THESIS

THE INFLUENCE OF DAPAGLIFLOZIN ON DIETARY MEDIATED PHYSIOLOGICAL AND BEHAVIORAL CHANGES

Submitted by

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ABSTRACT

THE INFLUENCE OF DAPAGLIFLOZIN ON DIETARY MEDIATED PHYSIOLOGICAL AND BEHAVIORAL CHANGES

The diabetes medication, Dapagliflozin, is a sodium-glucose co-transporter 2 (SGLT2) inhibitor. The mechanism of action is decreasing renal absorption of glucose, leading to glucosuria, and modest weight loss. We hypothesized that SGLT2 inhibition would potentiate the favorable influence of dietary counseling on body composition and physiological adaptations in overweight or obese adults. Fifty sedentary overweight/obese men (n = 12) and women (n = 38) were randomly assigned to 12 weeks of dietary counseling for weight loss, supplemented with daily ingestion of either placebo or Dapagliflozin (up to 10 mg/day); coded as Pill A and Pill B. Dietary counseling consisted of weekly, one-on-one, 30-minute meetings targeting modest calorie restriction. Before and after treatment, body composition, resting metabolic rate (RMR), insulin sensitivity, appetite and satiety were measured. Twelve weeks of dietary counseling decreased (P < 0.049) body mass, fat mass, and RMR; neither variable was influenced by pill assignment (interaction: P > 0.264). Dietary counseling also decreased lean mass (treatment main effect: P < 0.001), however the decrease in lean mass was greater in Pill B than in Pill A (interaction: P = 0.037). Neither dietary counseling nor SGLT2 inhibition influenced insulin sensitivity (P > 0.055). Overall, 12-weeks of dietary counseling leads to favorable modification of body mass and fat mass regardless of pill assignment. However, Pill A appears to reduce the dietary counseling mediated loss in lean mass. Except for lean mass, the effects of dietary counseling for weight loss were not influenced by SGLT2 inhibition.

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1. REVIEW OF LITERATURE

Introduction:

In the 45 minutes it takes to read this review 387 people in the United States will be diagnosed with Type-2-Diabetes (T2D). With over 415 million adults worldwide and 30.3 million people in the U.S.A. currently diagnosed with T2D (Ogurtsova et al., ADA, 2017), immediate curable and preventable action is required.

T2D results from a cocktail of behavioral and environmental risk factors, combined with genetic predisposition (Hamman, 1992; Neel, 1962; Ohlson et al., 1988; Tuomilehto et al., 2001). While the genetic foundation of T2D is yet to be fully described, there is overwhelming evidence that physical inactivity and obesity are major determinants (King et al., 1990; Lindström et al., 2003; Manson et al., 1991).

Clinically, T2D is diagnosed based on a fasting blood glucose concentration greater than 125 mg/dl (6.9 mmol/L). However, recently, a conservative approach is being adopted and people with pre-diabetes, characterized by a fasting blood glucose concentration between 100-125 mg/dl (5.6-6.9 mmol/L), are recommended to begin preventative pharmaceutical treatment and/or lifestyle changes. The progression toward T2D is associated with high plasma insulin due to insulin resistance developing in insulin responsive tissues. Insulin resistance results in chronic hyperglycemia followed by numerous late stage T2D complications including: decreased insulin secretion (pancreatic β-cell failure), accelerated lipolysis, dyslipidemia, incretin resistance (gastrointestinal tract), glycation of blood brain barrier (insulin resistance/leptin resistance) hyperglucagonemia, and increased renal glucose reabsorption (Florez, 2008; Fonseca, 2009; Hoerger et al., 2008; McCarthy, 2010; Wilding et al., 2014).

Unfortunately, the chronic hyperglycemia in T2D creates an environment that promotes protein glycation, covalent bonds between glucose and plasma proteins (Singh et al., 2014). Long term complications of advanced glycation end products include retinopathy, neuropathy, and cardiomyopathy (Ahmad et al., 2016; Donahoe et al., 2007; Ritz & Orth, 1999; Roger et al., 2011; Singh et al., 2014). Hence, patients must make lifestyle and/or pharmaceutical changes to prevent T2D symptom progression. Lifestyle regulates diabetes. Some beneficial lifestyle changes include, exercise and a low-calorie, low refined sugar, high fiber diet (Bhatt et al., 2017; Schwingshackl et al., 2017), and more recently a low carbohydrate, higher fat diet.

The American College of Sports Medicine (ACSM) has recommended 150 minutes of moderate aerobic exercise per week for diabetes prevention. However, recent studies support the efficacy of low-volume high-intensity interval training (HIIT) (Gibala et al., 2012; Little et al., 2011) and resistance exercise to improve glycemic control (Richards et al., 2010; van Dijk et al., 2012). While, in theory, regular exercise is beneficial for patients with T2D, there are numerous person limitations; these include poor habitual compliance due to the perceived time commitment, insufficient knowledge of proper exercise techniques, and the financial burden of equipment and gym membership. Pharmaceuticals provide an alternative option for hyperglycemia treatment. However, despite the reasonable success of current anti-diabetes drugs, side effects and complications exist (Opie et al., 2011).

One of the earlier pharmaceutical therapies was Metformin. This medication is arguably the most commonly prescribed treatment for diabetes and has been recommended as the first course of action by the American Diabetes Association (ADA). Metformin lowers hepatic gluconeogenesis (He et al., 2009; Hundal et al., 2000; Madiraju et al., 2014), decreases gastro-intestinal glucose absorption (Wu et al., 2017), and improves insulin sensitivity (Patanè et al.,

2000). However, the use of metformin has unfavorable side effects (DeFronzo et al., 2016). Additionally, not all patients with T2D tolerate Metformin supplementation (Rosenstock et al., 2017). If oral Metformin fails, insulin therapy is often supplemented as another pharmacological intervention for hyperglycemia (Inzucchi et al., 2014; Kilov et al., 2013). However, due to increasing insulin resistance, patients with T2D receiving insulin therapy, often require increased dosage. Higher insulin doses potentially lead to negative side effects including weight gain, fluid retention, and hypoglycemic events (Rosenstock et al., 2012; Stenlöf et al., 2013). Thus, there is a clear need for pharmacological therapy that will reverse hyperglycemia and prevent weight gain. In this regard, interest in a class of insulin independent drugs, sodium glucose cotransporter 2 (SGLT2) inhibitors, has flourished.

SGLT Proteins and Glucose Transport

Recent research has emphasized the importance of the kidney in glucose homeostasis. The kidneys filter approximately 160-180 grams of glucose per day (Gerich, 2010). Healthy individuals reabsorb almost all glucose that flows through the glomeruli. If glucose concentrations are too high, then the excess is not reabsorbed but rather excreted from the body via urine (glucosuria). The reabsorption of glucose is mediated by sodium glucose co-transporter (SGLT) proteins in an insulin independent process (Okabe et al., 2003).

