL), participate in CHC interactions ($p = 0.453$).

Subject selection and results of analysis of the change in proinsulin concentration. The initial number of available subjects for proinsulin was 18,788. Subjects that did not fast for at least 8 hours were excluded from the analysis of change in proinsulin concentration. Subjects that did not have both an initial and follow-up measurement of proinsulin were also excluded. Additionally, 51 subjects had results below the AMR and were excluded. After these exclusions, 2,356 subjects remained with 282 having CHC interactions and 2,074without CHC interactions. From this sample of subjects, 17 subjects were eliminated for BMI < 52.26. Z scores were generated to identify outliers yielding 18 subjects with change in proinsulin concentration corresponding to a z scores >3.3, and 18 with z scores < -3.3. These subjects were eliminated prior to random selection of subjects without CHC interactions. After randomizations, 523 subjects remained. To eliminate multivariate outliers, a MD was generated using study number as the DV and age, sex, initial BMI, the number of CHC Interactions, initial proinsulin, and difference in BMI as the IVs. The cutoff for chi square table with 6 degrees of freedom is 22.46, indicating that a MD > 22.46 would be considered an outlier (Pearson and Hartly, 1958). Based upon the MD generated, 11 subjects were considered multivariate outliers with MD > 22.46.

These 11 subjects were removed and analysis ran. Table 26 describes the patient demographics for the proinsulin biomarker sample.

Table 26: Demographics and Change in Proinsulin Concentrations for Subjects With and Without CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial Proinsulin (pmol/L)	Change in Proinsulin (pmol/L)
Yes						
n= 274	Mean	55.5	33% M	30.1	14.72	-1.17
	SD	14.4	67% F	6.2	10.47	7.92
No						
n=249	Mean	53.0	44% M	28.9	13.28	0.23
	SD	14.8	56% F	6.2	9.42	7.71
Total						
n =523	Mean	54.3	38%M	29.5	14.03	-0.50
	SD	14.6	62% F	6.2	10.00	7.84

Statistical analysis using a t-test in SPSS revealed a statistically significant difference in the mean change in proinsulin concentration between those that utilized CHC interactions and those who did not ($p = 0.042$).

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown in Table 27. The final model includes, initial proinsulin, sex, age, and initial BMI as significant CVs, and the DV change in proinsulin. Initial proinsulin concentration accounted for 11.4% of the variance in the model, while sex, age and initial BMI accounted for 1.3, 0.9 and 0.7% respectively. After adjusting for initial proinsulin, sex, age, and initial BMI, linear regression confirmed no statistically significant difference was detected for the change in proinsulin concentration between those who did (-1.17 pmol/L), and those who did not (0.23 pmol/L), participate in CHC interactions ($p = 0.104$).

Table 27: Significance of Covariates and CHC Interactions on the Mean Change in Proinsulin Concentration

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial Proinsulin	.338	.114	.114	67.084	< 0.001
2	Initial Proinsulin Sex	.357	.127	.013	7.983	0.005
3	Initial Proinsulin Sex Start Age	.370	.137	.009	5.605	0.018
4	Initial Proinsulin Sex Start Age Initial BMI	.379	.143	.007	4.042	0.045
5	Initial Proinsulin Sex Start Age Initial BMI CHC Y/N	.385	.148	.004	2.654	0.104

Subject selection and results of analysis of the change in BMI. Subjects that did not have both an initial and follow-up BMI were excluded. After exclusion, 88,747 subjects remained for BMI analysis with 4,029 having CHC interactions and 84,718 without CHC interactions. For BMI, randomization occurred before univariate outlier investigation, generating 8072 total subjects. From this sample, 77 subjects were removed for having a BMI > 52.26. Z scores were generated to identify univariate outliers, yielding 74 subjects with a change in BMI corresponding to a z score < -3.3, and 67 with a z score > 3.3. These subjects were removed, leaving 7854 subjects for analysis.

To eliminate multivariate outliers, MD was generated using study number as the DV and age, sex, initial BMI, CHC yes or no, and difference in BMI as the IVs. The cutoff for chi square table with 5 degrees of freedom is 20.52, indicating that a MD > 20.52 would be considered an outlier (Pearson and Hartly, 1958). Based on the MD generated, 49 subjects were considered

multivariate outliers with MD > 20.52. These 49 subjects were removed and analysis ran. Table 28 describes the patient demographics for the proinsulin biomarker sample.

Table 28: Demographics and Change in BMI for Subjects With and Without CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Change in BMI
Yes					
n= 3893	Mean	57.9	52% M	30.0	-0.49
	SD	13.5	48% F	6.5	2.22
No					
n=3912	Mean	56.7	44% M	29.2	-0.20
	SD	14.0	56% F	6.2	2.16
Total					
n = 7805	Mean	57.3	48% M	29.6	-0.34
	SD	13.8	52% F	6.4	2.21

Statistical analysis using a t-test in SPSS revealed a statistically significant difference in the mean change in BMI between those that utilized CHC interactions and those who did not ($p < 0.001$).

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous VCs and the newly entered CV. The model summary table is shown as Table 29.

Table 29: Significance of Covariates and CHC Interactions on the Mean Change in BMI

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial BMI	.235[a]	.055	.055	456.735	< 0.001
2	Initial BMI CHC Y/N	.241[b]	.058	.003	21.938	< 0.001

The final model only included initial BMI as a significant CV, and the DV change in BMI. Initial BMI accounted for 5.5% of the variance explained. After adjusting for initial BMI, linear regression confirmed a statistically significant difference was detected for the change in

mean BMI between those who did (-0.49), and those who did not (- 0.20, participate in CHC interactions (p = < 0.001).

Specific Aim 2

The purpose of Specific Aim 2 was to determine if statistically significant differences exist in the change in glucose, %HgbA1c, and BMI health scores between subjects who did and those who did not interact with CHCs. Before statistical analysis was performed, the data set was reviewed for univariate and multivariate outliers. The follow section presents the results of data cleaning and subject exclusion for each marker and BMI.

For Specific Aim 2, regression was performed using the change in health score as the DV. The IV was the use or withholding of CHC interaction or no interaction, with BMI, sex, and age at the subjects' initial visit as potential CVs. The change in health score was determined by subtracting the subjects' marker health score at their follow-up blood draw from their marker health score at their initial blood draw. A negative change corresponds to an improvement in the health score for that particular marker, while a positive change corresponds to a worsening of the health score for that marker. The mean change in health score for %HbA1c, glucose, and BMI was calculated for both the CHC and non-CHC groups. Table 30 describes marker means for each group and the unadjusted t-test results of the comparison of change in health scores.

Table 30: Mean Changes in Glucose, %HbA1c, and BMI Health Scores in Subjects with and without CHC Interactions, and their Statistical Significance

Test	CHC[a]	Non-CHC[a]	F	df	p value
Glucose	-0.2	0.02	3.562	1	0.059
HbA1c	0.09	0.10	0.081	1	0.776
BMI	-0.07	-0.04	12.911	1	<0.001

a.)subjects who interacted with CHCs. b.)subjects who did not interact with CHCs

Table 31 describes the ANCOVA linear regression which revealed the significant CVs for each marker, as well as the significance of the CHC and non-CHC groups differences.

Table 31: The Significance of CHC Interactions After Adjusting for Covariates on the Difference Between the Change in Glucose, HbA1c, and BMI

Test	Mean Health Score Change CHC	Mean Health Score Change Non-CHC	Significant CVs	Significance of CHC Interactions (p value)
Glucose	-0.2	0.02	Initial glucose, age, sex	0.165
HbA1c	0.09	0.10	Initial HbA1c	0.949
BMI	-0.07	-0.04	Initial BMI	< 0.001

Subject selection and results of analysis of the change in glucose health score. For Specific Aim 2, the same 1944 subjects selected for the analysis of the changes in glucose concentration were examined for the changes in glucose health score. Twenty-five of these subjects exhibited a MD > 22.46, and were excluded from the analysis. The demographics of the subjects utilized for the analysis are presented in Table 32.

Table 32: Demographics and Change in Glucose Health Score for Subjects With and Without CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial Glucose (mg/dL)	Initial Health Score	Change in Health Score
Yes							
n= 958	Mean	56.1	33% M	30.1	97.9	0.40	-0.02
	SD	13.4	67% F	6.6	17.5	0.62	0.54
No							
n= 961	Mean	54.6	45% M	28.7	96.8	0.36	0.02
	SD	14.2	55% F	5.9	18.0	0.60	0.52
Total							
n = 1919	Mean	55.3	39% M	29.4	97.4	0.38	0.00
	SD	13.8	61% F	6.3	17.8	0.61	0.53

Statistical analysis using a t-test in SPSS revealed no statistically significant difference in the mean change in glucose health score for those that utilized CHC interactions and those who

did not (p = 0.059). Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. This analysis revealed that initial glucose, age, and sex were significant CVs. Initial glucose accounted for 7.4% of the variance, while age and sex explained 0.6% and 0.4% of the variance respectively. Engaging or not engaging in CHC interactions only accounted for 0.1% of the variance. After adjusting for initial glucose, age, and sex, linear regression confirmed that there was no statistically significant difference in the change in mean glucose health score between those who did (-0.2), and those who did not (0.02) participate in CHC interactions (p = 0.165). The model summary is shown in Table 33. The final model includes all significant CVs and the DV.

Table 33: Significance of Covariates and CHC Interactions on the Mean Change in Glucose Health Score

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial Glucose	.271	.074	.074	152.373	< 0.001
2	Initial Glucose Start Age	.283	.080	.006	13.426	< 0.001
3	Initial Glucose Start Age Sex	.290	.084	.004	8.229	0.004
4	Initial Glucose Start Age Sex CHC Y/N	.291	.085	.001	1.927	0.165

Based upon their assigned health score for glucose concentration, subjects were classified as normal, prediabetic, or diabetic. The distribution of these classifications between those who did or did not have CHC interactions in shown in Table 34. For the group with CHC interactions, the follow-up glucose health score showed a decrease in the number of subjects classified as diabetic by 17, an increase in the number classified as prediabetic by 11, and an increase in the

number classified as normal by six. For the group without CHC interactions, there was no change in the number of subjects classified as diabetic, and an increase of 21 classified as prediabetic.

Table 34: Classification of Subjects by Glucose Health Score

	Initial Classification			Follow-up Classification		
	Normal	**Prediabetic**	**Diabetic**	**Normal**	**Prediabetic**	**Diabetic**
CHC Interactions	644	246	68	650	257	51
No CHC Interactions	682	216	63	661	237	63
Total	1326	462	131	1311	494	114

Subject selection and results of analysis of the change in HbA1c health score. For Specific Aim 2, the same 2685 subjects selected for the analysis of the changes in %HgbA1c were examined for the changes in %HgbA1c health score. There were 45 subjects with a MD > 22.46. These subjects were excluded from the analysis. The demographics of the subjects utilized for the analysis are presented in Table 35.

Table 35: Demographics and Change in HbA1c Health Score for Subjects With and Without CHC Interactions

CHC Interactions		**Initial Age**	**Sex**	**Initial BMI**	**Initial HbA1c**	**Initial Health Score**	**Change in Health Score**
Yes							
n= 1330	Mean	45.3	45% M	30.4	5.6	0.43	0.09
	SD	14.4	55% F	6.8	0.75	0.66	0.48
No							
n= 1310	Mean	55.9	46% M	29.5	5.6	0.42	0.10
	SD	14.0	54% F	6.3	0.75	0.66	0.47
Total							
n = 2640	Mean	50.58	45% M	30.0	5.6	0.43	0.10
	SD	15.1	55% F	6.6	0.75	0.66	0.47

Statistical analysis using a t-test in SPSS revealed no statistically significant difference in the mean change in %HbA1c health score for those that utilized CHC interactions and those who did not (p = 0.776). Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous VCs and the newly entered CV. Only initial %HbA1c was a statistically significant CV in the best fit model, accounting for 1.8% of the variance. After adjusting for initial %HbA1c, linear regression confirmed no statistically significant difference in the change in mean %HbA1c health score between those who did (0.09), and those who did not (0.10) participate in CHC interactions (p = 0.949). The model summary is shown in Table 36. The final model includes all significant CVs and the DV.

Table 36: Significance of Covariates and CHC Interactions on the Mean Change in HbA1c Health Score

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial HbA1c	.134	.018	.018	48.568	< 0.001
2	Initial HbA1c CHC Y/N	.134	.018	.000	.004	0.949

Based upon their assigned health score for %HgbA1c, subjects were classified as normal, prediabetic, or diabetic. The distribution of these classifications between those who did or did not have CHC interactions in shown in Table 37.

