

MEDITERRANEAN-STYLE DIET AND EXERCISE IMPROVE PARAMETERS FOR
MANAGEMENT AND PREVENTION OF TYPE 2 DIABETES MELLITUS

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Stefani Ann Derrick
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COMMITTEE MEMBERSHIP

TITLE: Mediterranean-style Diet and Exercise Improve
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Type 2 Diabetes Mellitus

AUTHOR: Stefani Ann Derrick

DATE SUBMITTED: December 2022

COMMITTEE CHAIR: Angelos Sikalidis, Ph.D.
Associate Professor of Nutrition

COMMITTEE MEMBER: Scott Reaves, Ph.D.
Professor of Nutrition

COMMITTEE MEMBER: Aleksandra Kristo, Ph.D.
Lecturer of Nutrition

ABSTRACT

Mediterranean-style Diet and Exercise Improve Parameters for Management and Prevention of Type 2 Diabetes Mellitus

Stefani Ann Derrick

Type 2 diabetes mellitus (T2DM) is a chronic condition recognized as the inability to maintain glucose homeostasis, typically presenting with insulin resistance and systemic inflammation. With the prevalence of T2DM and major risk factors such as prediabetes and obesity increasing each year, there is a crucial need to identify strategies for the management and prevention of this condition. Addressing lifestyle-related risk factors through consumption of a well-balanced, nutritious diet and maintaining regular moderate- to high-intensity physical activity may provide a strategy for improving glycemic control, improving metrics of body composition, and decreasing the inflammatory response associated with metabolic dysregulation. Twenty-two overweight to obese adults with a medical diagnosis of T2DM, indicators of prediabetes, or who were metabolically healthy participated in Cal Poly's Nutrition and Exercise in Type 2 Diabetes (CPNET) study. The study protocol included adherence to a Mediterranean-style diet, daily consumption of a high-quality whey protein supplement, and adherence to the *Physical Activity Guidelines for Americans* for 16 weeks. Body composition data, via dual-energy X-ray absorptiometry (DXA), and fasting blood samples were collected at baseline and following the intervention. Due to restrictions associated with the global COVID-19 pandemic, only 13 participants were able to return for the second data collection following the 16-week intervention. The prediabetic and T2DM groups exhibited reductions in fasting plasma glucose to that of normal and prediabetic levels, respectively, while the T2DM group also showed improvement in hemoglobin A1c to the prediabetic level. Additionally, the metabolically healthy, overweight group demonstrated significant improvements in adiposity, while the obese prediabetic and T2DM groups showed non-significant improvements in all measured metrics of body composition. No changes were observed in inflammatory biomarkers. Thus, our results suggest that adherence to a well-balanced nutritious diet and regular physical activity may improve parameters of glycemic control and provide benefits to body composition that help manage and prevent the development of T2DM.

Keywords: Type 2 diabetes mellitus, prediabetes, Mediterranean diet, whey protein, physical activity, obesity, body composition, systemic inflammation.

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Chapter 1

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic dysregulation of glucose metabolism typically due to insulin resistance (IR) affecting millions of adults worldwide (American Diabetes Association [ADA], 2017; International Diabetes Federation [IDF], 2021). The prevalence of diabetes has steadily increased in the past few decades with over 536 million recorded cases of diabetes and 541 million recorded cases of prediabetes worldwide in 2021 (World Health Organization [WHO], 2021; IDF, 2021). Long-term uncontrolled hyperglycemia, as seen in T2DM, can injure the delicate vasculature of the eyes, kidneys, and nervous system, leading to complications commonly seen in diabetes such as blindness, chronic kidney disease (CKD), and, in advanced stage with poorly controlled diabetes, amputations (IDF, 2021). Damage can also occur to the macrovasculature, promoting the development of cardiovascular disease (CVD), rendering diabetes a cause for CVD and related death secondary to diabetes. These complications cost the United States approximately \$327 billion in 2017, mostly attributable to direct medical costs and loss of productivity in individuals with T2DM.

Risk factors for developing T2DM can be classified into two groups, namely non-modifiable and modifiable (ADA, 2017). Non-modifiable risk factors are factors typically beyond the control of the individual, including age, sex, genetic predisposition, and ethnicity. Conversely, modifiable risk factors can typically be modulated to a greater or lesser degree through lifestyle and behavior modifications, such as improved diet and increased physical activity. A major modifiable risk factor for developing T2DM is prediabetes, classified as elevated fasting blood glucose, impaired glucose tolerance, and/or elevated hemoglobin A1c. Prediabetes typically presents with other clinical signs of metabolic dysregulation collectively known as the metabolic syndrome (Harris, 2013). Individuals presenting with 3 or more indicators of the metabolic syndrome (MetSy) may be at higher risk for developing T2DM and CVD. These indicators include abdominal obesity, dyslipidemia, hypertension, and elevated fasting plasma glucose. The presence of obesity alone, especially abdominal obesity, has been shown to promote a state of low-grade, chronic inflammation and oxidative stress, increasing the risk of T2DM (Kahn, Hull, & Utzschneider, 2006). Obesity status is

commonly assessed by calculating body mass index (BMI) from an individual's height and body weight, with higher BMIs associated with greater risk for metabolic diseases, including T2DM and CVD (ADA, 2021). As BMI only utilizes height and body weight measurements, additional measurement of body fat percentage (BF%) and waist circumference are recommended to provide a more accurate assessment of obesity status (Unamuno et al., 2018).

White adipose tissue (WAT), one of the largest, metabolically active endocrine organs in the body, secretes proinflammatory cytokines (or "adipokines") to regulate whole-body homeostasis (Unamuno et al., 2018). Under conditions of obesity with excessive WAT stored in the abdominal region (visceral adipose tissue [VAT]), adipocyte hypertrophy leads to dysregulated WAT metabolism and altered adipokine secretion (Longo et al., 2019). High plasma levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) have been linked to IR, possibly through interference with insulin signal transduction (Dandona, Aljada, & Bandyopadhyay, 2004). A relationship has been observed between plasma high-sensitivity C-reactive protein (hs-CRP) and CVD risk, body weight, the MetSy, and fasting insulin concentrations, indicating a possible connection between inflammation and IR. There is strong evidence that weight loss of at least 5% of total body weight can improve biomarkers of metabolic dysfunction in T2DM and prevent the progression of prediabetes to T2DM (ADA, 2021). Although several medications exist to treat diabetes and reduce excess adiposity, lifestyle modifications through improved diet and increased physical activity remain the cornerstone of T2DM treatment and prevention (IDF, 2021).

Currently, there is no cure for diabetes (Kirkman et al., 2015). With the prevalence of T2DM and major risk factors such as prediabetes and obesity increasing each year, there is a crucial need to identify strategies for optimal management and prevention of this condition. Adherence to a healthy dietary pattern and performing regular physical activity may provide a strategy for increasing insulin sensitivity, decreasing adiposity, and decreasing the inflammatory response associated with metabolic dysregulation (ADA, 2021; Georgoulis, Kontogianni, & Yiannakouris, 2014). A Mediterranean-style diet (MSD) is a primarily plant-based diet derived from the populations residing in the regions that border the Mediterranean Sea (Trichopoulou et al., 2014; Salas-Salvadó et al., 2016; Georgoulis, Kontogianni, & Yiannakouris, 2014). Although MSDs differ

within the Mediterranean region, an MSD typically consists of high intakes of vegetables, fruits and nuts, whole grains, legumes, fish, and poultry. Monounsaturated fats from olive oil are the main source of dietary lipids and alcohol is consumed in low-to-moderate quantities, mostly as red wine. An MSD is typically low in red meat and non-fermented dairy products. Long-term adherence to an MSD has been shown to reduce the risk of T2DM, as well as CVD, various cancers, and all-cause mortality (Trichopoulou et al., 2014). Recently, there has been growing interest in the possible insulinotropic and appetite-suppressing effects of dietary whey protein for individuals with hyperglycemia and T2DM (Adams & Broughton, 2016; Jakubowicz & Froy, 2013). Whey protein is a good source of all nine essential amino acids and is absorbed quickly and efficiently in the small intestine making it a high-quality “complete” protein. Whey protein has been shown to promote anabolism and have rapid effects on glucose metabolism, preventing postprandial hyperglycemia and improving insulin sensitivity with similar efficacy to anti-diabetic medications.

A sedentary lifestyle has been shown to increase the likelihood of chronic diseases and all-cause mortality (U.S. Department of Health and Human Services [USDHHS], 2018). For significant improvements to overall health, the *Physical Activity Guidelines for Americans*, issued by the United States Department of Health and Human Services, provide recommendations for individuals of all ages and levels of health to increase their daily activity. The American Heart Association (AHA) and the American Diabetes Association (ADA) recommend moderate- to vigorous-intensity physical activity for patients with T2DM to improve glycemic control, reduce the risk of CVD, maintain a healthy weight, and promote longevity (Buse et al., 2007). Overall, current evidence strongly suggests that increased daily physical activity improves T2DM outcomes and reduces the occurrence of T2DM in individuals at high risk.

1.1 Research Objectives

Cal Poly’s Nutrition and Exercise in Type 2 Diabetes (CPNET) study was designed to assess whether changes in lifestyle can improve the metabolic status of individuals with T2DM and prediabetes. More specifically, the CPNET pilot study was conducted to evaluate the effectiveness of long-term adherence to an MSD, daily consumption of a high-quality whey protein supplement,

and regular physical activity on the parameters of glycemic control, the metrics of body composition, and biomarkers of systemic inflammation in obese individuals with T2DM and prediabetes. A novelty of our study is the inclusion of prediabetic participants, a currently understudied group. Throughout the 16-week intervention period, participants were instructed to adhere to the following protocol: consume a personalized nutrition plan based on a Mediterranean-style diet (with 500 kcal reduction in daily energy intake for those interested in weight loss), consume a 25 g whey protein supplement powder daily, and perform exercise in accordance with the *Physical Activity Guidelines for Americans*. At baseline and week 16, blood samples were collected to assess glycemic control and systemic inflammation. Additionally, anthropometric measurements and body composition data were collected at the indicated time points. The primary objective of the study was to investigate the effects of modifications to diet and physical activity level on markers of glycemic control, inflammation, and body composition in individuals with T2DM and prediabetes, as well as healthy individuals. Secondary objectives of the study were to determine which group demonstrated the most improvement and if improvements in measured outcomes occur in a progressive manner among the healthy, prediabetic, and T2DM groups.

1.2 Statement of Research Questions

The CPNET study was conducted to investigate the following research questions:

1. Do modifications to dietary pattern and daily physical activity impact glycemic control, systemic inflammation, and body composition in metabolically healthy, prediabetic, and T2DM populations?
2. If an individual's body composition metrics are improved, does this associate with improvements in their metabolic status as assessed by fasting plasma glucose, hemoglobin A1c, and fasting plasma insulin?
3. If an individual's markers of inflammatory status are improved, does this associate with improvements in their metabolic status as assessed by fasting plasma glucose, hemoglobin A1c, and fasting plasma insulin?

4. In which group is the intervention most effective – metabolically healthy, prediabetic, or T2DM groups?
5. Do improvements in measured outcomes occur in a progressive manner based on initial health status pertinent to glycemic control?

Chapter 2

BACKGROUND

2.1 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is a chronic condition recognized as the inability to maintain glucose homeostasis, typically presenting with insulin resistance (IR), chronic hyperglycemia, and systemic inflammation (ADA, 2017; IDF, 2021). As IR develops, peripheral tissues, such as skeletal muscle, become less efficient in engaging in glucose clearance, leading the pancreas to secrete greater amounts of insulin with subsequent hyperinsulinemia. Over time, this places undue burden on pancreatic β -cells, which progressively decreases their ability to produce insulin in response to elevations in blood glucose concentration (IDF, 2021). Type 1 diabetes mellitus (T1DM) differs from T2DM in that the former is characterized by autoimmune destruction of pancreatic β -cells resulting in dramatic decreases in insulin production and secretion, and the subsequent need for exogenous insulin administration. A T1DM diagnosis is typically accompanied by noticeable clinical signs and symptoms, such as weight loss, polydipsia, polyuria, polyphagia, and diabetic ketoacidosis. Conversely, individuals with T2DM often do not exhibit strong symptoms and, as they can retain some β -cell function, they can live undiagnosed for significant periods of time. In T2DM, IR is the main cause of chronic hyperglycemia, leading organs and delicate vasculature to become damaged over time from increased glycation phenomena. The consequent micro- and macrovascular complications can affect the heart, nervous system, kidneys, and eyes, which can lead to blindness, amputations, chronic kidney disease (CKD), cardiovascular disease (CVD), and even death (Babey et al., 2016).