Sodium glucose co-transporters couple the transfer of glucose against the concentration gradient with sodium along the concentration gradient (Chen et al., 2013; Mackenzie et al., 1996). There are three main SGLT proteins: SGLT1, SGLT2, and SGLT3. SGLT1 proteins are in the kidney and intestine and have a high affinity but low capacity to transport glucose. SGLT2 proteins are in the kidney and pancreas and, in contrast to SGLT1, have a low affinity but high capacity for sodium glucose transport. SGLT3 proteins are found in the small intestine and

skeletal muscle and have a moderate affinity for glucose to transport (Zhao et al., 2007). The focus of this review is specifically on the SGLT2 proteins.

The SGLT2 is in the proximal convoluted tubule of the kidney. Approximately ninety percent of the filtered glucose is reabsorbed from the kidney by SGLT2. The remaining ten percent is reabsorbed by SGLT1 in the distal portion of the proximal straight tubule (Kanai et al., 1994; Wright et al., 2011). A reduction in SGLT2 expression caused by either mutation or inhibition leads to glucosuria (Kanai et al., 1994). Therefore, SGLT2 inhibition provides a potential mechanism for reversing hyperglycemia and enhancing weight loss.

One important characteristic of SGLT2 proteins is that they are insulin independent. This is very different from the major glucose transport protein (GLUT4) in insulin-sensitive tissues, such as skeletal muscle and adipose tissue (Wright et al., 2011). Unlike GLUT4, SGLT2 proteins are membrane-bound proteins that actively transport glucose across the concentration gradient (Chen et al., 2013; Mackenzie et al., 1996; Wright et al., 2011). Additionally, because SGLT2 proteins are non-insulin dependent, they are a potentially effective therapeutic target in a disease in which insulin action/production is damaged.

SGLT2 Inhibition

Compared with metabolically health individuals, people with diabetes have elevated renal gluconeogenesis and glucose release (Gerich, 2010; Meyer et al., 1998; Meyer et al., 2004; Wilding et al., 2014). Hyperglycemia also occurs without the expected increase in glucosuria due to increased SGLT2 expression (Tabatabai et al., 2009). Expression of SGLT2 proteins increases transport maximum (absolute capacity of the kidneys to reabsorb glucose) approximately twenty percent higher in individuals with diabetes compared to their healthy counterparts (Mogensen, 1971). This increased expression of SGLT2 protein raises the renal threshold for glucose

excretion (RT_g) (Figure 1) (Liang et al., 2012; Rave et al., 2006; Ruhnau et al., 1997; Tabatabai et al., 2009; Wilding et al., 2014). In opposition, SGLT2 inhibition lowers RT_g. (DeFronzo et al., 2013).

While it is important for healthy individuals to reuptake glucose for energy use and storage, this increased RTg is maladaptive in patients with T2D and T1D (Abdul-Ghani et al., 2011; Nauck, 2014). In a person with diabetes, the reabsorption of glucose will lead to elevated blood glucose levels and contribute to hyperglycemia, glucose toxicity, and β-cell damage caused by lasting exposure to elevated glucose concentrations (Robertson et al., 2003).

Because of the maladaptive increased RTg caused by elevated SGLT2 expression, SGLT2 inhibition is emerging as a novel treatment for patients with T2D. Ideally, SGLT2 inhibition will increase urinary glucose disposal (glucosuria). Augmented glucosuria reduces fasting plasma glucose, improves glucose tolerance leading to reduced glucose toxicity (decreased chronic hyperglycemia), promotes calorie loss that can promote, weight loss, and finally improves β-cell function and insulin sensitivity (Chen et al., 2013; Idris et al., 2009).

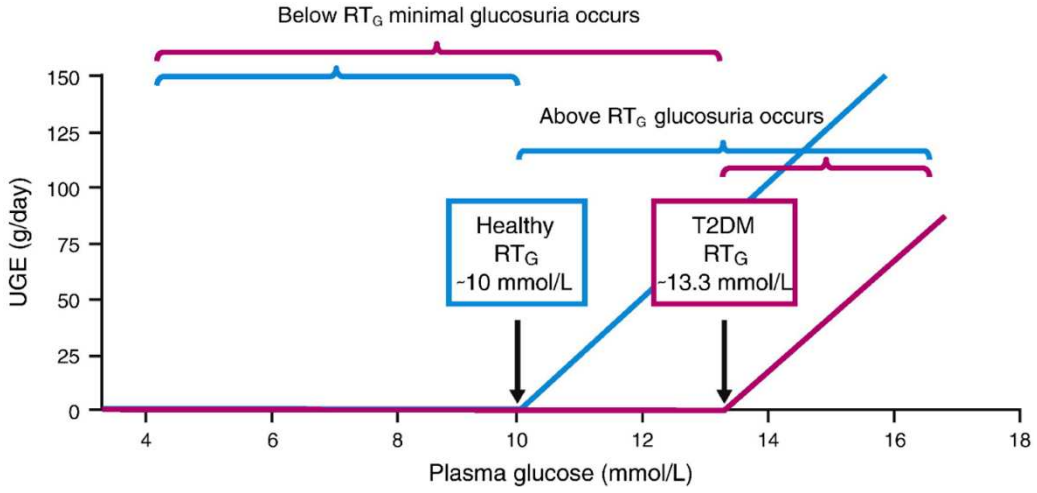

Figure 1: Wilding et al. 2014 - Linear Relationship between UGE and Plasma Glucose Concentration

Influence of Dapagliflozin on Weight Loss and Metabolic Health

Dapagliflozin is one of several SGLT2 inhibitors. As of 2012 in Europe and 2014 in the United States, dapagliflozin is approved for treatment of T2D. Following ingestion, the concentration of dapagliflozin peaks in plasma at two hours and has a half-life of fourteen hours (Obermeier et al., 2010). The pharmacokinetics of dapagliflozin in patients with T2D and in healthy participants are comparable (Komoroski, Vachharajani, Feng, et al., 2009). The recommended starting dosage of dapagliflozin is 5 mg/day, and increases to 10 mg /day in patients able to tolerate the higher dose or who require additional glycemic control.

The Effect of Dapagliflozin on Weight Loss

Weight gain is a common result of some diabetic medications such as thiazolidinediones (Fonseca, 2003). Numerous studies have observed weight loss in patients with T2D using dapagliflozin monotherapy (Bailey et al., 2012; Ferrannini et al., 2010; Kaku et al., 2013; Merovci et al., 2014). By inhibiting SGLT2 proteins and decreasing reabsorption and enhancing glucosuria, substantial calories (glucose) should be lost in the urine. In theory, this effect may lead to cumulative loss of calories, which should result in weight loss. Dapagliflozin induced glucosuria in diabetic patients leads to a net caloric loss of up to 200-300 kcal/day (List et al., 2009).