Table 37: Classification of Subjects by %HgbA1c Health Score

	Initial Classification			Follow-up Classification		
	Normal	**Prediabetic**	**Diabetic**	**Normal**	**Prediabetic**	**Diabetic**
CHC Interactions	885	316	129	761	439	130
No CHC Interactions	884	303	123	778	385	147
Total	1769	619	252	1539	824	277

For the group with CHC interactions, the follow-up glucose health score showed a decrease in the number of subjects classified as normal by 124, with an increase in the number classified as prediabetic by 123. For the group without CHC interactions, there was a decrease in the number of subjects classified as normal by 106, with increases of 82 classified as prediabetic and 24 classified as diabetic.

Subject selection and results of analysis of the change in BMI health score. For Specific Aim 2, the same 7854 subjects selected for the analysis of the changes in BMI were examined for the changes in BMI health score. There were 61 subjects with a MD > 20.52. These 61 subjects were excluded prior to analysis. The demographics of the subjects utilized for the analysis are presented in Table 38.

Table 38: Demographics and Change in BMI Health Score for Subjects With and Without CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial Classification	Change in Classification
Yes						
n= 3900	Mean	57.9	52% M	30.0	1.26	-0.07
	SD	13.5	48% F	6.6	0.79	0.42
No						
n= 3893	Mean	56.7	44% M	29.2	1.17	-0.04
	SD	14.1	456% F	6.3	0.79	0.41
Total						
n = 7793	Mean	57.3	48% M	29.6	1.21	-0.05
	SD	13.8	52% F	6.4	0.80	0.42

Statistical analysis using a t-test in SPSS revealed that there was a significant difference in the mean change in BMI health scores between subjects who had CHC interactions and those who did not ($p < 0.001$). Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. Initial BMI was the only significant CV, accounting for 0.4% of the variance, while CHC interactions accounted for 0.6%. After adjusting for initial BMI, a statistically significant difference in the change in

mean BMI health scores remained, between those who did (-0.07), and those who did not (-0.04), participate in CHC interactions (p =0.001). The model summary table is shown as Table 39. The final model includes all significant CVs and the DV.

Table 39: Significance of Covariates and CHC Interactions on the Mean Change in BMI Health Score

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial BMI	.066	.004	.004	33.800	< 0.001
2	Initial BMI CHC Y/N	.075	.006	.001	10.562	< 0.001

Based upon their assigned health score for BMI, subjects were classified as normal, overweight, or obese. The distribution of these classifications between those who did or did not have CHC interactions in shown in Table 40.

Table 40: Classification of Subjects by BMI Health Score

	Initial Classification			Follow-up Classification		
	Normal	**Overweight**	**Obese**	**Normal**	**Overweight**	**Obese**
CHC Interactions	840	1222	1838	945	1286	1669
No CHC Interactions	935	1345	1613	987	1383	1523
Total	1775	2567	3451	1932	2669	3192

For the group with CHC interactions, the follow-up BMI health score showed a decrease in the number of subjects classified as obese by 169, with an increase in the number classified as normal by 105, and an increase in the number classified as overweight by 64. For the group without CHC interactions, there was a decrease in the number of subjects classified as obese by 90, with increases of 52 classified as normal and 38 classified as diabetic.

Specific Aim 3

The purpose of Aim 3 was to determine the relationship between the number of CHC interactions and magnitude of the change in blood glucose concentration, %HbA1c, blood 1,5-AG, blood insulin concentration, blood C-peptide concentration, blood proinsulin concentration, and BMI. Subjects were grouped according to whether they had 1, 2, 3, or $\geq$4 CHC interactions. Before statistical analysis was performed, the data set was reviewed for univariate and multivariate outliers. The sections following Table 41 describe how subjects were eliminated, and the results of data cleaning for each marker and BMI.

Linear regression was performed using the change in each marker as the DV, and the number of CHC interactions as the IV. A negative change in mean marker values would indicate an improvement in that particular marker, with the exception of 1,5-AG. As with Specific Aims 1 and 2, initial BMI, sex, and age at the subject's initial visit were used as potential CVs. Linier regression provided an ANCOVA table. However, since the number of CHC visits were an interval level of measurement, statements of significance of biomarker difference between those who and did not utilize CHCs could not be provided. Linear regression could only determine the significance of the number of CHC interactions on the change in the DV. In addition to linear regression, the number of interactions were considered as a categorical variable and the significance of the differences in the changes in marker results between the number of CHC interactions was tested using ANOVA followed by a paired one way t-test. Results are reported as Bonferroni post hoc mean comparisons to investigate the mean differences between the CHC interaction groups. Table 41 provides a summary of results and between-group differences.

Table 41: The Significance of the Number of CHC Interactions on the Change in Markers of Glucose Homeostasis

Marker	Best Model Including Significant CVs	Significance of the Number of CHC Interactions (p value)	Significance of Between Number of CHC Interaction Differences (p value)
Glucose (mg/dL)	Initial Glucose Start Age Sex Initial BMI # of CHC Interactions	No p = 0.971	No p > 0.05
HbA1c (%)	Initial %HbA1c # of CHC Interactions	Yes p = 0.029	No p > 0.05
1,5-AG (µg/mL)	Initial 1,5-AG # of CHC Interactions	No p = 0.229	No p > 0.05
Insulin (µU/mL)	Initial Insulin Initial BMI Sex # of CHC Interactions	Yes p = 0.010	No p > 0.05
C-Peptide (ng/mL)	Initial C-peptide Initial BMI Start Age # of CHC Interactions	No p = 0.435	Yes Between Groups 1-2 P = 0.035
Proinsulin (pmol/L)	Initial Proinsulin # of CHC Interactions	No p = 0.976	No p > 0.05
BMI	Initial BMI # of CHC Interactions	Yes p < 0.001	Between Groups 1-4 p < 0.001

Subject selection and results of analysis of the change in glucose concentration by number of CHC interactions. There were 969 subjects that had at least one CHC interaction. Mahalanobis distances were generated, replacing only CHC Y/N with the number of interactions. Thirty-one subjects were identified with a MD > 22.46. These subjects were excluded prior to the statistical analyses. To ensure that all CHC interaction groups had at least 10% of the number of subjects in the largest group, subjects with four or more interactions were combined into one group. Table 42 details CHC interaction group demographics and change in glucose concentration.

Table 42: Demographics and Change in Glucose Concentration for Subjects by Number of CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial Glucose (mg/dL)	Change in Glucose (mg/dL)
1						
n= 522	Mean	54.6	34% M	29.4	96.9	-0.51
	SD	13.4	66% F	6.4	16.0	11.70
2						
n= 219	Mean	56.8	34% M	30.0	98.4	-1.45
	SD	13.0	66% F	6.4	18.0	13.30
3						
n= 90	Mean	57.4	26% M	31.0	95.9	0.41
	SD	13.2	74% F	6.8	14.3	12.46
4 or more						
n= 107	Mean	59.4	30% M	32.0	99.0	-0.44
	SD	13.6	70% F	7.2	18.4	13.10
Total						
n = 938	Mean	55.9	33%M	30.0	97.4	-0.64
	SD	13.4	67% F	6.6	16.6	12.30

Linear regression revealed no significant relationship between the mean change in glucose concentration and the number of CHC interactions. Covariates were entered stepwise into the linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary is shown in Table 43. The final model includes all significant CVs and the DV. For the change in glucose, the initial glucose accounted for 24.8% of the variance. Age, sex and initial BMI explained 1.6%, 0.8 and 0.3% of the variance respectively. The number of CHC interactions did not statistically account for any of the variance. After adjusting for initial glucose, age, sex, and initial BMI, there was no statistically significant relationship between the mean change in glucose and the number of CHC interactions ($p = 0.971$).

Table 43: The Effects of Covariates and the Number of CHC interactions on the Significance of Mean Change in Glucose

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial Glucose	.498	.248	.248	308.817	< 0.001
2	Initial Glucose Start Age	.514	.264	.016	20.222	< 0.001
3	Initial Glucose Start Age Sex	.522	.272	.008	10.627	0.001
4	Initial Glucose Start Age Sex Initial BMI	.525	.276	.003	4.471	0.035
5	Initial Glucose Start Age Sex Initial BMI # of CHC Interactions	.525	.276	.000	.001	0.971

Graphical representation (Figure 3) suggests a decrease in mean change in glucose between CHC interaction groups one and two, but an increase with CHC interaction group three.

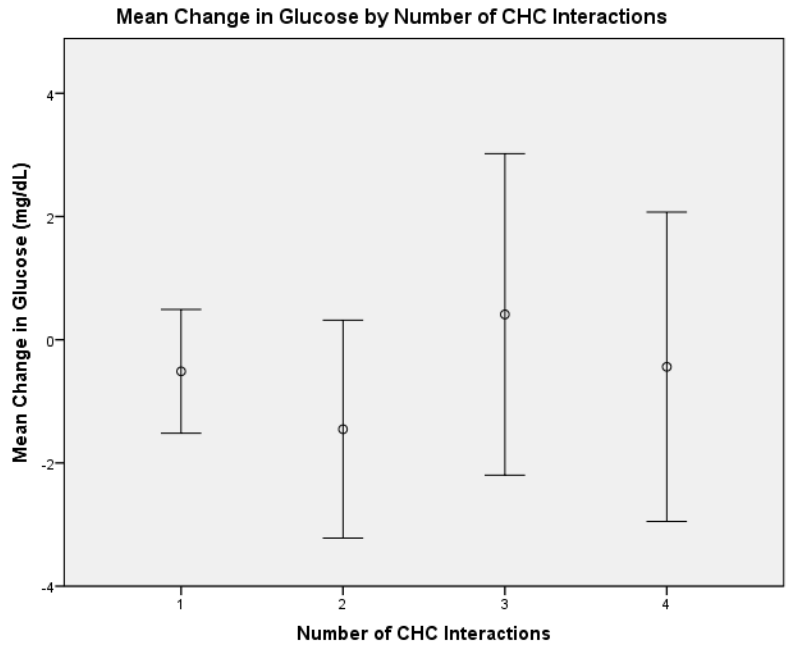

Figure 3: Mean Change in Glucose by Number of CHC Interactions
Error bars represent 95% Confidence Intervals

A Bonferroni multiple comparison table (Table 44) confirms that there was no statistically significant difference in the mean change in glucose between CHC interactions groups, ($p < 0.05$).

Table 44: Bonferroni Multiple Comparison for Glucose and Number of CHC Interactions

# of CHC Interactions	Additional Visit	Mean Difference	Standard Error	p value	95% CI of the Difference	
					Upper	Lower
1	2.00	.939	0.991	1.000	3.56	-1.68
	3.00	-.925	1.406	1.000	2.79	-4.64
	4.00	-.074	1.307	1.000	3.38	-3.53
2	1.00	-.939	0.991	1.000	1.68	-3.56
	3.00	-1.863	1.542	1.000	2.21	-5.94
	4.00	-1.013	1.452	1.000	2.83	-4.85
3	1.00	.925	1.406	1.000	4.64	-2.79
	2.00	1.863	1.542	1.000	5.94	-2.21
	4.00	.850	1.761	1.000	5.51	-3.81
4	1.00	.074	1.307	1.000	3.53	-3.38
	2.00	1.013	1.452	1.000	4.85	-2.83
	3.00	-.850	1.761	1.000	3.81	-5.51

Subject selection and results of analysis of the change in %HbA1c by number of CHC interactions. There were only 1,357 subjects that had at least one CHC interactions. Mahalanobis distance was generated replacing only CHC Y/N with the number of CHC Interactions. Fifty-one subjects were identified with a MD > 22.46. These subjects were excluded prior to the statistical analysis. To ensure all groups had at least 10% of the largest group, subjects with more than four interactions were combined in to one group, four or more. Table 45 details CHC interaction group demographics and change in %HbA1c.

Table 45: Demographics and Change in %HbA1c for Subjects by CHC Number of Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial HbA1c (%)	Change in HbA1c (%)
1						
n= 736	Mean	45.2	42% M	29.4	5.54	0.12
	SD	14.3	58% F	6.6	0.65	0.37
2						
n=298	Mean	45.2	48% M	31.3	5.71	0.08
	SD	14.2	52% F	6.8	0.81	0.42
3						
n=124	Mean	45.7	44% M	31.0	5.69	0.03
	SD	14.5	56% F	6.7	0.68	0.43
4 or more						
n=148	Mean	46.2	55% M	32.9	5.61	0.04
	SD	15.5	45% F	7.2	0.67	0.46
Total						
n =1306	Mean	45.3	45%M	30.4	5.61	0.09
	SD	14.4	55% F	6.8	0.70	0.40

Linear regression revealed there was a significant relationship between the mean change in %HbA1c and the number of CHC interactions.