In 2021, the global prevalence of diabetes in adults ages 20-79 years was estimated at 10.5% (536.6 million people), with an additional 541 million adults living with prediabetes (IDF, 2021). These numbers are expected to increase by 46% by the year 2045. In the United States alone, 34.1 million (13%) adults had diagnosed diabetes in 2018, while an additional 7.3 million (2.8%) adults were estimated to be clinically diabetic but remain undiagnosed (Centers for Disease Control and Prevention [CDC], 2020). The increased need for medical care and hospitalizations in

those living with diabetes and associated complications have widespread and costly implications to public health. Furthermore, there are indirect costs of T2DM that can also have a negative economic impact, such as loss of productivity, absence from work and school, disability, and premature death (Saeedi et al., 2020). In 2017 in the US alone, this translated to an estimated \$90 billion in lost productivity and \$237 billion in direct medical costs, totaling \$327 billion (ADA, 2018). Globally, the economic burden was \$966 billion in 2021, an amount projected to rise to \$1.03 trillion by 2030 (IDF, 2021).

2.1.1 Etiology and Diagnosis

Although the specific cause of T2DM remains unknown, the decrease in insulin secretion of pancreatic β -cells has been correlated with a genetic predisposition to pancreatic insufficiency, as well as chronic inflammation and metabolic stress (ADA, 2017). Unlike T1DM, there is no autoimmune destruction of β -cells in T2DM and individuals with the condition do not normally present with other known etiologies. However, individuals with T2DM typically present with overweight or obesity, predominantly in the abdominal region (i.e., excessive visceral adipose tissue). Living in a long-term state of obesity has been shown to increase low-grade systemic inflammation and some insulin resistance, factors in the pathogenesis of many chronic diseases (O'Rourke, 2009).

As mentioned, individuals with early to moderate stages of T2DM do not typically present with symptoms; however, some may experience symptoms of hyperglycemia like T1DM, including polydipsia (increased thirst), polyphagia (increased hunger), and polyuria (increased urination) as blood glucose levels rise and glucose is not being utilized by peripheral tissues (IDF, 2021). As chronic hyperglycemia typically develops over time and does not present with symptoms early on, T2DM frequently goes undiagnosed, demonstrating a need for routine testing to detect preclinical disease beginning at age 45 (ADA, 2017). The tests commonly used to diagnose T2DM are based on the identification of impaired glucose tolerance, including fasting plasma glucose (FPG), 2-h plasma glucose (2-h PG) following an oral glucose tolerance test (OGTT) utilizing 75 g of glucose, and measurement of glycated hemoglobin in red blood cells through the hemoglobin A1c (HbA1c)

assay. The HbA1c assay provides an indirect representation of average blood glucose levels over the previous 3-4 months making it superior to the other tests, as it is less affected by day-to-day variations in blood glucose due to stress or illness and does not require fasting. Although it is considerably more convenient than other diagnostic tests, measuring HbA1c can be more costly, may not be readily available in developing countries, may not accurately represent average glucose levels in certain individuals, and does not take age, ethnicity, or anemia-induced variations into account. Furthermore, HbA1c levels may appear higher in Black Americans versus non-Hispanic white Americans despite comparable FPG and 2-h PG levels (ADA, 2017).

T2DM is diagnosed through one or more of the following criteria: elevated FPG (≥ 126 mg/dL or 7.0 mmol/L), impaired glucose tolerance (2-h PG following 75 g OGTT ≥ 200 mg/dL or 11.1 mmol/L), and/or HbA1c $\geq 6.5\%$ (48 mmol/mol) (Table 1; ADA, 2017). Additionally, the presence of hyperglycemia in the absence of hyperinsulinemia, seen in more advanced cases of T2DM, can be indicative of insufficient β -cell functioning, as insulin levels would be expected to rise in tandem with blood glucose levels under normal metabolic circumstances and physiological glycemic response. In all cases, the American Diabetes Association (ADA) recommends a second T2DM test before a diagnosis can be confirmed (ADA, 2017).

Table 1. NIDDK guidelines for the classification of type 2 diabetes mellitus and prediabetes.

Diagnosis	Hemoglobin A1c (HbA1c, %)	Fasting Plasma Glucose (FPG, mg/dL) ^a	Oral Glucose Tolerance Test (OGTT, mg/dL) ^b	Random Plasma Glucose test (RPG, mg/dL) ^c
Normal	< 5.7	≤ 99	≤ 139	-
Prediabetes	5.7 - 6.4	100 - 125	140 - 199	-
Diabetes	≥ 6.5	≥ 126	≥ 200	≥ 200

^aPerformed following a fast of at least 8 hours. ^b2-hour plasma glucose measured following a bolus of glucose equal to 75 g anhydrous glucose dissolved in water. ^cPerformed in patients experiencing hyperglycemic crisis or who present with classic symptoms of hyperglycemia. NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases. Source: NIDDK, 2022.

2.1.2 Risk Factors

Various risk factors for developing T2DM have been identified (ADA, 2017). The term “prediabetes” is used to identify individuals at high-risk for developing T2DM and is characterized by one or more of the following parameters detected through the T2DM diagnostic tests mentioned

previously: elevated FPG (100-125 mg/dL or 5.6-6.9 mmol/L), impaired glucose tolerance (2-h PG following 75 g OGTT of 140-199 mg/dL or 7.8-11.0 mmol/L), and/or HbA1c value of 5.7-6.4% (39-47 mmol/mol) (Table 1). The ADA recommends screening for prediabetes at a minimum of every 3 years in asymptomatic adults \geq 45 years of age who are overweight or obese (BMI \geq 25, or \geq 23 in Asian Americans) (ADA, 2017).

Individuals with prediabetes often present with dyslipidemia, hypertension, and abdominal obesity, conditions associated with the metabolic syndrome (Harris, 2013). The metabolic syndrome, also called "syndrome X", can be classified as a combination of risk factors for developing cardiovascular disease (CVD) and T2DM. The metabolic syndrome (MetSy) is typically indicated when an individual exhibits 3 or more of the following risk factors: abdominal obesity (waist circumference \geq 102 cm or 40 inches for men and \geq 88 cm or 35 inches for women), dyslipidemia indicated by elevated triglycerides (TG; \geq 1.7 mmol/L) and/or reduced high-density lipoprotein cholesterol (HDL-c; $<$ 1.0 mmol/L for men and $<$ 1.3 mmol/L for women), hypertension (HTN; \geq 130 mm Hg systolic or \geq 80 mm Hg diastolic blood pressure), and elevated FPG ($>$ 99 mg/dL or 5.5 mmol/L). The underlying pathophysiology of MetSy is thought to result from the combination of insulin resistance and abdominal obesity (Harris, 2013).

Other major risk factors for developing T2DM can be classified into two groups: non-modifiable and modifiable (ADA, 2017). Non-modifiable risk factors are typically inherent (beyond the control of the individual) and cannot be modified through pharmaceutical therapy or behavior change. Men, women with a history of gestational diabetes, individuals with a genetic predisposition and/or family history of T2DM, and those over the age of 40 years (even greater risk over the age of 60 years) are at the greatest risk of developing T2DM. Ethnicity has also been found to put individuals at greater risk, with Blacks, Native Americans, Asians, Hispanics, and Pacific Islanders demonstrating the greatest risk for T2DM. Modifiable risk factors are those factors that can be managed or reversed through lifestyle and behavior modifications. Lifestyle-related conditions such as non-alcoholic fatty liver disease (NAFLD), the MetSy, and morbid obesity (BMI \geq 40) can put individuals at risk for developing T2DM (ADA, 2017; Chalasani, 2012; Papatheodorou et al., 2015). Additionally, the use of some prescription medications, such as atypical antipsychotics, thiazide

diuretics, statin drugs, and glucocorticoids, are known to increase blood glucose concentrations, potentially putting the patient at risk for T2DM with use over time. However, the risk factors that remain under the greatest control of the individual are arguably consumption of a poor diet and lack of physical activity.

2.1.3 Obesity

Obesity is associated with the development of insulin resistance (IR) and is one of the major modifiable risk factors for T2DM (Kahn, Hull, & Utzschneider, 2006). In 2020, the prevalence of obesity in the United States was 41.9%, an increase from 30.5% in the year 2000, with an estimated \$173 billion in associated annual medical expenses in 2019 (Stierman et al., 2021). Factors such as genetics, epigenetics, and overnutrition via excess energy consumption and lack of physical activity have been shown to result in an excessive energy surplus resulting in obesity (Kahn, Hull, & Utzschneider, 2006; Longo et al., 2019). Metabolic abnormalities other than T2DM may result from obesity-induced IR, such as non-alcoholic fatty liver disease (NAFLD), hypertension, dyslipidemia, stroke, and coronary heart disease (Longo et al., 2019). A common clinical anthropometric measurement to assess obesity status is the body mass index (BMI), an index representative of the relationship between height and body weight (ADA, 2021). BMI classifications are as follows: 18-24.9, normal; 25-29.9, overweight; 30-34.9, obese class I; 35-39.9, obese class II; and ≥ 40 , obese class III or morbid obesity. A higher BMI is associated with greater risk for metabolic diseases, including T2DM. As BMI only utilizes height and body weight, this index may not accurately represent body composition in all individuals; therefore, measurement of body fat percentage (BF%) is recommended, when possible, to assess an individual's obesity status and risk of metabolic complications more accurately (Unamuno et al., 2018). It should be noted that not all obese individuals develop IR or metabolic comorbidities; the induction of IR and progression to T2DM appear to be more a consequence of the distribution of body fat than the quantity alone (Longo et al., 2019).

Previously considered to be an inert tissue mainly utilized for long-term energy storage, white adipose tissue (WAT) is now recognized as a dynamic endocrine organ participating in whole-

body metabolic homeostasis and active cellular reactions (Unamuno et al., 2018). Adipocytes have been shown to participate in glucose and lipid metabolism, the inflammatory response via secretion of adipokines, and appetite regulation. Prolonged positive energy balance can promote WAT dysfunction resulting in the inability to store excess energy, leading to “lipotoxicity” and ectopic lipid accumulation in organs responsible for glucose homeostasis (Longo et al., 2019). Excess energy intake can also result in either healthy or unhealthy adipocyte expansion. Subcutaneous adipose tissue (SAT) responds to excess energy intake through adipocyte hyperplasia, leading to healthy adipocyte expansion, increased insulin sensitivity, and decreased inflammation (Figure 1). When the storage capacity of SAT depots is limited, further excess energy intake leads to ectopic lipid accumulation in the liver, heart, and skeletal muscle, as well as around internal organs in visceral adipose tissue (VAT). Adipocytes associated with VAT tend to expand their storage capacity via hypertrophy, promoting complications associated with metabolic dysfunction such as increased secretion of proinflammatory adipokines, infiltration of macrophages, and decreased insulin sensitivity. Additionally, VAT is more lipolytic and less sensitive to insulin than SAT, releasing more non-esterified fatty acids (NEFAs) into circulation and potentially increasing exposure of the liver to NEFAs (Kahn, Hull, & Utzschneider, 2006). There is strong evidence of an association between central adiposity and metabolic diseases; therefore, clinical measurement of waist circumference (WC) is utilized to assess abdominal obesity and VAT mass (Longo et al., 2019).