However, Dapagliflozin induced weight loss appears to plateau approximately one year after initiation of treatment (Wilding et al., 2012). This plateau might be explained, in part, by a decrease in resting metabolic rate (Frey-Hewitt et al., 1990)The implication for the patient wishing to maintain weight loss is that to continue to sustain negative energy balance caloric intake must be decreased and/or energy expenditure must be increased.

Administration of dapagliflozin over 24-102 weeks has been reported to decrease total body mass, fat mass, visceral adipose tissue, subcutaneous adipose tissue, waist circumference, and the concentration of glycated hemoglobin (hemoglobin A1C), and improve glycemic control in patients with T2D (Bolinder et al., 2012; Wilding et al., 2012). Additionally, Dapagliflozin supplementation stabilizes insulin dosing (less volatile blood glucose concentrations) (Wilding et al., 2012). Furthermore, long-term observations indicate that sustained weight loss occurs with major decreases in mean arterial pressure and death due to cardiovascular events and stroke. These beneficial effects continue for up to two years without any increased rate of hypoglycemia (Bolinder et al., 2012; Wilding et al., 2012).

Noteworthy, much of the dapagliflozin induced weight loss can be attributed to loss of fat mass (Bolinder et al., 2012). Excess adipose tissue is associated with ectopic lipid accumulation resulting in lipotoxicity, the damaging effects of excess fat accumulation on glucose metabolism (DeFronzo, 2010; Unger, 2003). Lipotoxicity partially mediates insulin resistance by phosphorylating the serine residue on IRS-1, inhibiting GLUT4 translocation and halting insulin dependent glucose uptake into cells. Additionally, lipotoxicity initiates increased release of inflammatory cytokines (TNF alpha and IL6). Therefore, by decreasing fat mass through SGLT2 inhibition, dapagliflozin could, in theory, reverse lipotoxicity and potentially restore insulin sensitivity while lowering inflammation (Katsiki et al., 2010; Tang et al., 2017).

Numerous studies have investigated dapagliflozin use by healthy diabetes-free adults; namely, pharmacokinetic characteristics (Komoroski, Vachharajani, Feng, et al., 2009; Yang et al., 2013), influence on appetite (Bertran et al., 2018), and co-administration with regular exercise (Newman et al., 2018). However, no study has specifically tested the influence of short-term dapagliflozin on weight loss in overweight/obese, but otherwise healthy adults.

Other Effects of Dapagliflozin on Metabolic Function

The Effect of Dapagliflozin on β-Cell Function

Dapagliflozin reduces transport maximum of glucose by fifty-five percent (DeFronzo et al., 2013). Thus, dapagliflozin lowers RTg to levels below fasting glucose levels. Therefore, glucosuria occurs at lower glucose concentrations after SGLT inhibition in both patients with T2D and their healthy counterparts (DeFronzo et al., 2013). Increased glucosuria leads to improvements in β-cell function (Merovci et al., 2015; Solomon et al., 2012).

Insulin resistance, lipotoxicity, and chronic elevation of plasma glucose, are just a few of the factors that contribute to β-cell dysfunction. For example, in normal glucose-tolerant individuals a small elevation in 24-hour plasma glucose concentration can lead to a twenty-four percent decrease in β-cell function (Solomon et al., 2012). Alternatively, lowering plasma glucose concentration with insulin therapy in T2D improves insulin secretion, reflective of properly functioning β-cells (Mayorov et al., 2005; Scarlett et al., 1982). Specifically, two weeks of glucose administration followed by dapagliflozin supplementation lowered blood glucose concentrations and improved β-cell function (Merovci et al., 2015). Thus, circulating glucose concentration influences β-cell function, based on evidence of the inverse association of prolonged glucose concentration and β-cell function.

The Effect of Dapagliflozin on Pancreatic Alpha-Cells

Dapagliflozin improves skeletal muscle insulin sensitivity but enhances endogenous glucose production independent of any decrease in plasma glucose concentration due to elevated plasma glucagon concentration (Merovci et al., 2015). It is currently believed that the elevated endogenous glucose production is due to inhibition of SGLT2 proteins expressed in the glucagon-secreting alpha-cells of the pancreatic islets (Bonner et al., 2015). Therefore,

dapagliflozin instigates glucagon secretion and enhanced endogenous (hepatic) glucose production. However, the glucose production is modest. For example, 10 mg/day of dapagliflozin increased circulating glucagon concentrations by 91% but had no effect of circulating blood glucose concentrations (Bonner et al., 2015). This direct effect of SGLT2 inhibition on alpha-cells may counter the hyperglycemia lowering abilities of dapagliflozin.

Effects of Dapagliflozin on Blood Pressure

In addition to the maintenance of fluid and electrolyte homeostasis, the kidneys also regulate blood pressure. In healthy individuals, kidneys increase excretion of salt and water as blood pressure increases; consequently, lowering blood volume and blood pressure back to baseline (Oliva et al., 2014). Hypertension is another negative outcome of T2D. Over seventy-five percent of adults with diabetes are either using antihypertensive drugs or have systolic blood pressures (SBP) greater than 140 mmHg (Proctor 2019).

Data from a recent meta-analysis demonstrated that SGLT2 inhibitors reduce mean arterial blood pressure due to enhanced sodium excretion in patients with T2D (Baker et al., 2014; Oliva & Bakris, 2014; Tikkanen et al., 2015), suggesting that SGLT2 inhibition not only improves glycemic control but also has a diuretic-like ability to lower BP (Lambers Heerspink et al., 2013). In rodent models, evidence suggests that the sodium loss from increased diuresis may help counteract some of the hypertension in diabetes (Maliha et al., 2014). However, diuresis can only account for blood pressure reduction in the short term. Rodent studies found that sodium levels in the distal convoluted tubule of the glomerulus normalize after 10-12 days of dapagliflozin administration (Thomson et al., 2012). Similar observations are seen in patients with T1D, in which hyperfiltration decreased allowing for increased diuresis, sodium excretion, and decreased short term blood pressure. (Cherney et al., 2014).

SGLT2 inhibition mediates blood pressure, part of which is due to increased sodium loss and decreased arterial stiffness with SGLT2 inhibition. As sodium levels decrease, plasma volume will decrease, and SBP should theoretically drop. In support, SGLT2 inhibition decreases risk of cardiovascular events and mortality (Birkeland et al., 2017). This is important because the presence and duration of diabetes leads to increased arterial stiffness and consequently elevated rates of cardiovascular, renal, retinal, and autonomic disease (Theilade et al., 2013).

Risks of SGLT2 Inhibitors and Dapagliflozin

Hypoglycemia

Hypoglycemia is the condition of very low blood glucose concentrations. The risk for hypoglycemia is quite low when taking SGLT2 inhibitors. There is no increased risk of hypoglycemic episodes when dapagliflozin is used as a monotherapy; however, there is an increased hypoglycemic risk if dapagliflozin is taken in conjunction with sulfonylurea or insulin (exogenous) (Strojek et al., 2011; Vasilakou et al., 2013; Wilding et al., 2012). Insulin stimulates glucose uptake, while SGLT2 inhibitors increase glucosuria. These effects potentially compound one another leading to hypoglycemia (Wilding et al., 2012a). Therefore, decreased insulin dosage is required when supplemented with an SGLT2 inhibitor.