Covariates were entered stepwise into the linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary is shown in Table 46. The final model includes all significant CVs and the DV. For Change in HbA1c, initial HbA1c accounted for 20.1% of the variance explained. The addition of the number of CHC

interactions into the model was also significant, explaining 0.3 % of variance. After adjusting for initial %HbA1c, analysis confirmed a statistically significant relationship between the mean change in %HbA1c and the number of CHC interactions (p = 0.029).

Table 46: The Effects of Covariates and the Number of CHC interactions on the Significance of Mean Change in %HbA1c

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial HbA1c	.448	.201	.201	327.027	< 0.001
2	Initial HbA1c # of CHC Interactions	.451	.203	.003	4.752	0.029

Graphical representation (Figure 4) suggests a decrease in the mean change in HbC1a as the number of CHC interactions increases until three CHC interactions.

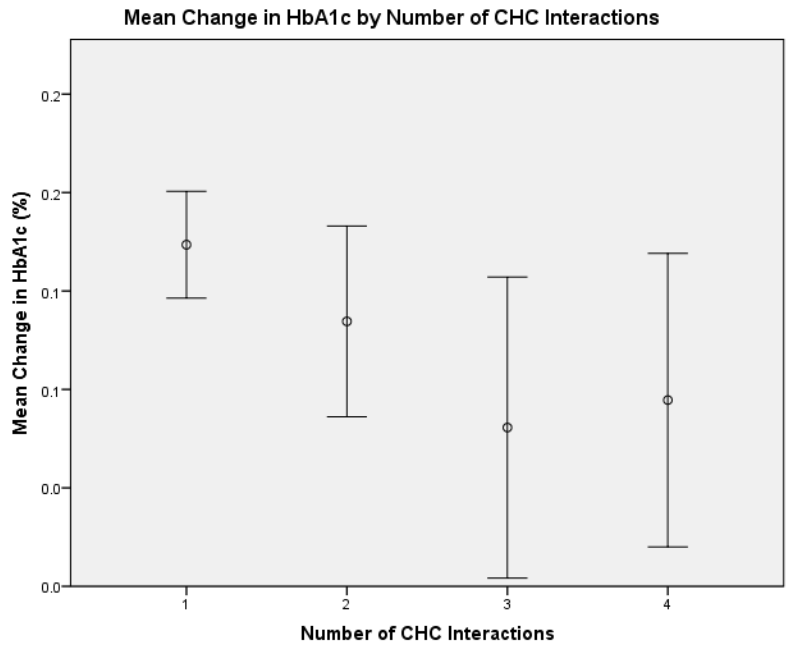

Figure 4: Mean Change in HbA1c by Number of CHC Interactions
Error bars represent 95% Confidence Intervals

A Bonferroni multiple comparison table (Table 47) confirms that there was no statistically significant difference in the mean change in HbA1c between CHC interactions groups, ($p > 0.05$).

Table 47: Bonferroni Multiple Comparison for HbA1c and Number of CHC Interactions

# of CHC Interactions	Additional Visit	Mean Difference	Standard Error	p value	95% CI of the Difference	
					Upper	Lower
1	2.00	.039	.028	0.952	0.111	-0.034
	3.00	.093	.039	0.105	0.196	-0.010
	4.00	.079	.036	0.177	0.175	-0.017
2	1.00	-.039	.028	0.952	0.034	-0.112
	3.00	.054	.043	1.000	0.167	-0.059
	4.00	.040	.040	1.000	0.147	-0.067
3	1.00	-.093	.039	0.105	0.010	-0.196
	2.00	-.054	.043	1.000	0.060	-0.167
	4.00	-.014	.049	1.000	0.115	-0.143
4	1.00	-.079	.036	0.177	0.017	-0.174
	2.00	-.040	.040	1.000	0.067	-0.147
	3.00	.014	.049	1.000	0.143	-0.115

Subject selection and results of analysis of the change in 1,5-AG by number of CHC interactions. There were 25 subjects that had at least one CHC interactions. Mahalanobis distance was generated replacing only CHC Y/N with the number of CHC Interactions. To ensure all groups had at least 10% of the largest group subjects with three or more interactions were combined. Table 48 describes the demographics and change in 1,5-AG concentration for each CHC interaction group.

Table 48: Demographics and Change in 1,5-AG Concentration for Subjects by Number of CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial 1,5-AG (µg/mL)	Change in 1,5-AG (µg/mL)
1						
n= 12	Mean	45.2	33% M	26.2	16.34	-0.98
	SD	14.6	67% F	5.9	8.57	2.10
2						
n=7	Mean	52.9	29% M	30.5	14.63	-0.80
	SD	14.3	71% F	5.9	7.02	1.01
3						
n=6	Mean	62.8	50% M	30.3	16.07	1.33
	SD	10.8	50% F	3.1	12.91	7.01
Total						
n =25	Mean	51.6	36%M	28.4	15.80	-0.37
	SD	15.0	64% F	5.6	9.02	3.67

Linear regression revealed no significant relationship between the mean change in 1,5-AG concentration and the number of CHC interactions.

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 49. The final model includes all significant CVs and the DV. For change in 1,5-AG, initial 1,5-AG accounted for 21.8% of the variance explained. The number of CHC interactions did not statistically account for any variance explained. After adjusting for initial 1,5AG, there was no statistically significant relationship between the mean change in glucose and the number of CHC interactions ($p = 0.229$).

Table 49: The Effects of Covariates and the Number of CHC interactions on the Significance of Mean Change in 1,5-AG

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial 1,5-AG	.467	.218	.218	6.427	0.018
2	Initial 1,5-AG # of CHC Interactions	.519	.269	.051	1.530	0.229

Graphical representation (Figure 5) suggests a trending increase in the mean change of 1,5-AG between CHC interaction groups one and three.

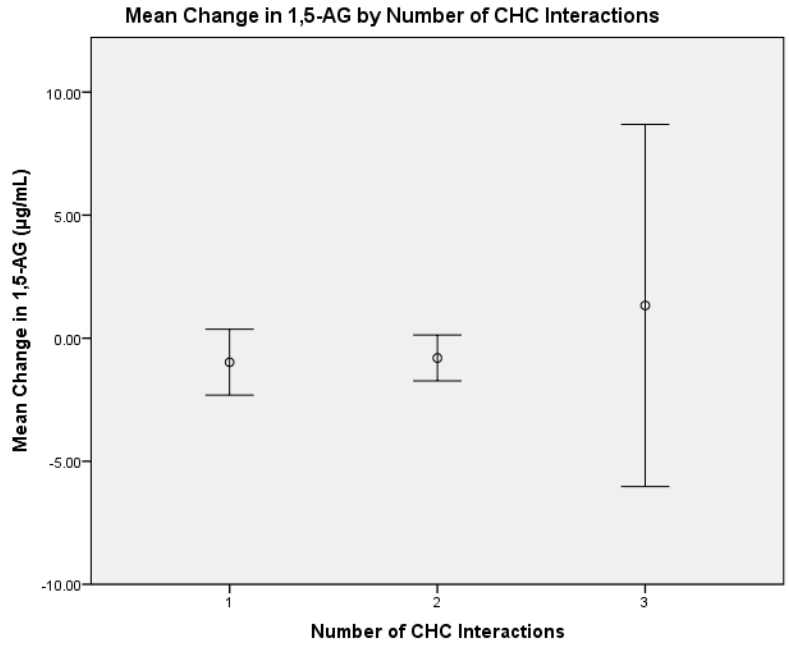

Figure 5: Mean Change in 1,5-AG by Number of CHC Interactions
Error bars represent 95% Confidence Intervals

A Bonferroni multiple comparison table (Table 50) confirms no statistically significant difference in the mean change in 1,5-AG between CHC interactions groups, ($p > 0.05$).

Table 50: Bonferroni Multiple Comparison for 1,5-AG and Number of CHC Interactions

# of CHC Interaction	Additional Visit	Mean Difference	Standard Error	p value	95% CI of the Difference	
					Upper	Lower
1	2.00	-.171	1.759	1.000	4.386	-4.728
	3.00	-2.305	1.849	0.677	2.486	-7.096
2	1.00	0.171	1.759	1.000	4.728	-4.386
	3.00	-2.134	2.057	0.932	3.196	-7.465
3	1.00	2.305	1.849	0.677	7.096	-2.486
	2.00	2.134	2.057	0.932	7.465	-3.196

Subject selection and results of analysis of the change in insulin concentration by number of CHC interactions. For insulin, there were 1,031 subjects that had at least one CHC interactions. Mahalanobis distance was generated replacing only CHC Y/N with the number of CHC Interactions. Thirty-one subjects were identified with a MD > 22.46. These subjects were removed prior to the statistical analysis. To ensure all groups had at least 10% of the largest group, subjects with more than four interactions were combined in to one group, four or more. Table 51 describes the demographics and change in insulin concentration for each CHC interaction group.

Table 51: Demographics and Change in Insulin Concentration for Subjects by Number of CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial Insulin) (μU/mL)	Change in Insulin (μU/mL)
1						
N= 577	Mean	54.4	36% M	29.6	11.6	-0.28
	SD	13.1	64% F	6.38	7.7	5.93
2						
N =288	Mean	56.4	36% M	29.8	12.1	-1.11
	SD	13.2	64% F	6.08	6.7	5.92
3						
N =105	Mean	57.6	27% M	31.0	12.6	-1.49
	SD	13.0	73% F	6.89	8.7	4.61
4 or more						
N =110	Mean	57.8	30% M	32.1	12.8	-1.50
	SD	13.3	70% F	6.55	7.2	5.36
Total						
N =1000	Mean	55.5	34%M	30.0	11.9	-0.73
	SD	13.2	66% F	6.43	7.5	5.76

Linear regression revealed a significant relationship between the mean change in insulin concentration and the number of CHC interactions. Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 52. The final model includes all significant CVs and the DV. For insulin, initial insulin, accounted for 17.5% of the variance explained. Initial BMI and sex were also explained 2.2%, and 0.4% of the variance respectively. After adjusting for initial insulin, initial BMI, and sex, analysis confirmed a statistically significant relationship between the mean change in insulin and the number of CHC interactions ($p = 0.01$).

Table 52: The Effects of Covariates and the Number of CHC interactions on the Significance of Mean Change in Insulin

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial Insulin	.418	.175	.175	211.510	< 0.001
2	Initial Insulin Initial BMI	.444	.197	.022	27.735	< 0.001
3	Initial Insulin Initial BMI Sex	.448	.201	.004	4.702	0.030
4	Initial Insulin Initial BMI Sex # of CHC Interactions	.454	.206	.005	6.660	0.010

Graphical representation (Figure 6) of the mean change in insulin suggests a downward trend with each additional CHC interactions.

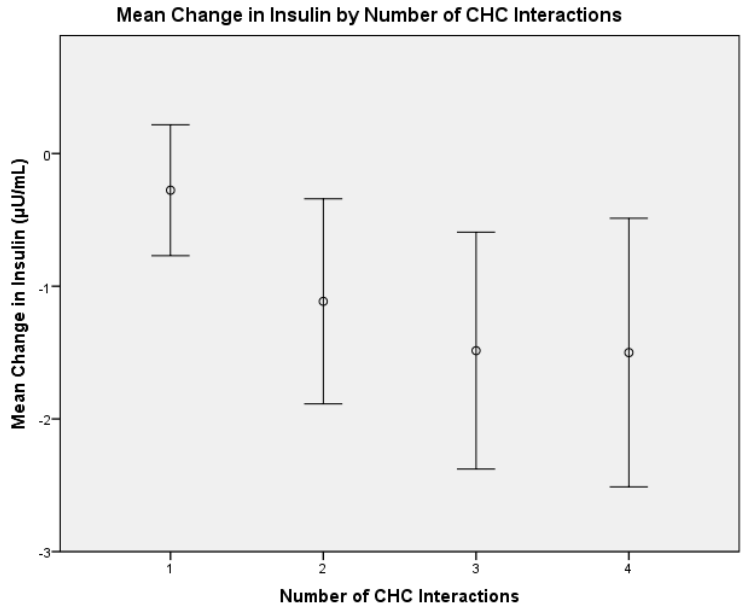

Figure 6: Mean Change in Insulin by Number of CHC Interactions
Error bars represent 95% Confidence Intervals

A Bonferroni multiple comparison table (Table 53) confirms no statistically significant difference in the mean change in proinsulin between CHC interactions groups, ($p > 0.05$).