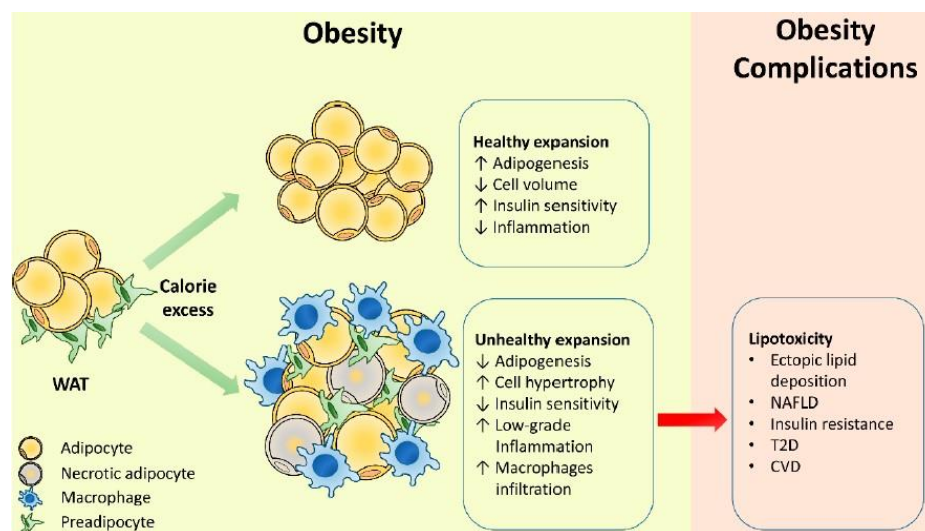


Figure 1. Healthy versus unhealthy adipocyte expansion in white adipose tissue (WAT). Source: Longo et al., 2019.

Although the exact mechanism by which excessive WAT leads to T2DM is unknown, it is proposed that excess NEFAs released by dysfunctional adipocytes result in a progressive decline in pancreatic β -cell functioning (Kahn, Hull, & Utzschneider, 2006). Increased circulating NEFA levels are commonly observed in individuals with obesity, IR, and T2DM, and may compete with glucose for substrate oxidation in peripheral tissues. Moreover, impairments in β -cell functioning can lead to decreased insulin secretion, causing multiple metabolic complications. First, decreased circulating insulin leads to increased blood glucose levels through decreased glucose uptake by peripheral tissues and increased glucose release from the liver. Second, low blood insulin concentrations can lead to impairments in fat cell metabolism, further increasing blood NEFA and glucose concentrations, leading to further β -cell destruction from the glucotoxic effects. And third, as insulin acts as a signaling agent in the hypothalamus regulating body weight, decreased insulin levels may lead to increased adiposity. Over time, this cyclic process can lead to impairments in blood glucose regulation, IR, and T2DM (Kahn, Hull, & Utzschneider, 2006).

The chronic low-grade inflammation typically observed in obesity is believed to lead to adipose tissue dysfunction and play a role in the development and progression of T2DM (Dandona, Aljada, & Bandyopadhyay, 2004; Longo et al., 2019). Increased plasma concentrations of the proinflammatory cytokines C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α), and interleukin-6 (IL-6) are associated with obesity and are predictive of the development of T2DM. Obesity's role in the development of IR, specifically, may be due to adipocyte- and macrophage-released TNF- α , IL-6, and monocyte chemoattractant protein-1 (MCP-1), as well as other products of increased macrophage recruitment to hypertrophic adipocytes (Kahn, Hull, & Utzschneider, 2006; Longo et al., 2019). Moreover, VAT may be more susceptible to infiltration by macrophages compared with SAT, leading to increased risk of inflammation and IR in individuals with excessive VAT (Unamuno et al., 2018). Another proposed mechanism suggests that oxidative stress caused by prolonged overnutrition may lead to a chronic proinflammatory state in the body (Dandona, Aljada, & Bandyopadhyay, 2004). Additionally, persistently elevated concentrations of TNF- α and IL-6 may suppress insulin signal transduction in peripheral tissues associated with glucose disposal, as well as interfere with insulin's anti-inflammatory effects, therefore promoting more

inflammation. Genetic predisposition may also play a role, as promoter polymorphisms in the genes for TNF- α and IL-6 have been observed to double the risk of IR in obese individuals with impaired glucose tolerance. Additionally, elevated plasma CRP, an acute-phase protein, and IL-6 concentrations greatly increase the risk for atherosclerosis, an inherently inflammatory disease that leads to CVD, the major cause of death for populations afflicted with obesity, IR, and T2DM (Dandona, Aljada, & Bandyopadhyay, 2004).

The inflammatory process can be identified and monitored through clinical measurement of proinflammatory cytokines in the blood (Pearson et al., 2003). Plasma high-sensitivity CRP (hs-CRP) concentration is associated with CVD risk, body weight, MetSy, and fasting insulin concentrations, indicating a possible relationship between inflammation and IR (Dandona, Aljada, & Bandyopadhyay, 2004; Pearson et al., 2003). Current evidence supports the use of hs-CRP as an indicator for CVD risk, with classification of risk as follows: <1.0mg/L, low risk; 1.0-3.0 mg/L, average risk; >3.0 mg/L, high risk) (Pearson et al., 2003). Incidentally, weight loss and increased activity or endurance exercise are associated with decreases in hs-CRP levels. Plasma IL-6 concentrations show similar correlations to the risk of CVD events as hs-CRP and are associated with obesity-induced chronic inflammation (Dandona, Aljada, & Bandyopadhyay, 2004; Pearson et al., 2003). Plasma TNF- α concentrations have been found to be significantly correlated with BMI and observed to decrease following weight loss from dietary restriction (Dandona, Aljada, & Bandyopadhyay, 2004). Obesity and chronic, low-grade inflammation, therefore, are clinical parameters that should be assessed by healthcare providers to establish the risk for and progression of T2DM, and associated complications, in at-risk populations.

2.1.4 Complications

Complications of T2DM are typically associated with a long-term glycemic burden on organs and tissues and can develop even in the undiagnosed (Papatheodorou et al., 2015). The overabundance of reactive oxygen species (ROS), creating an environment afflicted with oxidative stress and inflammation, and chronic hyperglycemia paired with deficient insulin secretion can lead to excessive glycation phenomena that damage tissues and organs. Microvascular complications

arise when damage is incurred by the delicate tissues of the nervous and vascular systems. These complications include neuropathy, nephropathy, and retinopathy which can lead to blindness. Macrovascular complications result from damage to the veins and arteries of the vascular system and can lead to atherosclerosis, CVD, and heart failure. Other complications associated with T2DM include postprandial dyslipidemia, diabetic foot ulcers with the possibility of amputations, poorly controlled infections, and increased risk of cancer (Papatheodorou et al., 2015).

2.1.5 Treatment

Currently, there is no cure for diabetes; therefore, individuals diagnosed with T2DM are typically prescribed multiple medications for the management of blood glucose and comorbidities (Kirkman et al., 2015). Metformin, a biguanide commonly utilized as the initial prescription treatment for the management of T2DM, is an inexpensive treatment with demonstrated effectiveness in reducing diabetes-related complications that has been labeled safe by the Food and Drug Administration of the United States (FDA). For patients with severe hyperglycemia, combination therapies, which include prescription medications plus insulin therapy, are typically prescribed to manage blood glucose and insulin concentrations. As an adjunct to metformin or other anti-diabetic medications, basal insulins can be administered to maintain euglycemia between meals and overnight, prandial insulins can be administered to control blood glucose levels at mealtimes, or premixed insulins, a combination of both basal and prandial insulins, can be utilized to control blood glucose concentrations. Unfortunately, there are several drawbacks to utilizing pharmacological treatments for T2DM. First, the cost of anti-diabetic medications and insulin therapies are continually on the rise and may decrease accessibility for low-income individuals or those on a fixed income, such as the elderly. Second, self-administered insulin injections increase the risk of improper dosing, which can increase hypoglycemic episodes, and older or disabled patients may have trouble administering the medication alone. Third, the continuously expanding list of anti-diabetic medications comes with a flood of concerns regarding their safety and efficacy and questions as to whether the risks are worth the benefits (Gionfriddo et al., 2014). Lastly, there is a wide range of adherence to oral anti-diabetic medications (from 36-93%) and injectable insulin

therapies (~ 63%), demonstrating the difficulty some individuals may experience managing their blood glucose concentrations through pharmaceutical means (Kirkman et al., 2015).

Although a plethora of pharmaceutical medications exists to treat the symptoms of T2DM and prevent its complications, lifestyle modifications remain the foundation of T2DM management and first line of defense against progression of the disease (IDF, 2021). Smoking cessation, maintaining a healthy body weight, regular physical activity, and eating a healthy, nutritious diet remain the top recommendations for both prevention and management of T2DM.

2.2 Diet Composition and Type 2 Diabetes Mellitus

There is strong evidence that moderate, sustained weight loss can be beneficial in both treating T2DM and preventing the progression of prediabetes to T2DM (ADA, 2021). More specifically, obesity management through dietary energy restriction has been shown to significantly reduce fasting blood glucose concentrations, improve glycemic control, reduce HbA1c, and reduce the need for anti-diabetic medications. Current trends in chronic disease research favor studying the effects of dietary patterns versus the effects of single nutrients alone, with several dietary patterns showing promise for the prevention and management of T2DM (Georgoulis, Kontogianni, & Yiannakouris, 2014). A systematic review and meta-analysis by Ajala, English & Pinkney (2013) found that high-protein, Mediterranean, low-carbohydrate, and low-glycemic index dietary patterns each significantly lowered HbA1c by 0.12-0.5% in diabetics, comparable to reductions observed with pharmaceutical treatment. A clinical trial conducted in 2002, the Diabetes Prevention Program (DPP) study, provided evidence that prevention of T2DM in prediabetics was significantly more effective with multifactorial lifestyle interventions (weight loss, increased physical activity, and improved diet) than treatment with metformin (Knowler et al., 2002). A randomized clinical trial (RCT) with a 6-year follow-up period in Da Qing, China conducted by Pan et al. (1997) demonstrated that diet, exercise, or combined diet and exercise protocols lead to significant reductions in the development of non-insulin dependent diabetes mellitus (NIDDM) in individuals with impaired glucose tolerance. These results demonstrate the potential impact of dietary pattern and physical activity on the management and prevention of T2DM without the high cost or risk of

side effects typically associated with pharmaceutical medications. The Mediterranean diet, a plant-based dietary pattern, is specifically celebrated for its association with low incidences of CVD and reduced all cause- and disease-related mortality (Georgoulis, Kontogianni, & Yiannakouris, 2014).

2.2.1 The Mediterranean Diet

Dietary patterns of populations living amongst the regions that border the Mediterranean Sea, mainly in Greece and Southern Italy, in the late 1950s and early 1960s are collectively known as the “Mediterranean diet” (Trichopoulou et al., 2014). This pattern of eating varied across the diverse cultures of the region, influenced by the local climate, agriculture, and economic hardship the countries were experiencing at the time, causing expensive meats, such as red meat, to be scarce. The Seven Countries Study conducted by Ancel Keys and colleagues in the 1950s brought the Mediterranean diet into the forefront after demonstrating a correlation between the low saturated fat intake in Mediterranean regions and the extremely low incidence of CVD (Trichopoulou et al., 2014; Georgoulis, Kontogianni, & Yiannakouris, 2014). Unfortunately, the rising influence of the “Western-style” diet, high in saturated fats and simple carbohydrates, and fast food during the past 50 years has led to increases in obesity and adverse health outcomes in these communities.

There has been much debate over the exact definition of the Mediterranean diet due to the diversity of the cultures residing in the Mediterranean region (Trichopoulou et al., 2014; Georgoulis, Kontogianni, & Yiannakouris, 2014). Because of this, a modernized Mediterranean diet, or Mediterranean-style diet (MSD), provides researchers and individuals with a basic framework of the traditional Mediterranean diet as a general guideline to follow. In an MSD, fruits, vegetables, whole grains, legumes, nuts, and olive oil are consumed in high amounts, while fish, poultry, red wine, and fermented dairy products are consumed in more moderate amounts. High-fat meats, other than fish, non-fermented dairy products, and foods with added sugars are consumed in low amounts (Trichopoulou et al., 2014; Salas-Salvadó et al., 2016; Georgoulis, Kontogianni, & Yiannakouris, 2014). The MSD shares similarities to other dietary patterns considered beneficial to overall health in that it is plant-based, with high intakes of fruits and vegetables, nuts, and legumes,

and contains low intakes of red meat, ultra-processed foods, and simple carbohydrates. An MSD is unique among diets with proposed health benefits in that it is considered a moderate- to high-fat diet, with intakes of approximately 30% and 40% of total energy in Italy and Greece, respectively, mostly due to the liberal use of olive oil. However, as most fats in the diet result from high amounts of olive oil, fatty fish, and tree nuts, the ratio of monounsaturated fatty acids (MUFAs) to saturated fatty acids (SFAs) tends to be high, a metric shown to be beneficial for cardiovascular health (Salas-Salvadó et al., 2016). Additionally unique to the MSD is the moderate consumption of alcohol, mostly in the form of red wine consumed with meals (Trichopoulou et al., 2014; Salas-Salvadó et al., 2016; Georgoulis, Kontogianni, & Yiannakouris, 2014).