Diabetic Ketoacidosis:

Diabetic ketoacidosis (DKA) is an acute and serious complication of diabetes (Ogawa et al., 2016). Ketoacidosis is the result of hyperglycemia and high acidic concentrations of ketone bodies. While DKA often stems from hyperglycemia and insulin resistance, it can occur with moderate healthy increases in blood glucose concentrations (Ogawa et al., 2016). Numerous cases of DKA have been found in patients with either T1D or T2D when treated with an SGLT2

inhibitor (Hayami et al., 2015; Hine et al., 2015; Peters et al., 2015). SGLT2 inhibition instigates DKA through a cascade of events. By increasing glucosuria, blood glucose levels decrease. Lower blood glucose levels decrease insulin levels, which in turn increase lipolysis and free fatty acid (FFA) production. Increases in FFAs augment β-oxidation; thus, creating more ketone body production. Additionally, amplified glucagon levels and decreased insulin levels will reduce acetyl-CoA carboxylase activity, followed by an increase in carnitine palmitoyltransferase-1 (CPT-1). Higher CPT-1 activity will instigate β-oxidation and ketone body production. Lastly, increased ketone body production will stimulate euglycemic DKA (Ogawa et al., 2016). Although DKA is a potential threat, there have been no reported cases of DKA in metabolically healthy individuals on SGLT2 inhibitors.

The Effects of SGLT2 Inhibition on Energy and Appetite

If the weight loss observed with SGLT2 inhibitors is less than hypothesized based on the glucosuria caloric loss, one would assume that SGLT2 inhibitors might increase hunger and appetite (Cefalu, 2014; Ferrannini et al., 2014; Zhang et al., 2014). It is likely that patients increase caloric intake rather than reduce energy expenditure when taking an SGLT2 inhibitor (Ferrannini et al., 2014). Evidence for increased caloric intake includes a shift in substrate oxidation from glucose to lipid but not accompanied by a change in energy expenditure when on SGLT2 inhibitors (Ferrannini et al., 2014).

However, there are conflicting data on whether SGLT2 inhibitors instigate increased appetite and caloric consumption. Studies have failed to find an SGLT2 influence on energy intake (Bertran et al., 2018), yet have found an increase in sugar intake in patients with T2D (Horie et al., 2018). Regardless of caloric intake, numerous studies see increased desire for salty

and sweet foods (Bertran et al., 2018; Horie et al., 2018). This appetite change likely matches the natriuretic and glucuretic effects of the drug.

The conflicting results provide a potential direction for nutritional intervention when supplementing weight loss with dapagliflozin. By knowing the appetite and consumption for sweet sugary foods will increase, nutrition counselors can directly limit sugar consumption successfully achieving desired weight loss. Further research must investigate sugar restricted, dapagliflozin supplemented dietary counseling in both healthy and patients with T2D.

Conclusion:

The use of SGLT2 inhibitors is associated with favorable modifications in body mass, beta-cell function, insulin sensitivity, and blood pressure. Dapagliflozin significantly influences weight loss in people with diabetes; however, the influence on metabolically healthy individuals is not heavily researched. If similar effects are seen in the metabolically healthy, SGLT2 inhibition may provide supplemental benefits to life style changes in the prevention of metabolic disease. Hence the purpose of this study was to determine if SGLT2 inhibition will augment the effects of 12-weeks of dietary counseling for weight loss while simultaneously comparing modifications in glucose tolerance, blood pressure, appetite and satiety in overweight and obese adults with or without supplemented SGLT2 inhibition (dapagliflozin). We hypothesized that SGLT2 inhibition would potentiate the favorable influence of dietary counseling on body composition and physiological adaptations in overweight or obese adults.

2. INTRODUCTION

Over 415 million adults worldwide and 30.3 million people in the U.S.A. are currently diagnosed with T2D (Ogurtsova et al., ADA, 2017). Long term complications of T2D include retinopathy, neuropathy, and cardiomyopathy (Ahmad et al., 2016; Donahoe et al., 2007; Ritz & Orth, 1999; Roger et al., 2011; Singh et al., 2014). There is overwhelming evidence that physical inactivity and obesity are major determinants of developing T2D (King et al., 1990; Lindström et al., 2003; Manson et al., 1991). Therefore, lifestyle changes and/or pharmaceutical interventions aid in attenuating T2D symptom progression. Lifestyle regulates diabetes.

One of the earlier pharmaceutical therapies and most commonly prescribed T2D drug is Metformin. If oral Metformin fails, insulin therapy is often supplemented as another pharmacological intervention for hyperglycemia (Inzucchi et al., 2014; Kilov et al., 2013). However, due to increasing insulin resistance, patients with T2D receiving insulin therapy often require increased dosage. Higher insulin doses potentially lead to negative side effects including weight gain, fluid retention, and hypoglycemic events (Rosenstock et al., 2012; Stenlöf et al., 2013). Hence, there is a clear need for pharmacological therapy that will improve hyperglycemia yet prevent weight gain. In this regard, interest in a class of insulin independent drugs, sodium glucose cotransporter 2 (SGLT2) inhibitors, has flourished.

Located in the proximal convoluted tubule of the kidney, SGLT2 regulates the reabsorption of glucose. A reduction in SGLT2 expression caused by either mutation or inhibition leads to glucosuria (DeFronzo et al., 2013; Kanai et al., 1994). By inhibiting SGLT2 proteins and enhancing glucosuria, substantial calories (glucose) should be lost in urine. In

theory, this effect may simultaneously lead to cumulative loss of calories, which should result in weight loss and improve insulin sensitivity.

Dapagliflozin is one of several SGLT2 inhibitors. Dapagliflozin induced glucosuria leads to a net caloric loss of up to 200-300 kcal/day (List et al., 2009). Administration of dapagliflozin over 24-102 weeks has been reported to decrease total body mass, fat mass, visceral adipose tissue, subcutaneous adipose tissue, waist circumference, and blood pressure, and improve glycemic control in patients with T2D (Bolinder et al., 2012; Wilding et al., 2012). Currently it is unclear whether these health benefits are due to SGLT2 inhibition or a secondary effect of weight loss.

There are conflicting data on whether SGLT2 inhibitors increase appetite and caloric consumption. Some studies have failed to find an influence on energy intake (Bertran et al., 2018), while others have found an increase in sugar intake in patients with T2D (Horie et al., 2018). Likely matching the natriuretic and glucuretic effects of the drug, numerous studies report an increased desire for salty and sweet foods (Bertran et al., 2018; Horie et al., 2018).