Table 53: Bonferroni Multiple Comparison for Insulin and Number of CHC Interactions

# of CHC Interaction	Additional Visit	Mean Difference	Standard Error	p value	95% CI of the Difference	
					Upper	Lower
1	2.00	.838	.452	0.384	2.03	-0.36
	3.00	1.209	.611	0.289	2.82	-0.41
	4.00	1.224	.599	0.249	2.81	-0.36
2	1.00	-.838	.452	0.384	0.36	-2.03
	3.00	.372	.677	1.000	2.16	-1.42
	4.00	.386	.667	1.000	2.15	-1.38
3	1.00	-1.209	.611	0.289	0.41	-2.82
	2.00	-.372	.677	1.000	1.42	-2.16
	4.00	.014	.784	1.000	2.09	-2.06
4	1.00	-1.224	.599	0.249	0.36	-2.81
	2.00	-.386	.667	1.000	1.38	-2.15
	3.00	-.014	.784	1.000	2.06	-2.09

Subject selection and results of analysis of the change in C-peptide concentration by number of CHC interactions. For C-peptide, 407 subjects had at least one CHC interactions. Mahalanobis distance was generated replacing only CHC Y/N with the number of CHC Interactions. Seven subjects were identified having a MD > 22.46 and removed prior to the statistical analysis. To ensure all groups had at least 10% of the largest group , subjects with more than four interactions were combined in to one group, four or more. Table 54 describes the demographics and change in C-peptide concentration for each CHC interaction group.

Table 54: Demographics and Change in C-peptide Concentration for Subjects by Number of CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial C-peptide	Change in C-peptide (ng/mL)
1						
n= 229	Mean	55.3	33% M	30.3	2.98	-0.02
	SD	13.5	67% F	6.6	1.36	0.91
2						
n=81	Mean	55.3	38% M	29.7	3.13	-0.33
	SD	14.7	62% F	4.8	1.37	0.76
3						
n=50	Mean	59.8	28% M	31.4	3.11	-0.10
	SD	13.4	72% F	7.5	1.33	0.69
4 or more						
n=40	Mean	59.3	20% M	30.5	3.16	-0.06
	SD	14.6	80% F	5.8	1.59	0.90
Total						
n =400	Mean	56.2	32%M	30.3	3.04	-0.10
	SD	13.9	68% F	6.3	1.38	0.86

Linear regression revealed no significant relationship between the mean change in C-peptide concentration and the number of CHC interactions

After adjusting for initial C-peptide, initial BMI, and age, no statistically significant effect was detected for the mean change in C-peptide and the number of CHC interactions.

Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 55. The final model includes all significant CVs and the DV. For C-peptide, initial C-peptide accounted for 11.7% of the variance explained. Initial BMI and age also explained 2.3%, and 1.0% of the variance respectively. The number of CHC interactions did not statistically account for any variance explained. After adjusting for initial C-peptide, initial BMI, and age, there was no statistically significant relationship between the mean change in C-peptide and the number of CHC interactions ($p = 0.435$).

Table 55: The Effects of Covariates and the Number of CHC interactions on the Significance of Mean Change in C-peptide

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial C-peptide	.342	.117	.117	52.659	< 0.001
2	Initial C-peptide Initial BMI	.374	.140	.023	10.595	0.001
3	Initial C-peptide Initial BMI Start Age	.387	.149	.010	4.514	0.034
4	Initial C-peptide Initial BMI Start Age # of CHC Interactions	.388	.151	.001	.612	0.435

Graphical representation (Figure 7) suggests a decrease in the mean change in C-peptide between CHC interaction groups one and two, with an increase noted with groups three and four.

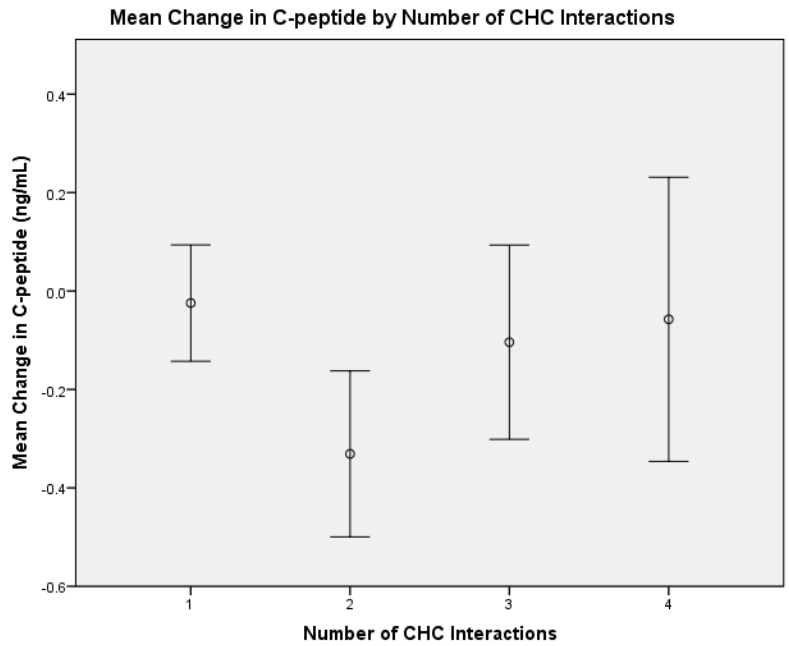

Figure 7: Mean Change in C-peptide by Number of CHC Interactions
Error bars represent 95% Confidence Intervals

A Bonferroni multiple comparison table (Table 56) confirms a statistically significant difference in change in C-peptide between CHC interactions groups. A statistically significant difference exist between groups one and two (p=0.035). No other statistically significant differences were observed between the number of CHC interaction groups (p > 0.05).

Table 56: Bonferroni Multiple Comparison for C-peptide and Number of CHC Interactions

# of CHC Interaction	Additional Visit	Mean Difference	Standard Error	p value	95% CI of the Difference	
					Upper	Lower
1	2.00	0.306	0.111	0.035	0.600	0.013
	3.00	0.080	0.134	1.000	0.434	-0.275
	4.00	0.033	0.147	1.000	0.422	-0.356
2	1.00	-0.306	0.111	0.035	-0.013	-0.600
	3.00	-0.227	0.154	0.848	0.181	-0.635
	4.00	-0.273	0.165	0.595	0.165	-0.712
3	1.00	-0.080	0.134	1.000	0.275	-0.434
	2.00	0.227	0.154	0.848	0.635	-0.181
	4.00	-0.047	0.182	1.000	0.435	-0.528
4	1.00	-0.033	0.147	1.000	0.356	-0.422
	2.00	0.273	0.165	0.595	0.712	-0.165
	3.00	0.047	0.182	1.000	-0.528	0.435

Subject selection and results of analysis of the change in proinsulin concentration by number of CHC interactions. There were 277 subjects that had at least one CHC interactions. Mahalanobis distance was generated replacing only CHC Y/N with the number of CHC Interactions with difference in health score. Seven subjects were identified with a MD > 22.46 and excluded prior to the statistical analysis. To ensure that all visit groups had at least 10% of the number of subjects in the largest group, subjects with more than four interactions were combined in to one group, four or more. Table 57 describes the demographics and change in Proinsulin concentration for each CHC interaction group.

Table 57: Demographics and Change in Proinsulin Concentration for Subjects by Number of CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Initial Proinsulin (pmol/L)	Change in Proinsulin (pmol/L)
1						
n= 161	Mean	56.1	35% M	30.065	14.88	-1.07
	SD	14.2	65% F	6.724	10.89	8.43
2						
n=56	Mean	53.4	37% M	29.346	13.98	-1.79
	SD	15.2	63% F	4.289	8.51	5.69
3						
n=29	Mean	55.4	31% M	30.104	12.59	-.07
	SD	13.6	69% F	6.8270	8.19	5.65
4 or more						
n=24	Mean	57.3	17% M	31.535	18.75	-2.25
	SD	15.0	82% F	5.4504	13.84	11.07
Total						
n =270	Mean	55.5	33%M	30.051	14.79	-1.21
	SD	14.4	67% F	6.19270	10.52	7.94

Linear regression revealed no significant relationship between the mean change in Proinsulin concentration and the number of CHC interactions. Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 58.

Table 58: The Effects of Covariates and the Number of CHC interactions on the Significance of Mean Change in Proinsulin

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial Proinsulin	.435	.189	.189	62.377	< 0.001
2	Initial Proinsulin # of CHC Interactions	.435	.189	.000	.001	0.976

The final model includes all significant CVs and the DV. For proinsulin, initial accounted for 18.9% of the variance explained. The number of CHC interactions did not statistically account for any variance explained. After adjusting for initial proinsulin, there was

no statistically significant relationship between the mean change in proinsulin and the number of CHC interactions ($p = 0.976$).

Graphical representation (Figure 8) suggests a decrease in the mean change in proinsulin between CHC interaction groups one and two and between groups three and four.

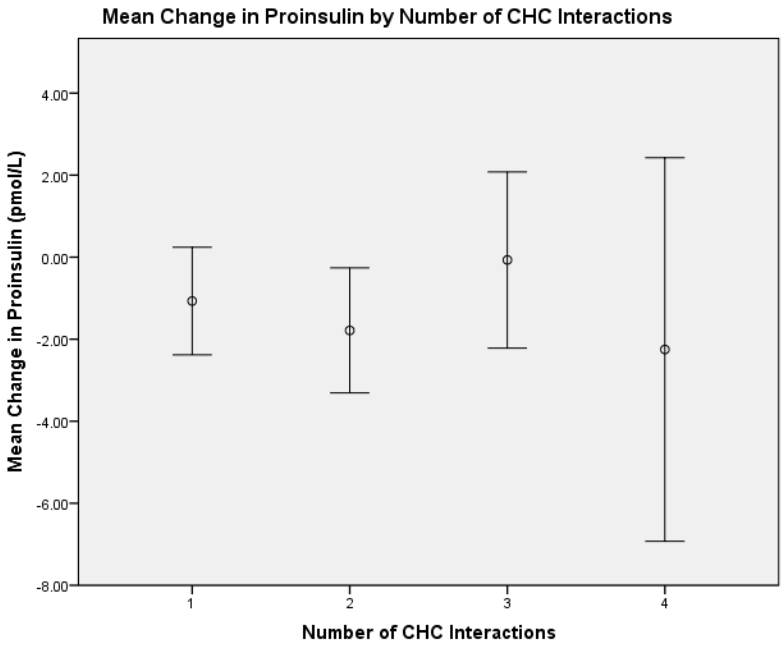

Figure 8: Mean Change in Proinsulin by Number of CHC Interactions
Error bars represent 95% confidence interval

A Bonferroni multiple comparison table (Table 59) confirms no statistically significant difference in the mean change in proinsulin between CHC interactions groups, ($p > 0.05$).

Table 59: Bonferroni Multiple Comparison for Proinsulin and Number of CHC Interactions

# of CHC Interactions	Additional Visit	Mean Difference	Standard Error	p value	95% CI of the Difference	
					Upper	Lower
1	2.00	717	1.235	1.000	4.00	-2.57
	3.00	-.999	1.606	1.000	3.27	-5.27
	4.00	1.182	1.742	1.000	5.81	-3.45
2	1.00	-.717	1.235	1.000	2.57	-4.00
	3.00	-1.717	1.821	1.000	3.12	-6.56
	4.00	.464	1.942	1.000	5.63	-4.70
3	1.00	.999	1.606	1.000	5.27	-3.27
	2.00	1.717	1.821	1.000	6.56	-3.12
	4.00	2.181	2.197	1.000	8.02	-3.66
4	1.00	-1.182	1.742	1.000	3.45	-5.81
	2.00	-.464	1.942	1.000	4.70	-5.63
	3.00	-2.181	2.197	1.000	-3.66	8.02

Subject selection and results of analysis of the change in BMI by number of CHC interactions. For BMI, 3,920 subjects had at least one CHC interaction. Mahalanobis distance was generated replacing only CHC Y/N with the number of CHC Interactions. Sixty-four subjects were identified with a MD > 20.52. These subjects were excluded prior to statistical analysis. To ensure all groups had at least 10% of the largest group, subjects with more than four interactions were combined in to one group, four or more. Table 60 describes the demographics and change in BMI concentration for each CHC interaction group.

Table 60: Demographics and Change in BMI for Subjects by Number of CHC Interactions

CHC Interactions		Initial Age	Sex	Initial BMI	Change in BMI
1					
n= 2142	Mean	57.9	51% M	29.4	-0.34
	SD	13.7	49% F	6.38	2.18
2					
n=919	Mean	57.7	54% M	30.2	-0.57
	SD	13.4	46% F	6.56	2.27
3					
n=376	Mean	57.3	53% M	30.8	-0.63
	SD	13.0	47% F	6.69	2.41
4 or more					
n=419	Mean	58.1	49% M	31.5	-0.94
	SD	13.7	51% F	7.00	2.35
Total					
n =3856	Mean	57.8	52%M	30.0	-0.49
	SD	13.5	48% F	6.56	2.25

Linear regression revealed no significant relationship between the mean change in BMI and the number of CHC interactions. Covariates were entered stepwise into a linear regression model, with each subsequent model including the previous CVs and the newly entered CV. The model summary table is shown as Table 61. The final model includes all significant CVs and the DV. For BMI, initial BMI accounted for 5.1% of the variance explained. The number of CHC interactions did statistically account for some variance explained, 0.4%. After adjusting initial BMI, analysis confirmed a statistically significant relationship between the mean change in BMI and the number of CHC interactions ($p < 0.001$).