The MSD is a nutrient-dense dietary pattern containing many bioactive constituents that work independently and synergistically to provide beneficial impacts to the various metabolic pathways of the human body (Trichopoulou et al., 2014; Salas-Salvadó et al., 2016). Diets high in SFAs have been shown to increase levels of triglycerides and low-density lipoprotein cholesterol (LDL-c) in the blood, increasing the risk for development of CVD. Replacing SFAs with MUFAs, as in an MSD, has been shown to decrease the risk for developing CVD, reduce central adiposity, and improve insulin sensitivity. Additionally, the amounts of essential alpha-linolenic acid provided in an MSD may be key to promoting health and lowering the risk of CVD. The MSD is rich in both soluble fibers (from fruits and vegetables) and insoluble fibers (from whole grains). High intake of dietary fibers is associated with a lower incidence of T2DM, likely from the delay in gastric emptying which reduces postprandial glycemic and insulin responses and improves insulin sensitivity. The reduced risk of insulin resistance and T2DM associated with an MSD may also be related to its low-glycemic index. The abundance of calcium, potassium, and magnesium paired with low sodium levels in the MSD may be protective against T2DM through optimizing intracellular glucose metabolism and improving insulin sensitivity. Olive oil and nuts contain high amounts of sterols, or phytosterols, compounds structurally similar to animal-derived cholesterol, which can lower blood cholesterol levels by inhibiting absorption of dietary cholesterol in the lumen of the small intestine. The high polyphenol content of the foods in the MSD (such as vegetables, seeds, fruits, red wine, and spices) provide potent antioxidant effects, and may even improve fasting glucose levels, reduce

postprandial glycemic response, reduce adiposity, provide anti-inflammatory benefits, improve lipid profiles, and reduce blood pressure. Interestingly, one of the major components of an MSD responsible for decreasing overall mortality appears to be moderate alcohol consumption. There is epidemiological evidence associating alcohol intake, of any kind, to increased HDL-c levels which are inversely correlated with CVD risk. Additionally, cohort studies have demonstrated the possible protective effects of moderate alcohol intake against the development of T2DM. Even though moderate intake of any type of alcohol confers these benefits, red wine and beer appear to be more protective against CVD and T2DM due to their polyphenol content (Trichopoulou et al., 2014; Salas-Salvadó et al., 2016).

Numerous epidemiological studies investigating the health of communities consuming an MSD have found strong associations with a low incidence of CVD, various cancers, T2DM, and decreased all-cause mortality (Trichopoulou et al., 2014). Randomized controlled trials (RCTs) have provided strong evidence for the ability of an MSD to provide vascular protection and improve CVD outcomes. Additionally, the protective effects of an MSD on the MetSy and T2DM have been extensively studied (Trichopoulou et al., 2014; Salas-Salvadó et al., 2016). A meta-analysis of 20 RCTs studying the effects of various diets (low-glycemic, high-protein, low-carbohydrate, and MSDs) showed significant improvements in glycemic control for all diets compared to control diets with the MSDs providing the largest effect compared with low-fat and control diets (Georgoulis, Kontogianni, & Yiannakouris, 2014). A few RCTs studying liver complications in T2DM patients have also demonstrated the ability of an MSD to significantly decrease liver transaminases in the blood, increase insulin sensitivity, and reduce the progression of NALFD to hepatic steatosis. Proposed mechanisms for the beneficial effects of MSDs suggest that there are both direct effects, through consumption of various beneficial nutrients that support metabolic health, and indirect effects, through the reduction of excess body weight. Furthermore, it has been found that there is a decreased likelihood of obesity and central adiposity in individuals who adhere to an MSD (Georgoulis, Kontogianni, & Yiannakouris, 2014).

2.2.2 Whey Protein Supplementation

There is growing interest in the possible insulinotropic and appetite-suppressing effects of dietary whey protein for individuals with hyperglycemia and T2DM (Adams & Broughton, 2016; Jakubowicz & Froy, 2013). Milk contains two major protein fractions that are separated during the cheese making process – soluble whey protein and insoluble casein protein, which precipitates at the isoelectric point (pI) of 4.6. Whey protein is a good source of all nine essential amino acids, classifying it as a “complete” protein, and is highly digestible and absorbable in the small intestine, making it a high-quality protein for humans and animals. It also contains a high amount of the branched-chain amino acids (BCAAs) valine, leucine, and isoleucine, which are known to exert signaling properties that impact various metabolic processes. Commercial whey protein as a dietary supplement is available in several forms including whey protein isolate (WPI), whey protein concentrate (WPC), and whey protein hydrolysates (WPHs) which have varying effects on glycemic control. Following hydrolysis by proteases located in the stomach and the proximal small intestine, individual amino acids and bioactive di- and tripeptides are liberated and absorbed. Whey is soluble and considered a “fast protein” as it typically escapes degradation by gastric proteases, quickly emptying into the intestinal lumen where it is then hydrolyzed and absorbed. This contrasts with casein, a “slow protein”, which is insoluble and precipitates when exposed to the acidic environment of the stomach, exposing it to gastric proteases and slowing gastric emptying. Whey protein’s fast rate of absorption has been shown to promote anabolism and have rapid effects on glucose metabolism (Adams & Broughton, 2016; Jakubowicz & Froy, 2013).

Whey protein supplementation has been demonstrated to prevent postprandial hyperglycemia and improve insulin sensitivity in individuals with T2DM with similar efficacy to anti-diabetic medications (Adams & Broughton, 2016; Jakubowicz & Froy, 2013). Whey protein extends significant insulinotropic effects when consumed both as a preload or during a meal, although the mechanisms by which it exerts these effects are not well understood. However, there are several mechanisms that have been proposed. First, increased gastric emptying following consumption of whey protein may lead to a rapid rise in amino acids, such as leucine, which promote secretion of insulin from the pancreas. As postprandial glycemia is important to blood glucose control and

management of T2DM, consuming whey protein as either a pre-meal load or during a meal may promote quicker secretion of insulin compared with other dietary proteins or meals devoid of protein. Second, the release of the incretin hormones gastric inhibitory peptide (GIP) and glucagon-like polypeptide-1 (GLP-1) may be stimulated by the bioactive amino acids and polypeptides of hydrolyzed whey protein. GIP acts to promote insulin secretion and has been found to be diminished in individuals with T2DM. GLP-1 is responsible for slowing gastric emptying, to slow carbohydrate absorption, and is not typically altered in T2DM. WPHs in the intestinal lumen also inhibit the action of dipeptidyl peptidase-IV (DPP-4) known to degrade and inactivate GIP and GLP-1. Positive correlations have been found between DPP-4 levels and HbA1c, although the exact mechanism is unclear. *In vitro* studies have shown WPHs to significantly reduce DPP-4 action when applied as a singular treatment and to increase the effectiveness of pharmaceutical DPP-4 inhibitors when utilized as a combined therapy. Whey protein supplementation may be beneficial in combating obesity as well by increasing postprandial thermogenesis and promoting satiety, through increased cholecystokinin (CCK) and peptide YY (PYY) release and suppression of ghrelin (Adams & Broughton, 2016; Jakubowicz & Froy, 2013).

A recent systematic review and meta-analysis of 22 RCTs investigating the effects of whey protein supplements on glycemic control in participants with the MetSy or associated conditions, including T2DM, found significant improvements in lipid profiles and significant reductions in HbA1c and homeostasis model assessment-estimated insulin resistance (HOMA-IR), with no effect on FPG (Amirani et al., 2020). The types of whey protein and dosages used in these studies varied widely, with the dosages ranging from 70 mg/d to 90 g/d. Whey protein supplements show promise as a safe and lower cost alternative to pharmaceutical therapies for the management of hyperglycemia in individuals with prediabetes or T2DM; however, more research is needed to establish the optimal delivery form, dose, and timing of intake.

2.3 Physical Activity and Type 2 Diabetes Mellitus

Physical activity has been shown to improve overall health and quality of life, as well as reduce the risk for developing chronic diseases in individuals of all ages (USDHHS, 2018). It is

well-established that physical activity provides acute and long-term health benefits, including improvements to overall energy, insulin sensitivity, sleep, activities of daily living, cognitive function, and overall wellbeing. However, recent studies indicate that only 19% of American women and 26% of American men perform enough physical activity to maintain optimal health. Alarming, the rise in sedentary behavior among Americans poses substantial impacts to public health due to a higher likelihood of CVD and all-cause mortality. Risks for conditions such as T2DM, CVD, obesity, hypertension, anxiety, depression, and Alzheimer's disease start to decline when an individual begins to incorporate even small amounts of physical activity into their daily life (USDHHS, 2018).

For significant improvements to overall health, the *Physical Activity Guidelines for Americans*, issued by the United States Department of Health and Human Services, recommend that adults perform aerobic exercise of moderate intensity for 150-300 minutes per week, or of vigorous intensity for 75-150 minutes per week, preferably dispersed throughout the weekdays (USDHHS, 2018). Physical activity beyond these recommendations, of at least moderate intensity, provides additional health benefits. Resistance training that includes all major muscle groups should also be performed at moderate or greater intensity for at least 2 days per week to maintain muscle mass and preserve bone density. Individuals with chronic health conditions are also recommended to follow these physical activity guidelines, albeit under supervision of a health care provider. Individuals with T2DM, specifically, can gain several benefits from incorporating physical activity into their daily lifestyle (USDHHS, 2018).

The American Heart Association (AHA) and the American Diabetes Association (ADA) independently, both recommend moderate- to vigorous-intensity physical activity, as outlined previously, for patients with T2DM to improve glycemic control, reduce the risk of CVD, maintain a healthy weight, and promote longevity (Buse et al., 2007). A prospective study and meta-analysis found that the risk of death in individuals with diabetes was greatly reduced with regular physical activity compared with inactive individuals (Sluik et al., 2012). A randomized crossover study conducted in 32 overweight to obese individuals with T2DM showed that a single session of 60-minute cycling exercise was able to significantly reduce the incidence of hyperglycemia for up to 48 hours post-exercise, compared with performing no exercise (van Dijk et al., 2012). The study

found no additional benefit to exercising daily and concluded that exercising every other day was sufficient to reduce hyperglycemia in T2DM. Lifestyle modification (including a low-fat, energy restricted diet and moderate-intensity exercise) sufficient to induce a loss of 7% of body weight was found to prevent the progression to T2DM in prediabetic individuals and was significantly more effective than treatment with metformin alone (Knowler et al., 2002). Overall, the current evidence suggests that increases in physical activity may not only prevent T2DM but also improve outcomes related to T2DM.

MATERIALS AND METHODS

3.1 Participants

Beginning in March 2019, individuals living in San Luis Obispo County, California were recruited to participate in Cal Poly's Nutrition and Exercise in Type 2 Diabetes (CPNET) study. E-mail advertisements and flyers were distributed to students, faculty, and staff at California Polytechnic State University in San Luis Obispo (Cal Poly), and advertisements were published in local newspapers, hospitals, and clinics. Eligible participants were men and women between the ages of 18 to 65 years with either a medical physician's diagnosis of T2DM, indicators of prediabetes, or who were metabolically healthy (indicated by fasting plasma glucose [FPG], hemoglobin A1C [HbA1C], and fasting plasma insulin [FPI] values within the normal physiological range). Participants were excluded if they were current smokers, had abnormally high blood pressure (systolic > 130 mm Hg, diastolic > 80 mm Hg), had received a diagnosis of a chronic medical condition other than T2DM, or were taking medications used to treat a significant health condition other than T2DM. Prior to beginning the study, each participant completed a health screening questionnaire via telephone with a member of the research team where they were required to confirm that they had been cleared for exercise by their physician. Any individual that could not confirm medical clearance for exercise was excluded from the study. Twenty-two adults (12 female, 10 male) were determined eligible to participate and provided written informed consent. All experimental procedures were approved by the Institutional Review Board for Human Subjects Research at Cal Poly (Protocol Number: 2018-131).