Several studies have investigated dapagliflozin in healthy diabetes-free individuals: pharmacokinetic characteristics (Komoroski et al., 2009; Yang et al., 2013), influence on appetite (Bertran et al., 2018), and exercise training (Newman et al., 2018). However, no study has specifically tested the influence of SGLT2 inhibition on weight loss in healthy individuals. Hence the purpose of this study is to determine if SGLT2 inhibition will augment the effects of 12-weeks of dietary counseling for weight loss while simultaneously comparing modifications in glucose tolerance, blood pressure, appetite and satiety in overweight and obese adults with or without supplemented SGLT2 inhibition (dapagliflozin). We hypothesized that SGLT2 inhibition

would potentiate the favorable influence of dietary counseling on body composition and physiological adaptations in overweight or obese adults.

3. METHODS

Study Design

A double-blind, repeated measures parallel design was employed incorporated with stratified random assignment. All procedures were approved by the Institutional Review Board at Colorado State University, Fort Collins in agreement with the principles originating from the Declaration of Helsinki.

Participants

Overweight and obese sedentary but otherwise healthy men and women volunteered and participated. Inclusion criteria included: provision of informed consent, aged between 18-65 years, body mass index (BMI) within the range of 27.5-45 kg/m^2, no significant exercise training (maximum of 3 physical activity sessions/week of less than or equal to 30 minutes per session over the previous year), negative pregnancy test for women of childbearing potential, no known metabolic diseases, and willingness to modestly reduce daily caloric intake for twelve weeks. Exclusion criteria were evidence of any significant disease that may interfere with study objectives, use herbal preparations or prescription drugs other than birth control, and/or medications to treat depression, current enrollment in another clinical study, habitual or recent use of tobacco in the last two years, history of hypersensitivity reaction to Dapagliflozin, severe renal impairment, pregnant, abnormal liver function, bilirubin > 2.0 mg/dL (34.2 umol/L), Hepatitis B or C antibodies or antigens, history of bladder cancer, recent cardiovascular events, or considered unsuitable for participation by medical monitor.

Protocol Overview

Following screening and prior to and after a 12-weeks of dietary counseling for weight loss supplemented with daily ingestion of either placebo or SGLT2 inhibitor (Dapagliflozin), participants were assessed for body mass and composition, resting metabolic rate, insulin sensitivity (oral glucose tolerance test), and hunger and appetite.

Table 1: Outline of Study Visits

Weeks -2 to 0			Weeks 1-12	Week 13	
Screening, ECG, and DEXA	OGTT, RMR, and Blood Pressure	Hunger and Appetite	Initiation of Treatment (Dietary counseling for weight loss and Placebo or Dapagliflozin supplementation)	OGTT, RMR and Blood Pressure	Hunger and Appetite, DEXA

ECG: Electrocardiogram
DEXA: Dual Energy X-ray Absorptiometry
OGTT: Oral Glucose Tolerance Test
RMR: Resting Metabolic Rate

Screening

Screening included: review of medical history, graded exercise stress test (rest to volitional fatigue) with 12-lead electrocardiogram, blood pressure assessment, blood sample (liver enzymes, fasting glucose concentrations, and hepatitis C). Peak oxygen consumption (VO_{2peak}) was measured during constant ramp protocol graded exercise stress test on stationary cycle ergometer (Dynafit Velotron; Racermate Inc., Seattle, USA) (Richards et al., 2010). With the goal of a 8-12 minute test, the specific ramp protocol was chosen at the researchers' initial impression of the participants' fitness. Wattage increases were between 15-30 Watts/min. Every two minutes heart rate, blood pressure, rating of perceived exertion (RPE) (Borg, 1982) were measured. The test was terminated at volitional fatigue or once pedal cadence fell below 40 revolutions/min. Expired gases and ventilation were measured continuously with a metabolic cart

(Parvo Medics, Sandy, Utah, USA). The highest four consecutive 15-s average VO_2 values were used to calculate VO_{2peak}.

Body Composition

Body composition was assessed using dual energy x-ray absorptiometry (DEXA: Hologic Discovery W, QDR Series, Bedford, MA, USA) (Scalzo et al., 2014). Body mass was assessed using a physician's digital scale. Waist circumference was measured midway between the lower border of the costal margin and the uppermost border of the iliac crest. Hip circumference was measured around widest portion of hips and gluteus maximus. Both waist and hip circumference was measured three times each and then averaged.

Resting Metabolic Rate & Oral Glucose Tolerance Test

Following a 12-hour fast and 24-hour abstention from exercise and caffeine, participants reported to the laboratory for assessment of resting metabolic rate (RMR) and completion of an oral glucose tolerance test (OGTT). On arrival, body mass was measured using a physician's digital scale. Participants were then placed in a dimly lit room and expired air was collected and used to calculate RMR and respiratory exchange ratio (RER) (Mass Spectrometry: MA Tech Services, INC. Saint Louis, Missouri, USA)) over a period of forty-five minutes (only the last 30 minutes of collection was used for RMR/RER calculations). Blood pressure was measured three times during the forty-five-minute period (once every 15 minutes) with accuracy of ± 3 mmHg (HEM-7113: Omron Healthcare Asia). RMR was calculated using the Weir Equation (RMR = $((3.9 \times VO_2) + (1.1 \times VCO_2)) \times 1.44$). Where VO_2 is the rate of oxygen consumption (ml/min) and VCO_2 is the rate of carbon dioxide production (ml/min).

After completion of the RMR, a venous catheter was placed in an antecubital or hand vein. Following baseline blood sampling (20 mL), participants ingested 75 grams of glucose

diluted in 300 mL water. Venous blood was sampled over the next two hours (20 mL at minutes 15, 30, 60, 75, 90, 120 & 1 mL at minutes 0, 5, 10, 20, 45, 105) for determination of circulating concentrations of glucose (2900 STAT Plus Glucose Lactate Analyzer, YSI Inc., Yellow Springs, Ohio, USA) and insulin (enzyme-linked immunosorbent assay; Crystal Chem, Inc., Elk Grove, Village, Illinois, USA) (Beals et al., 2017; Newman et al., 2018). Insulin sensitivity was estimated by Matsuda Index (10,000/square root of ((fasting glucose x fasting insulin) x (mean glucose x mean insulin during OGTT))) (Matsuda et al., 1999). 500 ul of serum and plasma were isolated and transferred into six phials each. Two phials containing plasma were cocktailed with protease inhibitor (10 ul DPP and 10 ul Sigma Pi) and 500 ul serum were immediately placed in a -80 C freezer.