Table 61: The Effects of Covariates and the Number of CHC interactions on the Significance of Mean Change in BMI

Model	Model Components	R	R^2	R^2 Change	F Change	p value
1	Initial BMI	.226	.051	.051	206.969	0.000
2	Initial BMI # of CHC Interactions	.234	.055	.004	15.104	0.000

Graphical representation (Figure 9) of the mean change in BMI suggests a downward trend in change in BMI with each additional CHC interaction.

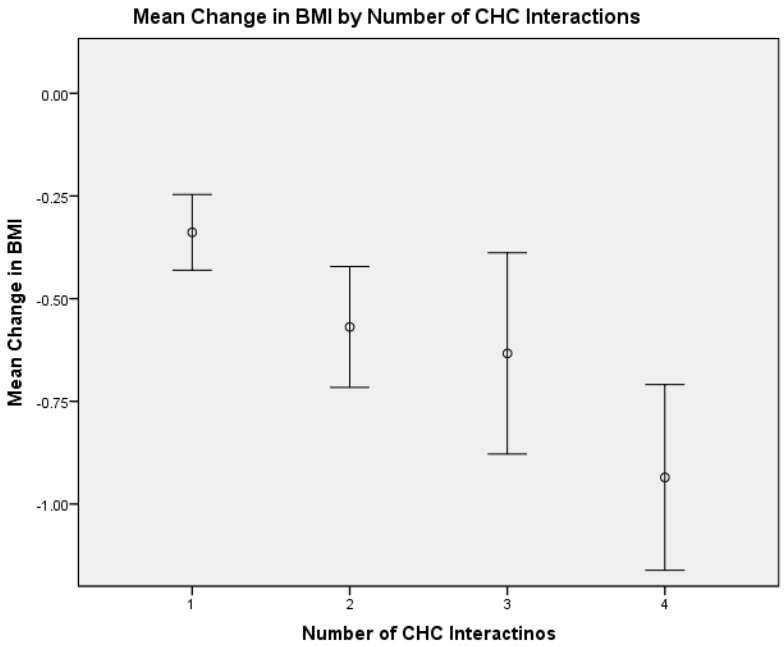

Figure 9: Mean Change in BMI by Number of CHC Interaction
Error bars represent 95% confidence interval

The Bonferroni multiple comparison table (Table 62) confirms a statistically significant difference in change in BMI between CHC interactions groups. A statistically significant difference in mean change was detected between CHC groups one and four, ($p < 0.001$) and between groups two and four ($p = 0.034$). Decreases in the mean change in BMI was also seen between groups one and two, between two and three, and between three and four, however the change was not significant, ($p > 0.05$).

Table 62: Bonferroni Multiple Comparison for BMI and Number of CHC Interactions

# of CHC Interactions	Additional Visit	Mean Difference	Standard Error	p value	95% CI of the Difference	
					Upper	Lower
1	2.00	0.230	0.089	0.056	0.464	-0.003
	3.00	0.295	0.126	0.114	0.626	-0.037
	4.00	0.597	0.120	0.000	0.913	0.280
2	1.00	-0.230	0.089	0.056	0.003	-0.464
	3.00	0.064	0.137	1.000	0.427	-0.299
	4.00	.366	0.132	0.034	0.716	0.017
3	1.00	-0.295	0.126	0.114	0.037	-0.626
	2.00	-0.064	0.137	1.000	0.299	-0.427
	4.00	0.302	0.160	0.351	0.723	-0.119
4	1.00	-0.597	0.120	0.000	-.280	-0.913
	2.00	-0.366	0.132	0.034	-0.017	-0.716
	3.00	-0.302	0.160	0.351	0.119	-0.723

Post-Hoc Analysis of Sample Size Requirements

Minimum sample size requirements are presented in Table 63. The mean changes and SDs of those changes, along with the number of subjects in each group were used in the determination of the Cohn's D for all biomarkers and BMI. Soper's online sample size calculator generates sample size estimates using the estimated effects size, the desired statistical power level, number of predictors, and a predetermined probability level. A power of 0.8 and probability of 0.05 was used for all sample size calculations. The number of predictors was determined by adding the number of CVs used in the ANCOVA to the DV. Table 63 describes the samples size requirements for each biomarker. These requirements were met for each of the analyses performed in this study.

Table 63: Post-Hoc Sample Size Determination

Test	Mean Change Non-CHC Mean (SD)	Mean Change CHC Mean (SD)	Non CHC n	CHC n	Cohen's D	Number of Predictors	Soper's Sample Size
Glucose	-0.08 (13.31)	0.57 (12.77)	964	962	0.049836	4	262
Hemoglobin A1c	-0.11 (.39)	-0.10 (0.40)	1295	1315	0.025314	2	378
1,5-AG	1.29 (3.64)	0.37 (3.67)	117	25	0.251708	2	41
Insulin	0.11 (5.31)	0.78 (5.65)	1010	973	0.122204	4	102
C-Peptide	0.03 (0.73)	0.11 (0.84)	422	401	0.101662	4	122
Proinsulin	-0.23 (7.71)	1.17 (7.92)	249	274	0.179127	5	77
BMI	0.20 (2.16)	0.49 (2.22)	3912	3893	0.132408	2	75

Chapter 5: Discussion

Chapter 5 provides an overview of the results stated in Chapter Four. This chapter discusses the three Specific Aim and markers investigated within each aim. Clinical implications and relation to the study hypotheses are presented. Finally, study limitations are discussed as well as suggestions for future studies.

Discussion of the Study

This study was conducted to determine if there is a relationship between the changes in subjects' biomarkers of glucose homeostasis and BMI, and their interactions with CHCs. Subjects' laboratory results, demographics, whether or not they engaged in CHC interactions, and if so, how many times, were obtained retrospectively from a laboratory in Richmond, VA. There are reports that CHCs are effective at improving BMI and %HbA1c; but research on their effectiveness at improving other markers of glucose homeostasis, such as insulin, C-peptide, proinsulin, and 1,5-AG has not been published. Additionally, research relating the magnitude of the change in BMI and biomarkers, and the number of CHC interactions, could not be found. This study compared the change in BMI and biomarkers over a one-year period for two groups, those who participated in CHC interactions, and those that did not.

Specific Aim 1

The results of this portion of the study confirmed a statistical difference between those who had CHC interactions and those who did not, but only for the average decrease in BMI. Reductions in the mean BMI were consistent with the findings of Apple et al., in which weight reductions were recorded over 24 months of various coaching styles (Apple et al. 2011). Clinically significant weight loss is defined as a 5% reduction in body weight (Stevens et al., 2006). In our study, the subjects' initial weight was not extracted from the records; but the mean change in BMI for the CHC interaction group was only - 0.49, suggesting that the changes were not clinically significant.

For all seven markers, the initial values explained more of the variance in every statistical model, than the use of CHCs, or any other CV. This suggests that initial marker value had the most influence of the variables used in this study in predicting the change in mean maker values. Random error accounted for the most variance. The only marker with a statistically significant difference was BMI. The difference in BMI could have been explained by the large sample size. BMI had almost three times the number of subjects than another marker. In addition, BMI was the only marker directly measured at the time of collection. The phlebotomist or nurse measured the patient's height and weight at the time of visit. The results of BMI were not affected by the same analytical sources of error as the other markers. Sources of error found with blood collection, sample integrity, or analytical variations on the instrument were not a potential source of error for the measurement of BMI. However, BMI could have been affected by variations in the scales used for measuring weight or variations with the techniques used to measure height.

The increase in mean %HbA1c is discordant with the findings of Wayne & Rivito 2014, Ko et al. 2007, and Battista et al., 2012. All three studies found that CHCs have a positive impact

on glucose homeostasis as evidenced by the improvement in %HbA1c in their patients. Wayne & Rivito utilized smartphone technology to accommodate subjects, and concluded that communication and support improved %HbA1c. Ko et al. concluded that coaching lowered %HbA1c. In addition, Battista et al. reported that dietitian education and guidance led to a 0.6% reduction in %HbA1c, a common topic discussed with CHC interactions. The CHCs utilized in this study had to be a registered dietician, exercise specialist, or registered nurse. The transition from the BioRad Turbo to the Trinity HPLCE methodology could explain the increase. As noted in the method comparison, the Trinity HPLC method did have a slightly positive bias compared to the BioRad Turbo. The combination of methodology change and variation in calibrator could be one factor for the increase. In addition, only a small percentage of variance was explained by the CVs and the IV, the majority of variance was unaccounted for as random error.

The effects of CHC therapies on changes in 1,5-AG, C-peptide, insulin, and proinsulin have not been published. As with the other markers, initial marker values accounted for the most variance of the variables used in statistical analysis. This would suggest that initial marker value, not the use of CHC interaction were more predictive of improvements in C-peptide, insulin, and proinsulin. However, the improvement of insulin, C-peptide, and proinsulin in the both the CHC and non-CHC groups could indicate an improvement of beta-cell health and therefore could improve overall glycemic control.

In addition to testing the statistical significance of CHC interactions on the mean change in markers, the clinical significance of the changes were also determined. Mean marker changes were compared to the range of biological change to determine clinical significance. The initial marker minimums and maximums were used to determine the absolute significant change by determining the percent change for the minimum and maximum values. The percent biological

variation for glucose, %HbA1c, insulin, and C-peptide were transformed to absolute values. The mean of the transformed values were compared to the mean change in the observed CHC interaction group. Clinically significant changes in biomarkers were not apparent for all subjects. The mean changes calculated for glucose, HbA1c, and C-peptide were less than the calculated mean absolute within marker changes (see Table 64). The changes in insulin, although not statistically significant, may be clinically significant.

Table 64: Relationship Between Within-Subject Biological Variation and the Observed Mean Change in Markers of Glucose Homeostasis for Subjects with CHC Interactions

Marker	CV_I (%)	Lowest Initial Result	Highest Initial Result	Absolute Mean Change CHC group	Change Required for Significance	Estimated Mean Range Change for Significance
Glucose (mg/dL)	5.6	52	331	0.57	5.8-37	10.7
Hemoglobin A1c (%)	1.9	4.1	9.9	0.10	0.16-0.38	0.14
Insulin (µU/mL)	14.6	1	46	0.78	0.3-13.26	0.50
C-Peptide (ng/mL)	16.6	0.5	8.9	0.11	0.16-7.7	2.0

CV_I = within-subject biologic variation
(Ricos, 2014)

Furthermore, the reagent lot-to-lot variation could be a source of variation. Table 65 describes the average bias of the reagent changes during the study timeframe. This could account for some of the unexplained variance in the biomarker ANCOVAs.

Table 65: Analytical Variation Observed with Reagent Change During the Study Timeframe

Test	Number of Reagent lot-to-lots	Date Range	Average Bias (%)
Glucose	30	2012-2016	0.7
Hemoglobin A1c- BioRad	34	2012-2014	-0.3
Hemoglobin A1c- Trinity	N/A	N/A	N/A
1,5-Anhydroglucitol	18	2013-2016	1
Insulin	7	2012-2016	-0.6
C-Peptide	7	2012-2016	-1.5
Proinsulin	13	2012-2016	-1

Specific Aim 2

The results for blood glucose concentration and %HgbA1c were assigned a health score based upon the established ranges for normal, prediabetes, and diabetes. Likewise, results for BMI were assigned a health score based upon the established ranges for normal, overweight, and obese.

Initial t-tests indicated that no statistical difference between the change in mean health score for those who did and did not participate in CHC interactions exists for glucose or HbA1c ($p > 0.05$). A statistically significant difference was found between the two groups for the unadjusted mean change in BMI health score ($p < 0.001$).

Statistically significant differences in the changes in mean BMI health scores were found between subjects with CHC interactions, and subjects without CHC interactions ($p = 0.001$). However, the differences in the changes in health scores for glucose and %HgbA1c were not significantly different between those groups of subjects. As was the case for the actual results for these markers, more of the variance in the changes in health scores was explained by the initial values for the markers. The low amount of variance accounted for by the CHC interaction group and the study CVs suggests that a majority of the difference is explained by random error, as

indicated by the analytical imprecision of the glucose and HbA1c assays and normal biological variation.