3.2 Experimental Protocol

All data for the CPNET pilot study were collected at two time points: at baseline and following the 16-week intervention period. Prior to beginning the study, each participant met with a member of the research team and was provided with a personalized nutrition plan based on a Mediterranean-style diet (MSD; guidelines presented in Appendix A) to be consumed for 16 weeks. The Elizabeth Stewart Hands and Associates (ESHA) software was utilized to calculate the daily

estimated energy requirement (EER) for each participant. If weight loss was a goal of the participant, the EER was reduced by 500 kcal per day. Additionally, participants were provided a whey protein powder supplement (supplied by Glanbia Nutritionals) with a shaker cup and instructed to consume 25 g per day of whey protein powder in 12 ounces of skim milk, almond milk, or water immediately following a bout of exercise. On days where no exercise was performed, the whey protein drink was to be consumed as a morning or afternoon snack.

During the intervention, participants were encouraged to adhere to the *Physical Activity Guidelines for Americans* (USDHHS, 2018). The participants were encouraged to perform aerobic exercise of moderate intensity for 150-300 minutes per week, or of vigorous intensity for 75-150 minutes per week, preferably dispersed throughout the weekdays. Additionally, resistance training involving all major muscle groups was encouraged to be performed at moderate or greater intensity for at least 2 days per week. Participants were instructed to perform 50% of their physical activity as aerobic exercise (e.g., running, cycling), and the remaining 50% as resistance training (e.g., weightlifting, yoga). Examples of light-intensity exercise included: walking, golfing, and stretching. Examples of moderate-intensity exercise included: carrying light loads, cycling at a regular pace, or doubles tennis. Examples of vigorous-intensity exercise included: lifting heavy weights, digging, aerobic exercise, running, or cycling at a fast pace. To assess physical activity status, each participant completed the International Physical Activity Questionnaire (IPAQ) at baseline and at week 16 (data not shown).

3.3 Analyses of Parameters of Glycemic Control and Systemic Inflammation

Following a 10-12 hour overnight fast, morning blood samples were collected from eligible participants at Pacific Diagnostic Lab in San Luis Obispo, California. Blood samples were drawn by venipuncture in the antecubital fossa by a licensed phlebotomist and analyzed for fasting plasma levels of glucose and insulin, and fasting serum levels of HbA1c, high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α). Due to technical issues, the laboratory was unable to calculate fasting plasma insulin (FPI) and TNF- α values for one female

participant in the control group at baseline, and the TNF- α value for one male participant in the prediabetic group post-intervention.

Participants were assigned to one of three groups based on FPG and HbA1c values following the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) guidelines for the classification of diabetes based on glycemic control (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], 2022) (Table 1): (1) the metabolically healthy group (control; n = 10); (2) the prediabetes group (preDM; n = 5); or (3) the type 2 diabetes mellitus group (T2DM; n = 7). Participants were characterized at baseline based on age, sex, and body mass index (BMI; Table 2).

Table 2. Characteristics of the study participants at baseline.

	Group			<i>p</i> -value
	Control n = 10	PreDM n = 5	T2DM n = 7	
Age (years) [†]	44.6 ± 10.3	51.8 ± 11.5	46.9 ± 14.8	0.565
Sex (% female)	60	40	57.1	0.781
Body Mass Index (BMI) [†]	28.6 ± 5.86	30.7 ± 6.46	31.6 ± 5.30	0.569

[†]Data presented as mean ± standard deviation (SD) obtained from one-way between-groups ANOVA with post-hoc Tukey HSD test.

3.4 Body Composition and Anthropometric Analyses

Following blood collection, body composition was analyzed via complete body scan using dual-energy X-ray absorptiometry (DXA) at Cal Poly's Food Science and Nutrition Department's Nutrition and Health Assessment Laboratory. Prior to the DXA scan, participants were provided with an appropriate DXA information sheet. All female participants were required to take a pregnancy test upon arrival to ensure negative pregnancy results prior to the scan. Complete body scans were obtained with the Lunar iDXA system (General Electric Company). Participants were apprised of their scan results and provided with a copy of the results/data. All DXA scans were performed by the same licensed technician following phantom spine calibration to ensure optimal performance of the instrument. Data collected from DXA scans utilized in this analysis include body fat percent region (BF%) and visceral adipose tissue mass (VAT, pounds [lbs]).

Anthropometrics for height (inches) and body weight (lbs) were measured with a beam scale and a stadiometer, and waist circumference (WC, inches) was measured using a standard, non-stretch measuring tape. Body mass index (BMI) was calculated using the formula $BMI = \frac{Weight(lbs) \times 703}{[height(inches)]^2}$.

3.5 Dietary Intake and Nutritional Analyses

Participants were provided with instructions for recording their dietary intake via a standardized 3-day food record (data not shown; an example record is presented in Appendix B) within one week of blood collection and DXA scan. Participants were instructed to document two weekdays and one weekend day to obtain a 3-day average of their typical eating habits. Data from the 3-day food records for all 22 participants were analyzed for average energy intake and macronutrient composition utilizing ESHA software (data not shown). An ESHA profile was created for each participant, which included age, sex, anthropometric measures, and physical activity level. ESHA software analyses for nutrient composition were conducted using the United States Department of Agriculture (USDA) Standard Reference database. Three-day food records were collected again following the 16-week intervention and an analysis of dietary intake was performed (data not shown).

3.6 Statistical Analyses

To detect differences between the control, preDM, and T2DM groups at baseline and following the 16-week intervention, FPG, HbA1c, FPI, hs-CRP, IL-6, TNF- α , body weight, WC, BMI, BF%, and VAT were analyzed using one-way between-groups analysis of variance (ANOVA) in SPSS 27 (IBM Corp.). Post-hoc comparisons using the Tukey HSD test were performed to correct for multiple comparisons and to identify where significant differences between the groups occurred. To detect changes from baseline to week 16 within each group (control, preDM, and T2DM), FPG, HbA1c, FPI, hs-CRP, IL-6, TNF- α , body weight, WC, BMI, BF%, and VAT were analyzed using paired-samples t-tests in SPSS 27 (IBM Corp.). All data are presented as mean \pm standard deviation (SD) and results with a *p*-value of less than or equal to 0.05 were considered significant.

Chapter 4

RESULTS

Twenty-two individuals (12 female, 10 male; control n = 10, preDM n = 5, T2DM n = 7) began the 16-week intervention at staggered time-points from March 2019 to March 2020 and were characterized at baseline based on age, sex, and BMI (Table 2). Recruitment for the study was curtailed by COVID-19 public health-related state mandates, thus significantly reducing the number of individuals able to participate in and complete the study. Nine individuals (4 from the control group, 2 from the preDM group, and 3 from the T2DM group) discontinued participation due to a variety of reasons, including COVID-19 restrictions (Figure 2). Thirteen participants (7 female, 6 male; control n = 6, preDM n = 3, T2DM n = 4) completed the 16-week intervention and returned for the final data collection.

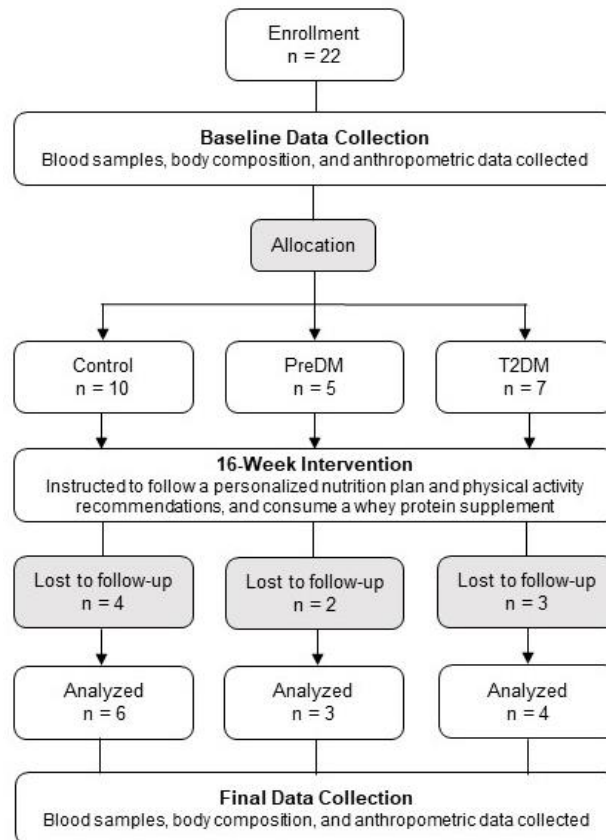


Figure 2. Flow-chart of the CPNET study. Includes participants enrolled at baseline (n = 22) and analyzed following the 16-week intervention (n = 13). The personalized nutrition plans were based on a Mediterranean-style diet (MSD). Physical activity recommendations were based on the *Physical Activity Guidelines for Americans*.

4.1 Parameters of Glycemic Control

At baseline, significant differences in fasting plasma glucose (FPG) were observed between the control and preDM groups ($p = 0.047$), and between the control and T2DM groups ($p < 0.0001$; Table 3; Figure 3A). These data confirm the allocation of participants into appropriate groups at baseline. Following the 16-week intervention, FPG was reduced in preDM and T2DM to the point where no significant differences persisted between any of the groups ($p > 0.05$). A decrease in FPG to normal levels within the PreDM group indicative of a trend with large effect size was observed (pre: 106.0 ± 9.11 mg/dL, post: 94.0 ± 7.55 mg/dL; $p = 0.065$, Cohen's $d = 2.144$), and the T2DM group showed a non-significant decrease in mean FPG to prediabetic levels (pre: 126.1 ± 26.98 mg/dL, post: 106.5 ± 31.17 mg/dL; $p = 0.232$). The results suggest that long-term adherence to the combined intervention tended to positively affect blood glucose regulation in those with preDM and T2DM.

Table 3. Parameters of glycemic control at baseline and the week 16 follow-up. †

		Group		
		Control	PreDM	T2DM
Fasting plasma glucose (FPG; mg/dL)	Baseline:	82.6 ± 7.66^a	106.0 ± 9.11^b	126.1 ± 26.98^b
	Week 16:	81.7 ± 5.72	94.0 ± 7.55	106.5 ± 31.17
	p -value ¹ :	0.790	0.065	0.232
Hemoglobin A1c (HbA1c; %)	Baseline:	5.2 ± 0.29^a	5.7 ± 0.30^a	6.8 ± 1.20^b
	Week 16:	5.1 ± 0.34^A	$5.7 \pm 0.06^{A,B}$	6.0 ± 0.75^B
	p -value ¹ :	0.224	1.000	0.391
Fasting Plasma Insulin (FPI; mU/L)	Baseline:	8.4 ± 4.35	10.7 ± 5.86	$17.5 \pm 12.27^{\$}$
	Week 16:	5.4 ± 2.93	7.0 ± 4.59	10.8 ± 7.25
	p -value ¹ :	0.162	0.209	0.216

†Data presented as mean \pm standard deviation (SD).

Different lowercase letters indicate a significant difference of $p \leq 0.05$ between groups at baseline ($n = 22$) and different uppercase letters indicate a significant difference of $p \leq 0.05$ between groups at week 16 ($n = 13$), obtained from one-way between-groups ANOVA with post-hoc Tukey HSD test.

^{\\$} $p < 0.1$ compared with control group at baseline ($n = 22$).

¹Within-group comparison of mean values at baseline versus week 16, obtained from paired-samples t-test.