Hunger and Appetite Assessment

Following a 12-hour fast and 24-hour abstention from exercise and caffeine, participants reported to the clinical laboratory and upon arrival, a venous catheter was placed in an antecubital or hand vein and 20 mL of blood was drawn for baseline measurements. Participants then ingested 1 kilocalorie/kg body mass of a small mixed macronutrient liquid meal, consisting of Ensure (24% fat, 60% carbohydrate, 16% protein). Sixty minutes after the liquid preload, participants selected food from an all-you-can-eat breakfast buffet. Participants were allowed 20 minutes to eat. The selected food choices (packaging included) were recorded and weighed before and after participant consumption. Remaining uneaten food was reweighed and participants' energy and macronutrient intakes were determined using the Food Intake Analysis System software (U Texas Health Sciences Center, Houston). Participants remained at the facility for the next three hours for follow up blood and satiety measurements. 20 ml of blood was draw 60 minutes prior to the priming meal and buffet, and then every 60 minutes for 3

hours. Blood was immediately assayed for concentrations of glucose (2900 STAT Plus Glucose Lactate Analyzer, YSI Inc., Yellow Springs, Ohio, USA). 500 ul of plasma cocktailed with protease inhibitor (10 ul DPP and 10 ul Sigma Pi) and 500 ul serum were immediately placed in a -80 C freezer.

At baseline, 0 and 60 minutes after liquid meal consumption, and 0, 60, 120, and 180 minutes after breakfast, participants completed a hunger and satiety survey. Surveys consisted of five questions: (1) How strong is your desire to eat right now? (2) How hungry do you feel right now? (3) How full do you feel right now? (4) How much do you think you could eat right now? (5) How thirsty do you feel right now? Subjects answered these questions by marking a vertical line on a 100 mm visual analog scale (Paris et al., 2016).

Treatment

Research participants were assigned, in a double-blind fashion, via stratified random sampling into one of two treatment groups: placebo or the SGLT2 inhibitor, Dapagliflozin, via coin flip until one treatment group had 25 participants, after which all remaining participants were assigned the other treatment. A stratified random assignment was used to balance sex distribution between groups. Oral ingestion of treatment or placebo coincided with the first day of counseling and ended on the last day of counseling. The dose of Dapagliflozin began at 5 mg/day for the first two weeks. The dose was increased to 10 mg/day in the absence of adverse events for the remainder of the study. An identical dosing procedure was used previously (Newman et al., 2018).

Dietary Counseling

Participants completed a three-day diet log before initiation of counseling. Diet logs and recalls included all food and drink consumed (ingredients and amounts), location of meal, time

of day, and hunger level before meal. Commercially available software (NutritionistPro, Axxya Systems, Redmond, WA) was used to determine the caloric and micro- and macro-nutrient make up of each item consumed. Dietary counseling consisted of weekly, one-on-one, 30-minute meetings targeting calorie restriction. Counseling focused on modest caloric restriction initially equal to resting metabolic rate multiplied by an activity factor of 1.2 and then lowered over to time to a kilocalorie target equal to the measured baseline resting metabolic rate. This dietary reduction was designed to reduce each participant's weight by approximately 0.5kg per week (Paris et al., 2016). Prescribed macronutrient intake comprised of approximately 50% carbohydrates, 30% fat, and 20% protein, while maintaining adequate micronutrient intakes (90-100% of recommended dietary allowance). Dietary counseling focused on methods of mindful eating while also teaching techniques to decrease energy density and intake of convenience commercially-prepared foods high in added sugar and fat, and to increase fiber intake and water consumption. Additionally, participants were instructed to maintain current activity levels and discouraged from beginning a program of exercise. During weeks 5 and 10, participants completed a 24-h diet recall under the supervision of counselor for the purpose of identifying strategies to enhance dietary objectives,

Blinding Procedures:

Pill bottles containing Dapagliflozin were labeled as "A" or "B". The clinical trials administrator was responsible for ensuring blinding of both participants and researchers. Neither researchers nor participants were unblinded until all data were analyzed and statistical analysis complete.

Statistical Analysis

Baseline (pre-counseling) differences between placebo and SGLT2 inhibition groups were determined via one-way Analysis of Variance (ANOVA). The influence of Dapagliflozin on physiological responses to dietary counseling was examined via two-way repeated measures ANOVA (placebo vs. Dapagliflozin x before vs. after dietary counseling). The influence of Dapagliflozin and counseling on dynamic changes to hunger, appetite, and oral glucose ingestion was examined using three-way ANOVA (placebo vs. Dapagliflozin x before vs. after dietary counseling x time). Pairwise comparisons were performed via the Tukey Method. Outliers were determined using Median Absolute Deviation (MAD) (Leys et al. 2013). Relationships of interest were calculated using a Pearson Correlation. The level of statistical significance was set a $P < 0.05$. Data were reported as mean and standard deviation, unless indicated differently. Calculations were performed using SigmaStat 3.0 (Systat Software Inc., San Jose, California, USA) and RStudio 3.5.1 (RStudio, Boston, MA, USA).

4. RESULTS

50 participants completed the study; baseline physiological characteristics are presented in Table 2. Consistent with exclusion and inclusion criteria, participants portrayed characteristics of sedentary overweight/obese, but otherwise healthy adults. There were no baseline differences in any primary variables between placebo and SGLT2 Inhibitor groups.

Body Composition

Body mass and composition are presented in Figure 2 and Table 3. Twelve weeks of dietary counseling decreased body mass, BMI, and fat mass ($P < 0.001$); neither variable was influenced by pill assignment (interaction: $P > 0.385$) (Figure 2A, B, C). Dietary counseling also decreased lean mass (treatment main effect: $P < 0.001$), however the decrease in lean mass was greater in Pill B than in Pill A (interaction: $P = 0.037$) (Figure 2D). Counseling increased bone mineral content (P=0.027); SGLT2 inhibition did not influenced this interaction (P=0.807) (Figure 2E).

Resting Metabolic Rate

RMR and RER data are presented in Figure 3 and Table 4. RMR and RER both decreased in response to dietary counseling (P<0.049); SGLT2 inhibition did not influence either outcome (P>0.397) (Figure 3A & 3B). ANCOVA results demonstrated the change in lean mass does not explain the change in RMR (P=0.8423). The changes in RMR and lean mass are not correlated (P=0.881 and r=0.022).

Glucose and Insulin

Glucose and Insulin data are presented in Figure 4 and Tables 1 and 4. Based on the Median Absolute Deviation (MAD) method (Leys et al. 2013) for determining outliers, baseline

insulin data were considered an outlier if greater than 17.21 mU/L. Four baseline insulin values (17.77, 19.64, 38.29, and 86.05 mU/L) were considered outliers and removed from all further insulin analysis. Any magnitude changes in baseline insulin from Week 0 to Week 12 over 5.82 mU/L for Pill A and 6.35 mU/L for Pill B were considered outliers. One delta value (7.40 mU/L) was removed from Pill A. A Matsuda Index value greater than 18.35 and a change in Matsuda Index greater than 9.64 for Pill A and 8.77 for Pill B constituted an outlier. Four Week 0 Matsuda scores were removed from analysis: two from Pill A (29.19 and 33.76) and two from Pill B (29.89 and 55.47). Five additional Matsuda scores were considered outliers because the magnitude of change exceeded the threshold criteria for being an outlier (Pill A: 24.02 and 13.15, Pill B: 10.04, 17.29, and 18.98).