There was a drop in the number of subjects classified as obese in both groups; but there were more subjects with CHC interactions who showed an improvement in BMI classification than those without CHC interactions. Health coach interactions are known to encourage weight loss (Leahey & Wing, 2013, Appel et al., 2011). Therefore, it was expected to observe improvements in weight and BMI that would result in a change in BMI health score. For the glucose health score, there was a small decrease in the number of subjects classified as diabetic in the group with CHC interactions, but no decrease in the group without CHC interactions. On the other hand, there was a decrease in the number of subjects classified as normal by their HgbA1c health score in both groups. One explanation could be the tendency of subjects to fast before their blood draws, without improving their day-to-day lifestyle. These variations in diet and activity can alter glucose results, potentially indicating an improved lifestyle. The HbA1c assay is not affected by these variations, producing a more accurate indication of the subject's diet. Furthermore, the decrease in the normal classification could be explained by the change in HbA1c assay methodology. The method comparison between the BioRad Turbo and Trinity Premier HbA1c assays indicated a slight positive bias with the Trinity assay. Since the subjects in the glucose, HbA1c, and BMI groups were different from the each other, comparison of the trends between CHC and non-CHC groups was not possible.

For all of the markers and health scores, the standard deviations in the changes in these parameters were much larger than the mean changes. This, along with the small percentage of the variances in the changes that was explained by CHC interactions, suggests that there were other factors that influenced the behaviors and changes in the markers of the subjects in both

groups. These factors could include prescribed medications, dietary habits, or exercise routines. All study subjects were provided with the same laboratory report, which included information on their overall health. The results of this study suggest that some individuals need CHCs for motivation to move through the stages of the TTM towards a healthier lifestyle, while others are self-motivated to do so.

Specific Aim 3

There was a statistically significant relationship between the magnitude of change in marker results and the number of CHC interactions, but only for insulin and BMI after the effects of the covariates were taken into account. The known relationship between increases in BMI and insulin resistance (Chung, Cho, Chung, & Chung, 2012) could explain why of the number of CHC interactions affected change in Insulin and BMI and not the other markers. However, not all subjects in the insulin marker group may have been in the BMI group and vise-versa. As with the changes in marker values and changes in health scores, the initial marker values explained more of the variance in those changes than any other covariate or CHC interactions.

In the case of the mean change in %HbA1c, there was an increase in %HbA1c regardless of the number CHC interactions, which is not consistent with the changes in insulin concentration and BMI. However, there was a transition from the BioRad Turbo Variant to the Trinity Premier Boronate affinity HbA1c testing platforms during the study period that could explain this observation.

Glucose and proinsulin are both sensitive to fasting status. If a subject ate or drank something during a fasting period, a falsely increased glucose or proinsulin could have been present. For not just glucose or proinsulin, if a subject misrepresented their fasting status or the duration of fasting, variations in all biomarkers with the exception of HbA1c and 1,5-AG could

be present as well. The inability to confirm fasting status in the study poses a limitation for all three Aims.

Conclusions

For all three Specific Aims in this study, there was a significant difference in the mean changes in BMI between patients who had CHC interactions and those who did not, as well as between patients with different numbers of CHC interactions. None of the other markers of glucose homeostasis showed consistent, significant differences between subjects in those groups. The initial BMI and initial biomarker values also showed consistent and significant effects across all three Specific Aims, and appear to be a more powerful motivators for change than CHCs alone.

Limitations

The first limitation of the study is the uncertainty of additional activities the subjects might have engaged in during the study timeframe. Neither the LIS database nor the CHC interaction database contains information on other resources/interventions the subjects might have utilized. Therefore, differences in training and credentials, coaching style, length of interactions, approachability, or recommended strategies specific to each coach may have affected the marker results.

Comorbidities in the study subjects were also not available for the data analysis. Diagnosis of diabetes or insulin resistance prior to being seen be the CHC could affect the study subjects' expectations or views of the CHC interactions. Subjects may have had diseases other than diabetes such as cardiovascular, liver, or kidney disease that could have affected their ability to control their glucose homeostasis. Liver and kidney diseases can also affect the clearance of

the markers and affected the results. In addition, mental and/or physical conditions could limit or inhibit the study participants' ability to adhere to CHC recommendations.

The inability to confirm that subjects followed the recommendations of the CHCs is also a limitation of this study. Whether or not subjects were actually fasting at the time of the blood collections could also not be confirmed. In addition, subjects' motivation to change their own behavior and improve glucose homeostasis was not measured. As mentioned earlier, a change in the methodology for measuring %HbA1c occurred during the study period, and may have introduced a bias in the follow-up results for some subjects.

Recommendations for Future Studies

The advantages of a retrospective such as reduced cost, availability of study subjects and data, and fewer IRB restrictions, also come with disadvantages. A prospective study could ensure that the sample size is the same across all biomarkers, as well as between the control and intervention groups. This would allow for multivariate analysis of the biomarkers to investigate the overall effect of the CHCs on changes within and across biomarker groups. Future studies should identify subjects with comorbidities, medications, or other therapies that could impact the measurement of the markers or changes in their results. Future studies should also standardize the CHC interactions, themselves. Finally, it would be recommended not include subjects whose study timeframe included a methodology change.

References

Akanuma, Y., Morita, M., Fukuzawa, N., Yamanouchi, T., & Akanuma, H. (1988). Urinary excretion of 1,5- anhydro- D- glucitol accompanying glucose excretion in diabetic patients. *Diabetologia; Clinical and Experimental Diabetes and Metabolism, 31*(11), 831-835. doi:10.1007/BF00277486

American Diabetes Association. (2013). Standards of medical care in diabetes- 2013. *Diabetes Care; Diabetes Care, 36,* S11-S66. doi:10.2337/dc13-S011

American Diabetes Association. (2018). Lifestyle management: *Standards of medical care in Diabetes—2018. Diabetes Care, 41*(Supplement 1), S38-S50. doi:10.2337/dc18-S004

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.) (DSM-5). Washington, DC: American Psychiatric Association.

Appel, L. J., Clark, J. M., Yeh, H., Wang, N., Coughlin, J. W., Daumit, G., . . . Brancati, F. L. (2011). Comparative effectiveness of weight- loss interventions in clinical practice. *The New England Journal of Medicine, 365*(21), 1959-1968. doi:10.1056/NEJMoa1108660

Armstrong, C. (2017). ADA updates standards of medical care for patients with diabetes mellitus. *American Family Physician, 95*(1), 40-43.

Balakhadze, M., Giorgadze, E., & Lomidze, M. (2016). The frequency of langerhans islets -cells autoantibodies (anti- GAD) in Georgian children and adolescents with chronic autoimmune thyroiditis. *International Journal of Endocrinology*, 2016 (6597091), 1-4. doi:10.1155/2016/6597091

Bandura, A. (1977). Self- efficacy: Toward a unifying theory of behavioral change. *Psychological Review, 84*(2), 191-215. doi:10.1037/0033-295X.84.2.191

Barrett, S., Begg, S., O'Halloran, P., Kingsley, M. (2018). Integrated motivational interviewing and cognitive behavior therapy can increase physical activity and improve health of adult ambulatory care patients in a regional hospital: the Healthy4U randomized controlled trial, *BMC Public Health,* 18(1), 1-11. doi: 10.1186/s12889-018-6064-7

Battista, M., Labonté, M., Ménard, J., Jean-Denis, F., Houde, G., Ardilouze, J., & Perron, P. (2012). Dietitian-coached management in combination with annual endocrinologist follow up improves global metabolic and cardiovascular health in diabetic participants after 24 months *Applied Physiology, Nutrition, and Metabolism,* 37(4), 610-620. doi:10.1139/h2012-025

Boden, G. (2008). Obesity and free fatty acids. *Endocrinology and Metabolism Clinics of North America, 37*(3), 635-46. doi:10.1016/j.ecl.2008.06.007

Bray, K., Turpin, R. S., Jungkind, K., & Heuser, G. (2008). Defining success in diabetes disease management: Digging deeper in the data. *Disease Management, 11*(2), 119-28. doi:10.1089/dis.2008.112722

Brownlee, M. (1995). Advanced protein glycosylation in diabetes and aging. *Annual Review of Medicine,* 46, 223-234. doi: 10.1146/annurev.med.46.1.223.

Brug, J., Spikmans, F., Aartsen, C., Breedveld, B., Bes, R., & Fereira, I. (2007). Training dietitians in basic motivational interviewing skills results in changes in their counseling style and in lower saturated fat intakes in their patients. *Journal of Nutrition Education and Behavior,* 39(1), 8-12. doi:10.1016/j.jneb.2006.08.010

Cefalu, W. T. (2017). American diabetes association: Standards of medical care in diabetes-2017. *Diabetes Care, 40*(1)

Centers for Disease Control and Prevention. (2014). National diabetes statistics report: Estimates of diabetes and its burden in the United States, 2014. Atlanta, GA: U.S. Department of Health and Human Services;. Retrieved from http://www.cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web.pdf

Centers for Disease Control and Prevention. (2017). National diabetes statistics report, 2017 Retrieved from https://www.cdc.gov/diabetes/data/statistics/statistics-report.html

Cerasi, E. (1992). *Aetiology of type II diabetes*. In F. M. Ashcroft, & S. J. H. Ashcroft (Eds.), *Insulin-molecular biology to pathology* , p. 352 Oxford: IRL Press.

Chan, S. J., Keim, P., & Steiner, D. F. (1976). Cell- free synthesis of rat preproinsulins: Characterization and partial amino acid sequence determination. *Proceedings of the National Academy of Sciences of the United States of America,* 73(6), 1964-1968.

Christy, A. L., Manjrekar, P. A., Babu, R. P., Hegde, A., & Rukmini, M. S. (2014). Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. *Iranian Biomedical Journal,* 18(2), 88-93. doi:10.6091/ibj.1257.2014

Chung, J.O., Cho, D.H., Chung, D.J., & Chung, M.Y. (2012). Associations among body mass index, insulin resistance, and pancreatic β-cell function in Korean patients with new-onset type 2 diabetes. *Korean Journal of Internal Medicine*, 24(1), 66-71. doi: 10.3904/kjim.2012.27.1.66

Clark, P. M. (1999). Assays for insulin, proinsulin(s) and C- peptide. *Annals of Clinical Biochemistry,* 36 (5), 541-564. doi: 10.1177/000456329903600501

Claxton, A. J., Cremer, J., & Pierce, C. (2001). A sysmatic review of the association between dose regimens and medication compliance. *Clinical Therapeutics,* 23(8), 1296-1310. doi:10.1016/S0149-2918(01)80109-0

Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults : The evidence report (1998). *National Heart, Lung, and Blood Institute in cooperation with the National Institute of Diabetes and Digestive and Kidney Diseases.* Bethesda, Md.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*, Hillsdale, N.J: L. Erlbaum Associates.

Cohen, R. M., Franco, R., Khera, P. K., Smith, E., Lindsell, C. J., Ciraolo, P., . . . Joiner, C. (2008). Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. *Blood,* 112(10), 4284-4291. doi:10.1182/blood-2008-04-154112

Cusi, K., Maezono, K., Osman, A., Pendergrass, M., Patti, M. E., Pratipanawatr, T., . . . Mandarino, L. J. (2000). Insulin resistance differentially affects the PI 3- kinase- and MAP kinase-mediated signaling in human muscle. *The Journal of Clinical Investigation,* 105(3), 311-320. doi: 10.1172/JCI7535

Dabelea, D., Rewers, A., Stafford, J. M., Standiford, D. A., Lawrence, J. M., Saydah, S., . . . Pihoker, C. (2014). Trends in the prevalence of ketoacidosis at diabetes diagnosis: The SEARCH for diabetes in youth study. *Pediatrics,* 133(4), e938-945. doi:10.1542/peds.2013-2795

DeFronzo, R. A., & Abdul-Ghani, M. (2011). Type 2 diabetes can be prevented with early pharmacological intervention. *Diabetes Care,* 34(S2), S202-S209. doi:10.2337/dc11-s221

Dungan, K. M., Buse, J. B., Largay, J., Kelly, M. M., Button, E. A., Kato, S., & Wittlin, S. (2006). 1,5-anhydroglucitol and postprandial hyperglycemia as measured by continuous glucose monitoring system in moderately controlled patients with diabetes. *Diabetes Care,* 29(6), 1214-1219. doi:10.2337/dc06-1910

Dunn, O. J. (1961). Multiple comparisons among means. *Journal of the American Statistical Association,* 56(293), 52–64.

Eakin, E. G., Lawler, S. P., Vandelanotte, C., & Owen, N. (2007). Telephone interventions for physical activity and dietary behavior change: A systematic review. *American Journal of Preventive Medicine,* 32(5), 419-434. doi:10.1016/j.amepre.2007.01.004

Ehrenberg, H. M., Dierker, L., Milluzzi, C., & Mercer, B. M. (2002). Prevalence of maternal obesity in an urban center. *American Journal of Obstertics and Gyneocology,* 187(5), 1189-1193. doi:10.1067/mob.2002.127125

Fairbanks, V. F. and Zimmerman, B. R. (1983). Measurement of glycosylated hemoglobin by affinity chromatography. *Mayo Clinical Proceedings*, 58(1), 770-773.