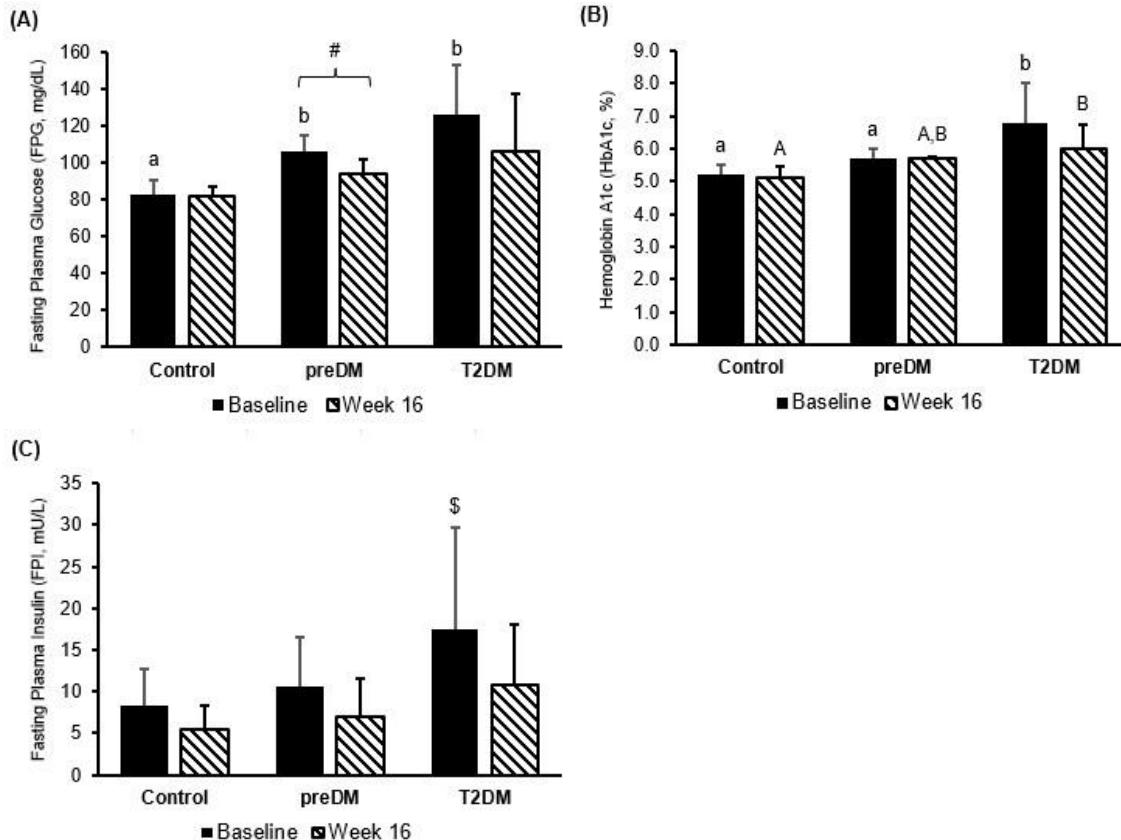


Figure 3. Parameters of glycemic control measured at baseline and following the 16-week intervention. (A) Significant differences in fasting plasma glucose (FPG) between the preDM and T2DM groups compared with control at baseline failed to persist following the intervention. A decrease in FPG indicative of a trend with large effect size was observed in the preDM group at week 16 ($p = 0.065$, Cohen's $d = 2.144$). (B) The T2DM group had significantly higher hemoglobin A1c (HbA1c) than the control and preDM groups at baseline and demonstrated a non-significant decrease to the prediabetic level following the intervention. (C) The T2DM group demonstrated a trend in higher fasting plasma insulin (FPI; $p = 0.093$) compared with control at baseline. At the week 16 follow-up, mean FPI in the T2DM group had decreased to levels comparable with the control and preDM groups. Different lowercase letters indicate a significant difference of $p \leq 0.05$ between groups at baseline ($n = 22$) and different uppercase letters indicate a significant difference of $p \leq 0.05$ between groups at the week 16 follow-up ($n = 13$) obtained from one-way between-groups ANOVA with post-hoc Tukey HSD test. $\$p < 0.1$ compared with control group at baseline ($n = 22$). $\#p < 0.1$ for within-group comparison of mean values at baseline versus week 16, obtained from paired-samples t-test. Data presented as mean \pm standard deviation (SD). preDM, prediabetes; T2DM, type 2 diabetes mellitus.

Significant differences in hemoglobin A1c (HbA1c) were observed between the control and T2DM groups ($p = 0.001$), and between the preDM and T2DM groups at baseline ($p = 0.044$; Table 3; Figure 3B), confirming initial allocation of participants into appropriate groups. Following the 16-week intervention, significant differences in HbA1c between the control and T2DM groups persisted

but were relatively less significant than at baseline ($p = 0.031$), and the mean HbA1c of the T2DM group had decreased to the point where there was no longer a significant difference between the preDM and T2DM groups post-intervention ($p > 0.05$). Although the decrease in mean HbA1c within the T2DM group was non-significant ($p = 0.391$), it was decreased from the diabetic to prediabetic level (pre: 6.8 ± 1.20 %, post: 6.0 ± 0.75 %). Thus, long-term adherence to the combined intervention may reduce the severity of glycation phenomena associated with T2DM.

There were no significant differences in mean fasting plasma insulin (FPI) levels observed between any groups at baseline; however, the T2DM group demonstrated a trend in higher FPI compared with control ($p = 0.093$; Table 3; Figure 3C). This trending difference between T2DM and control failed to persist at the week 16 follow-up due to a non-significant decrease in mean FPI within the T2DM group. Additionally, non-significant decreases in FPI were observed within all three groups from baseline to week 16 ($p > 0.05$). The results lend further support that the combined intervention may benefit glucose regulation through improvements in insulin sensitivity.

4.2 Systemic Inflammation

At baseline and the week 16 follow-up, there were no significant differences found between any of the groups for high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), or tumor necrosis factor-alpha (TNF- α ; Table 4; Figure 4). However, improvements in hs-CRP within the preDM group did suggest a possible benefit to the intervention, as the mean hs-CRP was reduced from levels indicating high risk for cardiovascular (CV) complications to average risk (pre: 3.6 ± 3.62 mg/L, post: 2.2 ± 3.02 mg/L; $p = 0.414$; Figure 4A). Although decreases in the mean hs-CRP were observed within the control and T2DM groups as well, the changes were non-significant and did not indicate improvements in risk for CV complications. Thus, the combined intervention was not able to significantly reduce inflammatory biomarkers within the time parameters of the study herein.

Table 4. Comparisons of inflammatory biomarkers at baseline and the week 16 follow-up. †

		Group		
		Control	PreDM	T2DM
High-sensitivity C-reactive protein (hs-CRP; mg/L)	Baseline:	2.4 ± 2.10	3.6 ± 3.62	5.7 ± 6.30
	Week 16:	1.5 ± 0.84	2.2 ± 3.02	4.0 ± 3.70
	<i>p</i> -value ¹ :	0.408	0.414	0.862
Interleukin-6 (IL-6; pg/mL)	Baseline:	1.8 ± 2.47	2.6 ± 1.74	3.8 ± 3.85
	Week 16:	1.2 ± 0.50	2.0 ± 1.39	4.1 ± 4.21
	<i>p</i> -value ¹ :	0.814	0.877	0.718
Tumor necrosis factor alpha (TNF-α; pg/mL)	Baseline:	1.0 ± 0.33	1.3 ± 0.19	1.4 ± 0.51
	Week 16:	1.2 ± 0.20	1.1 ± 0.28	1.3 ± 0.39
	<i>p</i> -value ¹ :	0.749	0.395	0.492

†Data presented as mean ± standard deviation (SD).

Different lowercase letters indicate a significant difference of $p \leq 0.05$ between groups at baseline ($n = 22$) and different uppercase letters indicate a significant difference of $p \leq 0.05$ between groups at week 16 ($n = 13$), obtained from one-way between-groups ANOVA with post-hoc Tukey HSD test.

¹Within-group comparison of mean values at baseline versus week 16, obtained from paired-samples t-test.

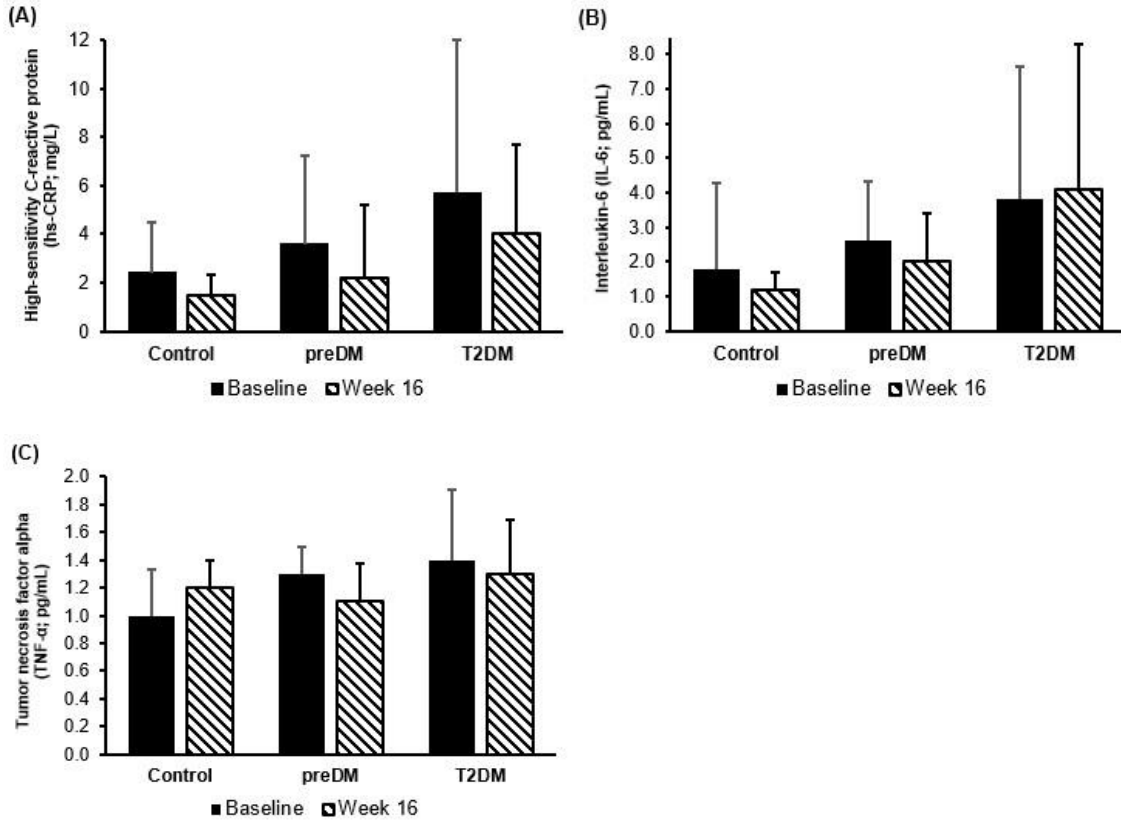


Figure 4. Inflammatory biomarkers measured in serum at baseline and following the 16-week intervention. No significant differences ($p > 0.05$) were observed between any groups at baseline or the week 16 follow-up, and no significant changes were observed within any groups due to the intervention for high-sensitivity C-reactive protein (A), interleukin-6 (B), or tumor necrosis factor-alpha (C). Comparisons between groups at baseline ($n = 22$) and between groups at the week 16 follow-up ($n = 13$) were obtained from one-way between-groups ANOVA with post-hoc Tukey HSD test. Within-group comparison of mean values at baseline versus week 16 were obtained from paired-samples t-test. Data presented as mean \pm standard deviation (SD). preDM, prediabetes; T2DM, type 2 diabetes mellitus.

4.3 Body Composition and Anthropometrics

At baseline and the week 16 follow-up, there were no significant differences found between any of the groups for body weight, waist circumference (WC), body mass index (BMI), body fat percent region (BF%), or visceral adipose tissue mass (VAT; Table 5; Figure 5). However, significant improvements were observed within the control group for several metrics of body composition. Following the intervention, mean body weight in the control group was significantly reduced from 191.7 ± 49.15 lbs to 167.1 ± 43.28 lbs with a large effect size ($p = 0.030$, Cohen's $d = 1.221$; Figure 5A), and mean BF% was significantly reduced from $36.6 \pm 6.46\%$ to $34.6 \pm 8.00\%$ with

a large effect size ($p = 0.003$, Cohen's $d = 2.204$; Figure 5D). Additionally, the control group demonstrated a significant decrease in mean BMI from 28.6 ± 5.86 to 26.2 ± 5.90 with a large effect size ($p = 0.043$, Cohen's $d = 1.104$; Figure 5C); however, classification based on BMI remained in the overweight category. The results provide evidence that long-term adherence to the combined intervention reduces adiposity in metabolically healthy, overweight adults, possibly preventing the development of metabolic dysfunction associated with T2DM.