Baseline fasting glucose and insulin were similar between placebo and SGLT2 inhibition groups (P>0.143) (Figure 4A & 4B). While there was no main effect of drug interaction on the circulating glucose response to glucose ingestion (P > 0.93), one-hour area under the curve (AUC) of circulating glucose concentrations decreased with dietary counseling (P = 0.036) (Figure 4C). There was no difference in glucose 2nd hour AUC (P = 0.14). Dietary counseling decreased circulating insulin concentrations and insulin AUC (P<0.009); SGLT2 inhibition had no influence on this outcome (P>0.089) (Figure 4B, 4D, & 4F). Neither dietary counseling nor SGLT2 inhibition influenced Matsuda Index (P > 0.055). Additionally, the amount of weight lost and magnitude of change in insulin sensitivity were not correlated (P=0.775 and r=0.048).

Hunger and Appetite

Circulating glucose concentrations, total caloric intake and the macronutrient distribution of food consumed over 20 minutes during the all-you-can-eat buffet, heart rate, and blood pressure responses during hunger and appetite assessment are all presented in Figure 5A, 5B, and

Table 5. Dietary counseling favorably modified glucose AUC (P=0.022) and heart rate at all time points (P<0.037), but no effect on SBP or DBP (P>0.056); SGLT2 inhibition did not influence these outcomes (P>0.233). However, SGLT2 inhibition did influence heart rate sixty minutes post buffet (P=0.036). At baseline, those taking Pill A consumed more total calories for fat, carbohydrate, and protein. (P<0.028). Dietary counseling decreased total calories consumed from fat (P=0.011); SGLT2 inhibition did not influence this outcome (Figure 5 & Table 5).

Participant satiety responses are present in Table 6 and Figure 6. Dietary counseling decreased perceived hunger and increased satiety 60 minutes post "Primer" meal (P<0.044); SGLT2 inhibition did not influence this outcome (P>0.526).

Diet Logs

Participant diet log and diet recall data are found in Table 7 and Figure 7. Dietary counseling decreased self-reported total caloric intake and carbohydrate and fat consumption (P<0.005); SGLT2 inhibition influenced total caloric intake and carbohydrate consumption (P<0.041). Post hoc Tukey analysis indicates SGLT2 inhibition influenced differences at Week 0 (P=0.013). Percentage of calories consumed by protein increased post counseling (P=0.017); SGLT2 inhibition did not influence this interaction (P=0.201). Percentage of calories from fat and carbohydrate consumption did not change post counseling (P>0.244).

Table 2: Selected Baseline Physiological Characteristics

	Pill A (Mean ±SD)	Pill B (Mean ± SD)	P-Value
Male/Female	7/18	5/20	-
Age (years)	35 ± 11	38 ± 11	0.362
Height (cm)	167 ± 9	169 ± 8	0.399
Body Mass (kg)	93.75 ± 20.43	96.47 ± 15.60	0.388
BMI (kg/m^2)	33.24 ± 5.61	33.50 ± 3.85	0.858
Body Fat (%)	39.80 ± 5.99	41.76 ± 5.16	0.222
Blood Pressure (mmHg)	112/69 ± 8/5	116/69 ± 10/8	0.066/0.819
VO_{2peak} (ml/kg/min)	24.75 ± 5.60	22.24 ± 4.97	0.100
Fasting Glucose (mg/dL	74.70 ± 6.93	76.08 ± 7.67	0.508
Fasting Insulin (mU/L)	5.12 ± 2.47	6.98 ± 3.90	0.120

Data: Mean ± SD
BMI: Body Mass Index
VO_{2peak}: Peak Oxygen Uptake

Table 3: Body Composition Derived Data Before and After 12-Weeks of Dietary Counseling Supplemented With or Without Sodium Glucose Co-Transport 2 Inhibition

	Pill A		Pill B		ANOVA P-Values		
	Pre-Counseling	**Post-Counseling**	**Pre-Counseling**	**Post-Counseling**	**Group**	**Counseling**	**Interaction**
Body Mass (kg)	93.75 ± 20.43	90.31 ± 19.72	96.47 ± 15.60	92.14 ± 16.73	0.660	**<0.001**	0.394
BMI (kg/m^2)	33.24 ± 5.61	32.00 ± 5.43	33.50 ± 3.85	31.97 ± 4.27	0.936	**<0.001**	0.446
WC (cm)	92.3 ± 10.2	90.7 ± 8.2	95.6 ± 9.0	94.2 ± 10.8	0.239	0.052	0.902
HC (cm)	115.8 ± 12.6	111.6 ± 9.4	119.9 ± 10.0	116.3 ± 10.3	0.066	**<0.001**	0.173
WHR	0.83 ± 0.93	0.81 ± 0.06	0.80 ± 0.73	0.81 ± 0.07	0.634	0.194	0.242
BMC (kg)	2.43 ± 0.37	2.46 ± 0.39	2.57 ± 0.32	2.59 ± 0.33	0.181	**0.027**	0.807
Fat Mass (kg)	36.95 ± 11.38	34.97 ± 11.23	40.13 ± 8.96	37.91 ± 10.26	0.306	**<0.001**	0.77
Lean Mass (kg)	52.83 ± 10.85	52.19 ± 11.10	52.97 ± 8.90	50.97 ± 9.04	0.848	**<0.001**	**0.037**
FFM (kg)	55.26 ± 11.17	54.59 ± 11.44	55.54 ± 9.16	53.56 ± 9.30	0.897	**<0.001**	**0.047**
Body Fat (%)	39.80 ± 5.99	38.73 ± 6.84	41.76 ± 5.16	41.13 ± 6.00	0.202	**0.001**	0.385

Data: Mean ± SD
WC: Waist Circumference
HC: Hip Circumference
WHR: Waist Hip Ratio
FFM: Fat Free Mass

Table 4: RMR and OGTT Derived Data Before and After 12-Weeks of Dietary Counseling Supplemented With or Without Sodium Glucose Co-Transport 2 Inhibition