Fajans, S., & Bell, G. (2011). MODY history, genetics, pathophysiology, and clinical decision making. *Diabetes Care*, 34(8), 1878-1884. doi:10.2337/dc11-0035

Forbes, J., & Cooper, M. (2013). Mechanisms of diabetic complications. *Physiological Review*, 93(1), 137-138. doi: 10.1152/physrev.00045.2011.

Funnell, M. M. (2006). The diabetes attitudes, wishes, and needs (DAWN) study. *Clinical Diabetes*, 24(4), 154-155. doi:10.2337/diaclin.24.4.154

Garde, A.H., Hansen, A. M., Skovgaard, L. T., Christensen, J. M. (2000) Seasonal and Biological Variation of Blood Concentrations of Total Cholesterol, Dehydroepiandrosterone

Sulfate, Hemoglobin A_{1c}, IgA, Prolactin, and Free Testosterone in Healthy Women. *Clinical Chemistry,* 46 (4), 551-559.

Godsland, I. F., Jeffs, J. A. R., & Johnston, D. G. (2004). Loss of beta cell function as fasting glucose increases in the non-diabetic range. *Diabetologia,* 47(7), 1157-1166. doi:-10.1007/s00125-004-1454-z

Gonzalez, J. S., Shreck, E., Psaros, C., & Safren, S. A. (2014). Distress and type 2 diabetes-treatment adherence: A mediating role for perceived control. *Health Psychology,* 34(5), 505-513. doi:10.1037/hea0000131

Haas, L., Maryniuk, M., Beck, J., Cox, C. E., Duker, P., Edwards, L., . . .Youssef, G. (2012). National standards for diabetes self-management education and support. *Diabetes Care,* 35(11), 2393-2401. doi:10.2337/dc12-1707

Haynes, R. B. (2001). Improving patient adherence: State of the art, with special focus on medication taking for cardiovascular disorders. In L. E. Burke, & I. S. Okenes (Eds.), *Patinet Compliance in Healthcare and Research: American heart association monograph series* (3rd ed., pp. 21). Armonk, NY: Futura Publishing Co.

Hedderson, M. M., Williams, M. A., Holt, V. L., Weiss, N. S., & Ferrara, A. (2008). Body mass index and weight gain prior to pregnancy and risk of gestational diabetes mellitus. *American Journal of Obstertics and Gynecology,* 198(4), 409 e.1-409.e7. doi:10.1016/j.ajog.2007.09.028

Heding, L. G., & Rasmussen, S. M. (1975). Human C-peptide in normal and diabetic subjects. *Diabetologia,* 11(3), 201-206.

Hills, C. E., & Brunskill, N. (2008). Intracellular signaling by C- peptide. *Experimental Diabetes Research* 2008(635158), 1-8, doi:10.1155/2008/635158

Hills, C. E., & Brunskill, N. J. (2009). C- peptide and its intracellular signaling. *The Review of Diabetic Studies,* 6(3), 138-137. doi:10.1900/RDS.2009.6.138

Hojat, M. (2007). Empathy in patient care : Antecedents, development, measurement, and outcomes. New York: New York : Springer.

Hojat, M., Louis, D. Z., Maio, V., & Gonnella, J. S. (2013). Empathy and health care quality. *American Journal of Medicial Quality,* 28(1), 6-7. doi:10.1177/1062860612464731

Honda, H., Igaki, M., Hatanaka, Y., Komatsu, M., Tanaka, S., Miki, T., . . . Hayashi, T. (2017). Repeated 3-minute stair climbing-descending exercise after a meal over 2 weeks increases serum 1,5- anhydroglucitol levels in people with type 2 diabetes. *Journal of Physical Therapy Science,* 29(1), 75-78. doi:10.1589/jpts.29.75

Horwitz, D. L., Starr, J. I., Mako, M. E., Blackard, W. G., & Rubenstein, A. H. (1975). Proinsulin, insulin, and C- peptide concentrations in human portal and peripheral blood. *The Journal of Clinical Investigation,* 55(6), 1278-1283.

IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

Insel, R. A., Dunne, J. L., Atkinson, M. A., Chiang, J. L., Dabelea, D., Gottlieb, P. A., . . . Ziegler, A. (2015). Staging presymptomatic type 1 diabetes: A scientific statement of JDRF, the endocrine society, and the american diabetes association. *Diabetes Care,* 38(10), 1964-1974. doi:10.2337/dc15-1419

International expert committee report on the role of the A1C assay in the diagnosis of diabetes. (2009). *Diabetes Care,* 32(7), 1327-1334. doi:10.2337/dc09-9033

Johnson, S. S., Paiva, A. L., Cummins, C. O., Johnson, J. L., Dyment, S. J., Wright, J. A., . . . Sherman, K. (2008). Transtheoretical model- based multiple behavior intervention for

weight management: Effectiveness on a population basis. *Preventive Medicine*, 46(3), 238-246. doi:10.1016/j.ypmed.2007.09.010

Jones, A. G., & Hattersley, A. T. (2013). The clinical utility of C- peptide measurement in the care of patients with diabetes. *Diabetes Medicine*, 30(7), 803-817. doi:10.1111/dme.12159

Jones, H., Edwards, L., Vallis, T. M., Ruggiero, L., Rossi, S. R., Rossi, J. S., . . . Zinman, B. (2003). Changes in diabetes self-care behaviors make a difference in glycemic control. *Diabetes Care*, 26(3), 732-737. doi:10.2337/diacare.26.3.732

Jones MB, Koler RD, Jones RT (1978). Micro-column method for the determination of hemoglobin minor fractions A1 (a + b) and A1c. *Hemoglobin*, 2, 53-58.

Juraschek, S. P., Steffes, M. W., Miller, E. R., & Selvin, E. (2012). Alternative markers of hyperglycemia and risk of diabetes. *Diabetes Care, 35*(11), 2265-270. doi:10.2337/dc12-0787

Kao, P.C., Taylor, R.L., Heser, D.W. (1992) C-peptide immunochemiluminometric assay developed from two seemingly identical polyclonal antisera. *Annual of Clinical Laboratory Science*, 22(5), 307-316.

Katz, R. J., Ratner, R. E., Cohen, R. M., Eisenhower, E., & Verme, D. (1996). Are insulin and proinsulin independent risk markers for premature coronary artery disease *? Diabetes*, 45(6), 736-741.

Kehler, D., Christensen, B., Lauritzen, T., Christensen, M. B., Edwards, A., & Risor, M. B. (2008). Ambivalence related to potential lifestyle changes following preventive cardiovascular consultations in general practice: A qualitative study. *BMC Family Practice*, 9(50), 1-10. doi:10.1186/1471-2296-9-50

Kjems, L.L., Røder, M.E., Dinesen, B., Hartling, S.G., Jørgensen, P.N., Binder, C. (1993) Highly sensitive enzyme immunoassay of proinsulin immunoreactivity with use of two monoclonal antibodies. *Clinical Chemistry,* 39(10), 2146-2150.

Klein, R. (1995). Hyperglycemia and Microvascular and Microvascular Disease in Diabetes. *Diabetes Care,* 18(2), 258-268.

Knowler, W.C., Barrett-Conner, E., Fowler, S.E., Hamman, R.F., Lachin, J.M., Nathan, D.M., Diabetes Prevention Program Research Group. (2002). Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin. *New England Journal of Medicine,* 346(6), 393-403.

Ko, S. , Song, K. , Kim, S. , Lee, J. , Kim, J. , Shin, J. , . . . Ahn, Y. . (2007). Long- term effects of a structured intensive diabetes education programme (SIDEP) in patients with Type 2 diabetes mellitus-a 4-year follow-up study. *Diabetic Medicine*, 24(1), 55-62. doi:10.1111/j.1464-5491.2007.02013.x

Krentz, A. J., Clark, P. M., Cox, L., & Nattrass, M. (1993). Hyperproinsulinaemia in impaired glucose tolerance. *Clinical Science*, 85(7), 97-100. doi:10.1042/cs0850097

Krebs, H.A. (1964). *Gluconeogenesis*. Proceedings of the Royal Society of London. Ser. B. 159, 545-564

Kubo, K. (2002). Effect of pioglitazone on blood proinsulin levels in patients with type 2 diabetes mellitus. *Endocrine Journal,* 49(3), 323-328. doi:10.1507/endocrj.49.323

Leahey, T. M., & Wing, R. R. (2013). A randomized controlled pilot study testing three types of health coaches for obesity treatment: Professional, peer, and mentor. *Obesity,* 21(5), 928-934. doi:10.1002/oby.20271

Li, C.I., Li, T.C., Liu, C.S., Lin, W.Y., Chen, C.C., Yang, S.Y., Lin, C.C. (2015). Extreme Values of Hemoglobin A1c Are Associated With Increased Risks of Chronic Obstructive Pulmonary Disease in Patients With Type 2 Diabetes. *Medicine*, 94(1), e367. doi:10.1097/MD.0000000000000367

Liao, H., Zhong, S., Li, P., Chen, W., Cheng, C., Wang, Y., . . . Xiao, C. (2015). Effects and mechanism of moderate aerobic exercise on impaired fasting glucose improvement. *Lipids in Health and Disease,* 14(157), 1-6 . doi:10.1186/s12944-015-0117-z

Liddy, C., Johnston, S., Nash, K., Ward, N., & Irving, H. (2014). Health coaching in primary care: A feasibility model for diabetes care. *BMC Family Practice,* 15(60), 1-8. doi:10.1186/1471-2296-15-60

Linden, A., Butterworth, S. W., & Prochaska, J. O. (2010). Motivational interviewing- based health coaching as a chronic care intervention. *Journal of Evaluation in Clinical Practice,* 16(1), 166-174. doi:10.1111/j.1365-2753.2009.01300.x

Little, R. R., Vesper, H., Rohlfing, C. L., Ospina, M., Safar-Pour, S., & Roberts, W. L. (2005). Validation by a mass spectrometric reference method of use of boronate affinity chromatography to measure glycohemoglobin in the presence of hemoglobin S and C traits. *Clinical Chemistry,* 51(1), 264-265. doi:10.1373/clinchem.2004.043141

Loveman, E., Frampton, G. K., & Clegg, A. J. (2008). The clinical effectiveness of diabetes education models for type 2 diabetes: A systematic review. *Health Technology Assessment,* 12(9), 1-116.

Mahalanobis, P.C. (1936) On the Generalized Distance in Statistics. *Proceedings of the National Institute of Science of India*, 2, 49-55.

Mann, D. M., Ponieman, D., Leventhal, H., & Halm, E. A. (2009). Predictors of adherence to diabetes medications: The role of disease and medication beliefs. *Journal of Behavioral Medicine,* 30, 278-284. doi:10.1007/s10865-009-9202-y

Marley, S. C., Carbonneau, K., Lockner, D., Kibbe, D., & Trowbridge, F. (2011). Motivational interviewing skills are positively associated with nutritionist self-efficacy. *Journal of Nutrition Education and Behavior,* 43(1), 28-34. doi:10.1016/j.jneb.2009.10.009

Miller, W. R. (. R. (2002). *Motivational interviewing: Preparing people for change* (2nd ed.. ed.). New York: New York : Guilford Press.

Mallia, A. K., Hermanson, G.T., Krohn, K.I., Fujimoto, E.K., & Smith, P.K. (1981). Preparation and use of a boronic acid affinity support for separation and quantitation of glycosylated hemoglobins. *Analytical Letters Part B: Clinical and Biochemical Analysis* 14(8), 649-661. doi: 10.1080/00032718108055476

Nagamatsu, S., Bolaffi, J. L., & Grodsky, G. M. (1987). Direct effects of glucose on proinsulin synthesis and processing during desensitization. *Endocrinology,* 120(4), 1225-1231.