Table 5. Comparisons of anthropometrics and body composition at baseline and the week 16 follow-up. [†]

		Group		
		Control	PreDM	T2DM
Body Weight (lbs)	Baseline:	191.7 ± 49.15	206.4 ± 41.31	198.9 ± 47.54
	Week 16:	167.1 ± 43.28	196.5 ± 40.82	171.8 ± 49.92
	p -value ¹ :	0.030	0.603	0.135
Waist Circumference (WC; inches)	Baseline:	39.8 ± 6.28	42.0 ± 7.89	42.7 ± 6.45
	Week 16:	37.2 ± 6.62	38.8 ± 8.01	37.4 ± 5.31
	p -value ¹ :	0.239	0.431	0.058
Body Mass Index (BMI)	Baseline:	28.6 ± 5.86	30.7 ± 6.46	31.6 ± 5.30
	Week 16:	26.2 ± 5.90	28.4 ± 5.19	28.6 ± 5.48
	p -value ¹ :	0.043	0.592	0.136
Body Fat (percent region, BF%)	Baseline:	36.6 ± 6.46	34.7 ± 8.40	38.4 ± 4.93
	Week 16:	34.6 ± 8.00	30.6 ± 7.73	36.3 ± 5.78
	p -value ¹ :	0.003	0.986	0.199
Visceral Adipose Tissue (VAT; mass, lbs)	Baseline:	2.5 ± 1.54	3.73 ± 2.49	4.35 ± 2.24
	Week 16:	1.83 ± 1.24	2.82 ± 2.92	2.99 ± 2.14
	p -value ¹ :	0.127	0.148	0.188

[†]Data presented as mean ± standard deviation (SD).

Different lowercase letters indicate a significant difference of $p \leq 0.05$ between groups at baseline ($n = 22$) and different uppercase letters indicate a significant difference of $p \leq 0.05$ between groups at week 16 ($n = 13$), obtained from one-way between-groups ANOVA with post-hoc Tukey HSD test.

¹Within-group comparison of mean values at baseline versus week 16, obtained from paired-samples t-test.

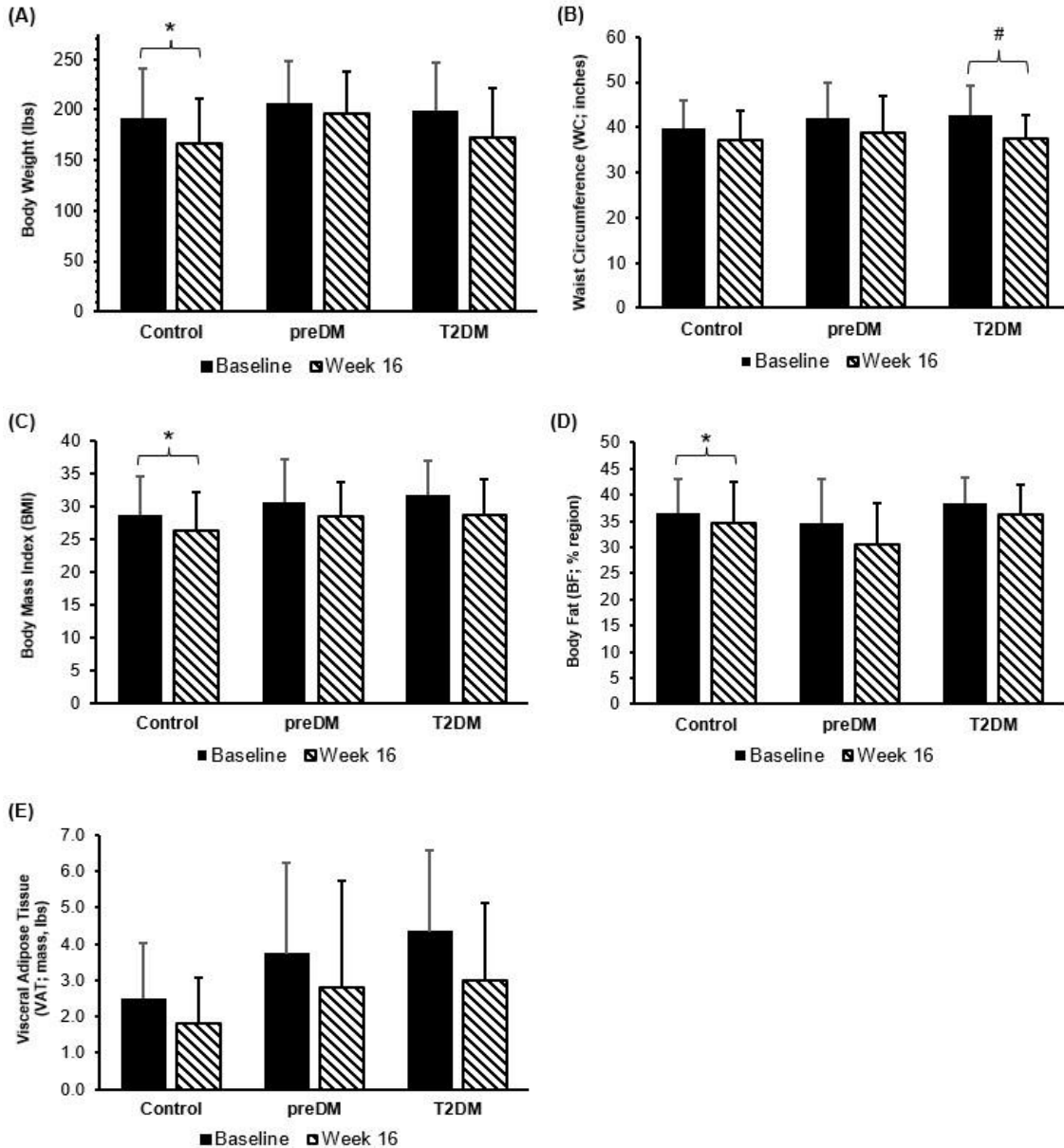


Figure 5. Measurements of anthropometric and body composition metrics at baseline and following the 16-week intervention. No significant differences were observed between any of the groups at baseline or the week 16 follow-up. Following the 16-week intervention, the control group exhibited a significant decrease with large effect sizes in body weight (A, $p = 0.030$, Cohen's $d = 1.221$), body mass index (C, $p = 0.043$, Cohen's $d = 1.104$), and body fat percentage (D, $p = 0.003$, Cohen's $d = 2.204$), and the T2DM group demonstrated a trend with large effect size in decreased waist circumference (B, $p = 0.058$, Cohen's $d = 1.500$). No significant changes were observed for any group for visceral adipose tissue (E, $p > 0.05$) following the 16-week intervention. Comparisons between groups at baseline ($n = 22$) and between groups at the week 16 follow-up ($n = 13$) were obtained from one-way between-groups ANOVA with post-hoc Tukey HSD test. $*p \leq 0.05$ and $\#p < 0.1$ for within-group comparison of mean values at baseline versus week 16, obtained from paired-samples t-test. Data presented as mean \pm standard deviation (SD). preDM, prediabetes; T2DM, type 2 diabetes mellitus; lbs, pounds.

In the preDM and T2DM groups, no significant changes to body weight, WC, BMI, BF%, or VAT were observed; however, slight improvements were found in each metric. In both groups, non-significant decreases were observed in mean body weight (Figure 5A), BF% (Figure 5D), and VAT (Figure 5E). Both groups also demonstrated non-significant decreases in mean BMI from the obese class I category to the overweight category (preDM: pre: 30.7 ± 6.46 , post: 28.4 ± 5.19 , $p = 0.592$; T2DM: pre: 31.6 ± 5.30 , post: 28.6 ± 5.48 , $p = 0.136$; Figure 5C). Additionally, the T2DM group demonstrated a trend in decreased mean WC from 42.7 ± 6.45 inches to 37.4 ± 5.31 inches with large effect size ($p = 0.058$, Cohen's $d = 1.500$; Figure 5B).

Chapter 5

DISCUSSION

Cal Poly's Nutrition and Exercise in Type 2 Diabetes (CPNET) study investigated the effects of lifestyle modification (as in diet and physical activity) on body composition and the regulation of glucose metabolism and systemic inflammation in overweight to obese healthy, prediabetic, and type 2 diabetic individuals. The combination of adherence to a Mediterranean-style diet (MSD), daily consumption of high-quality whey protein, and regular physical activity was associated with shifting the values of the assessed markers of glycemic control and insulin sensitivity of the T2DM group closer to that of the prediabetic group, while also reducing the prediabetic group's fasting plasma glucose to the normal range. Additionally, significant reductions to metrics of adiposity were observed in the metabolically healthy, overweight participants, while non-significant improvements were seen in all metrics of body composition measured in the study for the obese prediabetic and T2DM groups. These results imply a potential of the combined intervention to combat obesity, one of the major risk factors for T2DM. No improvements were observed in systemic inflammation; however, the prediabetic group did show a non-significant reduction in hs-CRP levels, potentially decreasing their risk for cardiovascular complications from high to average. Thus, our data suggest that consumption of an MSD and high-quality protein combined with regular physical activity may provide a strategy for effectively managing glucose metabolism in T2DM and mitigating major risk factors for T2DM in at-risk populations, potentially improving quality of life and increasing longevity.

Similar studies have been conducted to investigate the effectiveness of lifestyle modifications on the management and prevention of T2DM. The improvements to glycemic control observed in our study are consistent with findings in a systematic review and meta-analysis of studies investigating the effects of adherence to the Mediterranean diet (MD), compared with mainly low-fat control diets, conducted by Esposito et al. (2015). The meta-analysis found that the MD was effective at improving HbA1c and cardiovascular disease (CVD) risk factors in participants with T2DM, although the authors noted low availability of long-term randomized controlled trials (RCTs). Similarly, Huo et al. (2015) performed a meta-analysis of 9 RCTs assessing the impact of

an MSD versus control diet on glycemic control and body composition in participants with T2DM, concluding that an MSD is effective at reducing fasting glucose and insulin levels, HbA1c, body weight, and BMI, with additional improvements to lipid profiles. However, this meta-analysis was limited by variations in the control diets of the included studies and a low number of trials for some of the outcome variables. A network meta-analysis of 8 RCTs by Carter et al. (2014) also found that the MD was beneficial for reducing HbA1c in individuals with T2DM or at high-risk for T2DM and/or CVD, compared with usual care, but was not more effective than the Paleolithic diet. However, the researchers ultimately determined that the results were inconclusive as publication bias was not assessed due to the low number of available studies, narrow confidence intervals attributable to the method of network meta-analysis, and that several studies encouraged exercise and stress management in combination with the MD making it difficult to attribute the beneficial results to the dietary change alone. A meta-analysis of 16 RCTs by Esposito et al. (2011) investigating the effects of the MD on metrics of body composition found that the MD was effective at significantly reducing body weight and BMI, especially when consumed long-term, or when combined with energy restriction or exercise. High variability in the health status of the participants, however, limits the specificity of these findings. It is important to note that all the meta-analyses discussed herein mentioned a lack of homogeneity between the MD or MSD interventions utilized in the studies selected, although all of the intervention diets did contain the basic characteristics of the standard MD. It is important to note, however, that the way the MD is perceived in its originally derived regions (Greece and Southern Italy) is more of a lifestyle that, in addition to the dietary patterns, also includes physical activity, slow pace, afternoon rest, an attitude towards time that arguably renders less stress, and socialization with friends and family thus generating a solid support group.

A few observational studies have demonstrated a relationship between the consumption of an MD or MSD and improvements to glucose handling and risk factors for chronic diseases. A cross-sectional case-control study conducted by Murray et al. (2013) found that the diet of T2DM cases resembled that of a “Western-style” diet while non-T2DM controls had significantly higher Mediterranean diet (MD) scores and better overall quality of diet. This study was limited using 3-

day food records, which can lead to misreporting of dietary intake, small sample size, significant differences in BMI between the groups, and unbalanced ratios of men to women between cases and controls. Another observational study by Vitale et al. (2018) analyzed the baseline data from the Thiazolidinediones or Sulphonylureas and Cardiovascular Accidents Intervention Trial (TOSC.IT) in Italy. The study found associations between a high relative Mediterranean Diet score (rMED) score and improved glucose control, lower BMI, and lower prevalence of CVD risk factors in individuals with T2DM. Interestingly, the study found that the observed benefits corresponded to the MD pattern as a whole and not to individual nutrients contained in the MD.