National Diabetes Information Clearinghouse. (2014). Insulin resistance and prediabetes. (No. 14-4893). Bethesda MD: Retrieved from http://diabetes.niddk.nih.gov/dm/pubs/insulinresistance/index.aspx#resistance

National Institute of Health. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England Journal of Medicine,* 346(6), 393-403. doi:10.1056/NEJMoa012512

Nijpels, G., Popp-Snijders, C., Kostense, P., Bouter, L., & Heine, R. (1996). Fasting proinsulin and 2- h post- load glucose levels predict the conversion to NIDDM in subjects with

impaired glucose tolerance: The hoorn study. *Diabetologia; Clinical and Experimental Diabetes and Metabolism,* 39(1), 113-118. doi:10.1007/BF00400421

Nobel, J., & Valdes, A. (2011). Genetics of the HLA region in the Prediction of type 1 diabetics. *Current Diabetes Reports,* 11(6), 533-542. doi:- 10.1007/s11892-011-0223-x

Owen, O. E., Felig, P., Morgan, A. P., Wahren, J. Cahill, G.F. (1969). Liver and Kidney metabolism during prolonged starvation. T*he Journal of Clinical Investigation*, 48(3), 574-583. doi:10.1172/JCI106016

Patel, N., Taveira, T. H., Choudhary, G., Whitlatch, H., & Wu, W. (2012). Fasting serum C-peptide levels predict cardiovascular and overall death in nondiabetic adults. *Journal of the American Heart Association,* 1(6), 1-12. doi:10.1161/JAHA.112.003152

Patzelt, C., Labrecque, A. D., Duguid, J. R., Carroll, R. J., Keim, P. S., Heinrikson, R. L., & Steiner, D. F. (1978). Detection and kinetic behavior of preproinsulin in pancreatic islets. *Proceedings of the National Academy of Sciences of the United States of America*, 75(3), 1260-1264.

Pearson, E. S., Hartley, H. O. (1958). *Biometrika Tables for Statisticians*, Volume 1, (3rd ed.). (New York: Cambridge University Press.

Pettitt, C. (2013). Wellness coaching certifications: A new career frontier for personal trainers in health care. *Strength and Conditioning Journal,* 35(5), 63-64-67.

Pfützner, A., Kunt, T., Hohberg, C., Mondok, A., Pahler, S., Konrad, T., . . . Frost, T. (2004). Fasting intact proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. *Diabetes Care,* 27(3), 682-687.

Pfützner, A., Pfützner, A.,H., Larbig, M., & Forst, T. (2004). Role of intact proinsulin in diagnosis and treatment of type 2 diabetes mellitus. *Diabetes Technology & Therapeutics,* 6(3), 405-412. doi:10.1089/152091504774198124

Polit, D. F. (2008). *Nursing research: Generating and assessing evidence for nursing practice* (8th ed.). Philadelphia, Pennsylvania. Wolters Kluwer Health/Lippincott Williams & Wilkins.

Polonsky, K. S., Licinio-Paixao, J., Given, B. D., Pugh, W., Rue, P., Galloway, J., . . . Frank, B. (1986). Use of biosynthetic human C- peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. *The Journal of Clinical Investigation,* 77(1), 98-105.

Polonsky, W. H., & Henry, R. R. (2016). Poor medication adherence in type 2 diabetes: Recognizing the scope of the problem and its key contributors. *Patient Preference and Adherence,* 10, 12991307. doi.org/10.2147/PPA.S106821

Radin, M. S. (2014). Pitfalls in hemoglobin A1c measurement:When results may be misleading. *Journal of General Internal Medicine,* 29(2), 388. doi:- 10.1007/s11606-013-2595-x

Ramlo-Halsted, B. A., & Edelman, S. V. (1999). The natural history of tyep 2 diabetes: Implications for clinical practice. *Primary Care,* 26(4), 771-789.

Rice, B., Janssen, I., Hudson, R., & Ross, R. (1999). Effects of aerobic or resistance exercise and/or diet on glucose tolerance and plasma insulin levels in obese men. *Diabetes Care,* 22(5), 684-691.

Ricos, C. (2014). *Desirable biological variation database specifications*. Retrieved from https://www.westgard.com/biodatabase1.htm

Riddle, M. C. (2018a). Classification and diagnosis of diabetes: Standards of medical care in Diabetes-2018. *Diabetes Care*, 41(Supplement 1), S13-S27. doi:10.2337/dc18-S002

Riddle, M. C. (2018b). Standards of medical care in Diabetes—2018. *Diabetes Care*, 41(Supplement 1), S1-S150. doi:10.2337/dc18-Sint01

Riebe, D., Greene, G. W., Ruggiero, L., Stillwell, K. M., Blissmer, B., Nigg, C. R., & Caldwell, M. (2003). Evaluation of a Healthy-lifestyle approach to weight management. *Preventive Medicine,* 36(1), 45-55. doi:10.1006/pmed.2002.1126

Røder, M.E., Porte Jr, D., Schwartz, R.S., & Kahn, S.E. (1998). Disproportionately elevated proinsulin levels reflect the degree of impaired B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *The Journal of Clinical Endocrinology & Metabolism*, 83(2):604-608.

Saad, M. F., Kahn, S. E., Nelson, R. G., Pettitt, D. J., Knowler, W. C., Schwartz, M. W., . . . Porte, D. (1990). Disproportionately elevated proinsulin in pima indians with noninsulin-dependent diabetes mellitus. *The Journal of Clinical Endocrinology and Metabolism,* 70(5), 1247-1253. doi:10.1210/jcem-70-5-1247

Sapin, R., Le Galudec, V., Gasser, F., Pinget, M., Grucker, D. (2001) Elecsys Insulin Assay: Free Insulin Determination and the Absence of Cross-Reactivity with Insulin Lispro. *Clinical Chemistry*, 47(3), 602-605

Saltiel, A. R., & Kahn, R. C. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature,* 41, 799-806. doi:10.1038/414799a

Service, F., Rizza, R., Zimmerman, B., & Dyck, P. (1997). The classification of diabetes by clinical and C-peptide criteria: A prospective population-based study. *Diabetes Care,* 20(2), 198-201. doi:10.2337/diacare.20.2.198

Shamoon, H., Duffy, H., Fleischer, N., Engel, S., Saenger, P., Strelzyn, M., . . . Rand, L. (1993). The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-term Complication sin Insulin-Dependent Diabetes-Mellitus. *New England Journal of Medicine,* 329(14), 977-986.

Sinaiko, A. R., & Caprio, S. (2012). Insulin resistance. *The Journal of Pediatrics*, 161(1), 11-15. doi.org.proxy.library.vcu.edu/10.1016/j.jpeds.2012.01.012

Skovlund, S. E., & Peyrot, M. (2005). The diabetes atitude, wishes, and needs (DAWN) program: A new approach to improving outcomes of diabetes care. *Diabetes Spectrum,* 18(3), 136-142. doi:10.2337/diaspect.18.3.136

Skylar, J. S., Basris.G.L., Bonifacio, E., Darsow, T., Eckel, R. H., Groop, L., . . . Ratner, R. (2017). Differentiation of diabetes by pathology, natural history, and prognosis. *Diabetes,* 66(2), 241-255. doi:10.2337/db16-0806

Smith, U. (2001). Pioglitazone:Mechanism of action. *International Journal of Clinical Practice Supplement,* 121, 8-13.

Soper, D. S. (2018). A-priori sample size calculator for multiple regression [software]. Retrieved from http://www.danielsoper.com/statcalc

Starr, J. I., & Rubenstein, A. H. (1974). Metabolism of endogenous proinsulin and insulin in man. *The Journal of Clinical Endocrinology and Metabolism,* 38(2), 305-308.

Stein, M.W. (1965). *Clinical Methods of Enzymatic Analysis*, Cambridge Massachusetts: Academic Press.

Steiner, D. F., Cunningham, D., Spigelman, L., & Aten, B. (1967). Insulin biosynthesis: Evidence for a precursor. *Science,* 157(3789), 697-700.

Selvin, E., Lazo, M., Chen, Y., Shen, L., Rubin, J., McEvoy, J.W., … Coresh, J. (2014) Diaetes Mellitus, Prediabetes, and Incidences of Subclinical Myocardial Damage, *Circulation*. 130 (16), 1374-1382. doi:10.1161/CIRCULATIONAHA.114.010815/-/DC1

Stevens, J. , Truesdale, K.P., McClain, J.E., Cai, J. The definition of weight maintenance. (2006) *International Journal of Obesity*, 30, 391-399.

Stratton, I., Adler, A. I., Neil, H., Matthews, D., Manley, S., Cull, C., . . . Holman, R. (2000). Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): Prospective observational study. *British Medical Journal; Br.Med.J.*, 321(7258), 405-412.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using Multivariate Statistics* (5th ed.). New York: Allyn and Bacon.

The Diabetes Prevention Program Research Group. (2005). *Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program* doi:10.2337/diabetes.54.8.2404

The Expert Committee on the Diagnosis and Classification of,Diabetes Mellitus. (2003). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*, 26, S5-20.

Tirosh, A., Shai, I., Tekes-Manova, D., Israeli, E., Pereg, D., Shochat, T., Kochba, I., Rudich, A. (2005) Normal Fasting Plasma Glucose Levels and Type 2 Diabetes in Young Men. *New England Journal of Medicine*, 353(14): 1454-1462

Torjesen, P. A., Birkeland, K. I., Anderssen, S. A., Hjermann, I., Holme, I., & Urdal, P. (1997). Lifestyle changes may reverse development of the insulin resistance syndrome. The oslo diet and exercise study: A randomized trial. *Diabetes Care*, 20(1), 26-31

Törn, C., Landin-Olsson, M., Östman, J., Scherstén, B., Arnqvist, H., Blohmé, G., . . . Lernmark, Å. (2000). Glutamic acid decarboxylase antibodies (GADA) is the most important factor for prediction of insulin therapy within 3 years in young adult diabetic patients not classified as type 1 diabetes on clinical grounds. *Diabetes/Metabolism Research and Reviews,* 16(6), 442-447. doi:10.1002/1520-7560(2000)9999:9999<::AID-DMRR152>3.0.CO;2-T

Trapp, E. G., Chisholm, D. J., Freund, J., & Boutcher, S. H. (2008). The effects of high- intensity intermittent exercise training on fat loss and fasting insulin levels of young women. *International Journal of Obesity,* 32(4), 684-691. doi:10.1038/sj.ijo.0803781

Tukey, JW. (1977). Exploratory data analysis. Reading, Mass. :Addison-Wesely,

Vangipurapu, J., Stančáková, A., Kuulasmaa, T., Kuusisto, J., Laakso, M., & Kulkarni, R. (2015). Both fasting and glucose- stimulated proinsulin levels predict hyperglycemia and incident type 2 diabetes: A population- based study of 9,396 finnish men. *PLoS ONE,* 10(4), 1-11. doi:10.1371/journal.pone.0124028

Wayne, N., & Ritvo, P. (2014). Smartphone-enabled health coach intervention for people with diabetes from a modest socioeconomic strata community: Single-arm longitudinal feasibility study. *Journal of Medical Internet Research,* 16(6), e142-e161. doi:10.2196/jmir.3180

Weir, G. C., & Bonner-Weir, S. (2004). Five stages of evolving beta- cell dysfunction during progression to diabetes. *Diabetes,* 53(3), s16-s21.

Willard-Grace, R., DeVore, D., Chen, E. H., Hessler, D., Bodenheimer, T., & Thom, D. H. (2013). The effectiveness of medical assistant health coaching for low-income patients with uncontrolled diabetes, hypertension, and hyperlipidemia: Protocol for a randomized controlled trial and baseline characteristics of the study population. *BMC Family Practice,* 14(27), 1-10. doi:10.1186/1471-2296-14-27

Wolever, R. Q., Dreusicke, M., Fikkan, J., Hawkins, T. V., Yeung, S., Wakefield, J., . . . Skinner, E. (2010). Integrative health coaching for patients with type 2 diabetes. *The Diabetes Educator, 36*(4), 629-639. doi:10.1177/0145721710371523

Wolever, R. Q., Simmons, L. A., Sforzo, G. A., Dill, D., Kaye, M., Bechard, E. M., . . . Yang, N. (2013). A systematic review of the literature on health and wellness coaching: Defining a key behavioral intervention in healthcare. *Global Advances in Health and Medicine,* 2(4), 38-57. doi:10.7453/gahmj.2013.042

Wu, S. V., Lee, M., Liang, S., Lu, Y., Wang, T., & Tung, H. (2011). Effectiveness of a self-efficacy program for persons with diabetes: A randomized controlled trial. *Nursing & Health Sciences,* 13(3), 335-343. doi:10.1111/j.1442-2018.2011.00625.x

Yamanouchi, T., Akanuma, H., Asano, T., Konishi, C., Akaoka, I., & Akanuma, Y. (1987). Reduction and recovery of plasma 1,5-anhydro-D-glucitol levels in diabetes mellitus. *Diabetes,* 36(6), 709-715.

Yamanouchi, T., Ogata, N., Tagaya, T., Kawasaki, T., Sekino, N., Funato, H., . . . Miyashita, H. (1996). Clinical usefulness of serum 1,5-anhydroglucitol in monitoring glycaemic control. *The Lancet,* 347(9014), 1514-1518. doi:10.1016/S0140-6736(96)90672-8

Zavaroni, I., Deferrari, G., Lugari, R., Bonora, E., Garibotto, G., Dall'Aglio, E., . . . Gnudi, A. (1987). Renal metabolism of C- peptide in man. *The Journal of Clinical Endocrinology and Metabolism,* 65(3), 494-498.

Vita

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