Studies conducted in populations with prediabetes have found lifestyle modifications to provide a protective effect against the development of T2DM. The Diabetes Prevention Program (DPP) was a multicenter RCT conducted in the United States by Knowler et al. (2002) which assessed the effects of metformin treatment, lifestyle modification (a combination of a low-calorie, low-fat diet, 150 minutes of physical activity per week, and weight loss goal of 7% of body weight), or placebo on the incidence of T2DM in obese, prediabetic individuals. The study found that although metformin was effective at preventing T2DM, lifestyle modifications were significantly more effective than metformin alone; however, the study was unable to determine which aspects of the lifestyle modification were most effective at preventing T2DM. Pan et al. conducted an RCT in 1997 investigating the effects of improvements to diet alone (increased consumption of vegetables with less intake of simple sugars and alcohol), exercise alone, a combination of improved diet plus exercise, or no changes to diet or exercise on the development of non-insulin dependent diabetes mellitus (NIDDM) in participants with impaired glucose tolerance. The study found that NIDDM incidence was significantly lowered by either the diet or exercise interventions, with no additional benefit observed from combining diet and exercise. However, dietary change and energy restriction were not thoroughly recorded, interviewers were not masked, physical activity levels were not assessed at follow-up, and the participants were from a very specific region of China limiting the ability to generalize findings to broader populations. A systematic review and meta-analysis of 1 RCT, 9 prospective studies, and 7 cross-sectional studies performed by Kolooverou et al. (2014) evaluated the effects of the MD, specifically, on the incidence of T2DM,

concluding that the MD was effective at reducing T2DM incidence by 23%. However, a wide variation in the health status of the study participants, from apparently healthy individuals to individuals at high-risk for T2DM and/or CVD, contributing to high heterogeneity among the studies considered, and the inclusion of only one RCT limit the strength of these findings.

5.1 Strengths

A major strength of the CPNET study is the inclusion of individuals with prediabetes, an understudied population vital for developing strategies to combat the rise of T2DM diagnoses worldwide (Khan et al., 2019). Most studies conducted on diabetes thus far have included only T2DM populations; however, a few large studies targeting lifestyle-related factors such as diet and obesity, including the Diabetes Prevention Program (DPP), have demonstrated effective prevention of T2DM in prediabetic populations. Inclusion of healthy, prediabetic, and T2DM groups in the CPNET study allowed us to observe which groups specifically responded to the combined intervention and whether there was a progressive improvement in the study outcomes depending on initial metabolic status. Our results indicate that the combined intervention had the most significant effect on body composition in the metabolically healthy, overweight group. However, non-significant improvements in body composition did correspond with improvements to parameters of glycemic control in the prediabetic and T2DM groups. We also observed progressive improvement in glycemic control, as indicated by the reduction of fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) in the T2DM group to that of the prediabetic group, while FPG in the prediabetic group was decreased to normal levels following the intervention. These results support the inclusion of prediabetic individuals in T2DM research and suggest that improvement to lifestyle factors may provide a potential point of intervention to prevent T2DM in at-risk populations. In 2013-2016, 34.5% of the adult population of the United States was estimated to have prediabetes (CDC, 2020); therefore, focusing on prophylactic measures in the prediabetic population may provide a strategy for slowing the rate of T2DM incidence.

Other strengths of the CPNET study include addition of a high-quality whey protein supplement to the dietary intervention, to enhance muscle metabolism following exercise, and the

combination of long-term dietary and physical activity recommendations in the intervention protocol. Moreover, we were able to utilize well-established recommendations for improving multiple metrics of health in both healthy individuals and those diagnosed with chronic diseases by including the *Physical Activity Guidelines for Americans* in the CPNET study (USDHHS, 2018).

5.2 Limitations

The CPNET study was limited by the small sample sizes of the participant groups. This was mostly due to the constraints imposed by the global COVID-19 pandemic which limited recruitment, led to participant dropout during the intervention period, and resulted in early termination of recruitment for the study. Low response rates during recruitment may have also been due to the recruiting methods chosen for the study which mainly consisted of e-mails, flyer advertisements, and advertisements in local newspapers. Due to the resultant small sample sizes, our findings may be lacking in external validity and generalizability to the greater population. Additionally, low sample sizes can decrease the probability of identifying significant differences between the groups; however, this does strengthen the significant findings that were observed in our results. Furthermore, participant dropout during the study resulted in a lower number of participants completing the full intervention and returning for the week 16 measurements than began the study. Paired samples t-tests were utilized to detect changes from baseline to week 16 within each group; consequently, only participants that completed both pre- and post-assessments were able to be included in these analyses reducing the sample size even further. Therefore, due to the small sample sizes of the groups in our study, we are unable to conclude deterministic relationships between the combined treatment and outcome variables; rather our data are indicative of significant associations only.

Although 3-day food records were collected and analyzed for nutritional content at baseline, food records collected at week 16 were not analyzed. Analysis of 3-day food records at baseline and conclusion of the study, at minimum, would have provided a better indication of the actual intakes of participants during the study and would be more indicative of adherence to the study protocols. This also makes it difficult to discern which participants chose to implement a daily

caloric deficit and which did not. In addition, the participants were recommended to follow the *Physical Activity Guidelines for Americans* during the intervention period and an assessment of physical activity was performed at baseline and following the intervention; however, no analyses of these results were performed making it difficult to evaluate actual participant physical activity status during the intervention.

Other limitations include no stratification of data by sex, as there are typically differences in lean body mass, body fat percentage, and waist circumference between men and women, and the lack of data relating to duration of diabetes diagnosis. Additionally, markers for skeletal muscle metabolism (e.g., mitochondrial metabolites) were not measured as muscle biopsies were not obtained during the study. And lastly, utilizing high-sensitivity C-reactive protein (hs-CRP) as a marker of inflammation has its own constraints, as hs-CRP is an acute-phase protein which can have high within-person variability, thus requiring measurement at two separate time points to adequately reflect an individual's hs-CRP status (Pearson et al., 2003).

5.3 Future Directions

As the CPNET study had small sample sizes and did not measure adherence to the dietary protocols, we consider our study to be a preliminary pilot study demonstrating the potential of lifestyle modifications, specifically an MSD and increased physical activity, to manage and prevent T2DM. Future randomized, controlled trials that include larger sample sizes are necessary to increase statistical power and reveal deterministic relationships between modifications to diet and physical activity levels and parameters of T2DM. Therefore, these studies should focus on recruitment of participants with T2DM and prediabetes, as well as metabolically healthy individuals as a control. Additionally, inclusion of overweight and obese participants is crucial to explore how changes in various metrics of body composition affect glycemic control and levels of systemic inflammation. There is also a need to elucidate whether initially high levels of systemic inflammation in obese individuals with T2DM and prediabetes can be lessened by alterations to diet and physical activity and if this corresponds to improvements in glycemic control and insulin sensitivity. It will also be important for future studies to stratify participants based on sex, as waist circumference,

lean body mass, and body fat percentage and location typically vary between men and women. Future studies should also include muscle biopsies to measure skeletal muscle and mitochondrial proteins and metabolites, allowing observation of changes in muscle glucose and lipid metabolism following a long-term diet and physical activity intervention. It is also necessary to include validated measures of insulin resistance, such as homeostasis model assessment-estimated insulin resistance (HOMA-IR), to determine relationships between altered body composition and insulin sensitivity in the T2DM and prediabetic populations. And lastly, future studies should explore the effects of an MSD on the gut microbiota in obese T2DM and prediabetic populations to determine any causative links between altered body composition, impaired glycemic control, and gut health.

Chapter 6

CONCLUSION

The 16-week CPNET pilot study demonstrates the potential of a Mediterranean-style dietary pattern supplemented with whey protein and combined with regular physical activity to positively affect metrics of body composition, blood glucose regulation, and insulin sensitivity in obese individuals with prediabetes and type 2 diabetes mellitus (T2DM). The combined intervention was effective at decreasing markers of glycemic control in the prediabetic and T2DM participants, while having the most significant impact on body composition and adiposity in the metabolically healthy, overweight participants. Additionally, non-significant improvements to all utilized measures of body composition were observed in T2DM and prediabetic participants. These improvements may be beneficial to preventing and managing T2DM, and thus preventing the complications associated with T2DM. Our findings lend support to the use of diet and lifestyle modification recommendations by healthcare providers as a potential first line of defense against and as a treatment for T2DM.

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APPENDIX A

Dietary Guidelines for the Mediterranean-Style Diet

MEDITERRANEAN DIET
- GUIDELINES -

CARBOHYDRATES Whole Grains & Starchy Vegetables: 4-6 servings/day	PROTEIN Fish/Shellfish: 2-3 servings/week Poultry: 1-3 servings/week Red Meat: 4x/month	FAT Unsaturated fats: 4-6 servings/day
VEGETABLES Non-Starchy Vegetables: 4-8 servings/day	FRUIT All Fruit: 2-4 servings/day	DAIRY Low-Fat Dairy: 1-3 servings/day
NUTS & LEGUMES Nuts/Seeds/Legumes: 2-4 servings/day	SWEETS Eat sweets less often. Try fruit as dessert!	ALCOHOL Limit to one glass of wine a day or none at all!

SERVING SIZE

Whole Grains & Starchy Vegetables

1 slice whole wheat bread
½ cup potatoes, sweet potatoes, corn, peas
½ cup cooked oatmeal, quinoa, pasta,
brown rice, and barley

6 whole grain crackers
½ large whole grain bun
1 small whole grain roll

Protein

3 oz poultry, fish, shell fish and red meat
3 egg yolks per week (unlimited egg whites)

Fat

1 tsp. olive or canola oil
2 tsp light margarine
1 Tbsp of regular salad dressing
2 Tbsp of light salad dressing, made with oil

1 tsp regular mayonnaise
1/8 of an avocado
5 olives

Vegetables

1/2 cup cooked or 1 cup raw vegetables

Non-starchy vegetables include: artichoke, asparagus, beets, broccoli, brussels sprouts, cauliflower, cabbage, celery, carrots, tomatoes, eggplant, cucumber, onion, green beans, zucchini, turnips, peppers, salad greens and mushrooms

Fruit

1 small fresh fruit
½ cup juice or
¼ cup dried fruit

Dairy

1 cup of skim milk, non fat yogurt
1 oz of low fat cheese

Nut & Legumes

2 Tbsp. sunflower or sesame seeds
1 Tbsp. peanut butter
7-8 walnuts or pecans
20 peanuts
12-15 almonds

APPENDIX B

Example of 3-Day Food Record

In order for your diet analysis to be accurate, you must do the best job possible of accurately writing down what you eat.

- **We also encourage you to take pictures of meals and labels** because this can help our accuracy. Just email us the photos with info regarding when it was eaten.
- **Questions - Ask Dr. Reaves by text or email.**

Instructions for the Food Record:

- You will be recording everything you eat for three days. You will record **two weekdays** and **one weekend day**.

Tips for recording accurately:

- Record each individual food item plus any supplements you take. For example, if you ate oatmeal, fruit and coffee for breakfast, the meal would be represented as shown below:

Time of Day	Portion Size (amount)	Food Description including location/brand	Preparation Method
7:00 am Breakfast (B)	½ cup dry	Quaker Oats, old fashioned, dry	As instructed
B	2 teaspoons	Smart Balance light buttery spread with flax	
B	1 large	Egg	scrambled
B	1 cup	Lactaid milk, 1%	
B	1 tablespoon	Brown sugar	
B	½ cup	Blueberries, fresh	
B	1 cup	Brewed drip coffee	As instructed
B	1 tablespoon	Land O'Lakes Nonfat half and half	
B	1 tablet	Men's One-A-Day vitamin and mineral supplement	

Please notice all details about the oatmeal including added foods (buttery spread, milk, brown sugar), brand names if applicable (Quaker, Smart Balance, Land-o-Lakes), descriptions of the food products ("light," "1%") and specific amounts ("2 teaspoons," "1/2 cup") are provided. Please be as specific as possible ("fresh," "frozen," "blanched," "fried," "steamed") so proper assessment of the quality of your diet can be evaluated.

- If you don't know the exact portion, revert to the Food Portions Guide as a tool to estimate your portion size. Some examples are provided in the table below:

Time of Day	Portion Size (amount)	Food Description including location/brand	Preparation Method
8:00 pm Dinner (D)	1 deck of cards = 3 oz	Safeway Select Chicken breast, boneless skinless	Sautéed w/ canola oil and salt then finished in the oven
D	1 ½ quarters = ½ tablespoon	Canola oil	
D	¼ teaspoon	Salt	
D	1 fist full = 1 cup cooked	Brown rice, long-grain	As instructed