	Pill A		Pill B		ANOVA P-Values		
	Pre-Counseling	Post-Counseling	Pre-Counseling	Post-Counseling	Group	Counseling	Interaction
RMR	1636 ± 282	1543 ± 262	1610 ± 235	1572 ± 310	0.982	**0.049**	0.397
RER	0.90 ± 0.05	0.86 ± 0.07	0.88 ± 0.05	0.84 ± 0.06	0.228	**0.002**	0.898
GLUCOSE							
Fasting Glucose	74.70 ± 6.93	75.00 ± 7.84	76.08 ± 7.67	72.59 ± 5.54	0.857	0.512	0.261
AUC	12966 ± 2703	12419 ± 2431	12711 ± 1968	12111 ± 1974	0.625	0.062	0.93
AUC 0-1 Hr.	6295 ± 955	5966 ± 854	6216 ± 731	5977 ± 885	0.871	**0.036**	0.735
AUC 1-2 Hr.	6672 ± 1853	6453 ± 1737	6494 ± 1401	6133 ± 1322	0.545	0.14	0.713
INSULIN							
Fasting Insulin	5.12 ± 2.47	4.76 ± 2.43	6.98 ± 3.90	5.62 ± 3.33	0.134	**0.009**	0.125
AUC	6331 ± 4724	5510 ± 3232	8292 ± 5062	5694 ± 3375	0.356	**0.002**	0.089
AUC 0-1 Hr.	2960 ± 2539	2390 ± 1500	3729 ± 2280	2666 ± 1822	0.376	**<0.001**	0.254
AUC 1-2 Hr.	3371 ± 2328	3121 ± 1909	4564 ± 3157	3028 ± 1744	0.390	**0.009**	0.056
MATSUDA INDEX	8.94 ± 4.27	8.61 ± 3.48	6.27 ± 3.66	7.84 ± 3.16	0.135	0.209	0.055

Data: Mean ± SD
RMR: Resting Metabolic Rate
RER: Respiratory Exchange Ratio
Units for RMR: kcal/day
Units for glucose: mg/dL
Units for Insulin: mU/L
Glucose area under the curve units: mg/dL/min
Insulin area under the curve units: mU/L/min

<u>Table 5A:</u> Hunger and Appetite Test Glucose and Energy Intake Derived Data Before and After 12-Weeks of Dietary Counseling Supplemented With or Without Sodium Glucose Co-Transport 2 Inhibition

	Pill A		Pill B		ANOVA P-Values		
	Pre-Counseling	Post-Counseling	Pre-Counseling	Post-Counseling	Group	Counseling	Interaction
<u>Glucose (mg/dL)</u>							
Baseline	77.68 ± 7.68	76.24 ± 8.96	77.29 ± 7.32	74.24 ± 6.18	0.513	0.058	0.49
Pre-Buffet	79.85 ± 13.80	78.39 ± 16.79	79.43 ± 14.05	77.63 ± 11.58	0.867	0.402	0.928
Post-Buffet (0)	87.93 ± 14.45	84.73 ± 13.98	89.52 ± 14.60	80.55 ± 10.18	0.688	**0.005**	0.165
Post Buffet (60)	83.82 ± 20.31	88.58 ± 20.07	87.26 ± 19.18	80.04 ± 14.79	0.495	0.816	0.051
Post-Buffet (120)	85.17 ± 17.38	78.97 ± 15.19	80.52 ± 11.86	75.75 ± 7.82	0.241	**0.006**	0.714
Post-Buffet (180)	81.98 ± 12.32	78.65 ± 13.42	79.11 ± 8.22	73.88 ± 6.49	0.145	**0.006**	0.524
AUC Post Buffet	15236 ± 2611	14954 ± 2424	15127 ± 2252	13980 ± 1474	0.333	**0.022**	0.16
<u>Energy Intake</u>							
Fat	163 ± 70	132 ± 64	122 ± 99	92 ± 64	**0.028**	**0.011**	0.977
CHO	426 ± 187	431 ± 235	345 ± 130	297 ± 141	**0.018**	0.389	0.29
Protein	119 ± 38	114 ± 62	96 ± 51	82 ± 33	**0.022**	0.169	0.512
Total	693 ± 208	660 ± 313	549 ± 209	458 ± 184	**0.004**	0.059	0.381

Data: Mean ± SD
Units for glucose: mg/dL
Units for energy intake: kcal

<u>Table 5B:</u> Hunger and Appetite Test Cardiovascular Derived Data Before and After 12-Weeks of Dietary Counseling Supplemented With or Without Sodium Glucose Co-Transport 2 Inhibition

	Pill A		Pill B		ANOVA P-Values		
	Pre-Counseling	Post-Counseling	Pre-Counseling	Post-Counseling	Group	Counseling	Interaction
<u>Heart Rate</u>							
Pre-Buffet	69 ± 8	64 ± 10	67 ± 7	63 ± 7	0.446	**0.002**	0.802
Post-Buffet (0)	70 ± 10	66 ± 10	71 ± 8	66 ± 7	0.89	**<0.001**	0.551
Post Buffet (60)	78 ± 10	72 ± 16	73 ± 7	70 ± 7	0.14	**<0.001**	**0.036**
Post-Buffet (120)	73 ± 10	72 ± 11	70 ± 6	67 ± 7	**0.047**	**0.037**	0.477
Post-Buffet (180)	71 ± 9	68 ± 10	69 ± 9	65 ± 5	0.271	**0.007**	0.716
<u>SBP</u>							
Baseline	117 ± 9	119 ± 8	122 ± 11	122 ± 10	0.086	0.376	0.821
Pre-Buffet	117 ± 7	120 ± 9	121 ± 12	119 ± 11	0.509	0.768	0.262
Post-Buffet (0)	121 ± 9	123 ± 7	125 ± 11	124 ± 11	0.195	0.686	0.449
Post Buffet (60)	119 ± 9	115 ± 10	122 ± 10	119 ± 9	0.099	0.061	0.494
Post-Buffet (120)	118 ± 8	117 ± 9	121 ± 10	119 ± 14	0.242	0.560	0.983
Post-Buffet (180)	118 ± 8	117 ± 10	123 ± 13	122 ± 12	0.057	0.568	0.815
<u>DBP</u>							
Baseline	74 ± 6	75 ± 7	76 ± 12	74 ± 11	0.705	0.819	0.377
Pre-Buffet	74 ± 6	74 ± 7	75 ± 9	72 ± 9	0.935	0.393	0.286
Post-Buffet (0)	73 ± 6	76 ± 5	74 ± 9	74 ± 9	0.774	0.260	0.233
Post Buffet (60)	72 ± 7	69 ± 8	73 ± 9	72 ± 9	0.318	0.086	0.373
Post-Buffet (120)	71 ± 8	72 ± 7	70 ± 8	73 ± 8	0.948	0.079	0.299
Post-Buffet (180)	73 ± 6	73 ± 6	73 ± 11	75 ± 9	0.654	0.276	0.621

Data: Mean ± SD
Units for Heart Rate: Beats/Min
SBP/DBP: Systolic Blood Pressure/Diastolic Blood Pressure
Units for SBP & DBP: mmHg

Table 6: Satiety Questionnaire Derived Data Before and After 12-Weeks of Dietary Counseling Supplemented With or Without Sodium Glucose Co-Transport 2 Inhibition

	Pill A		Pill B		ANOVA P-Values		
	Pre-Counseling	Post-Counseling	Pre-Counseling	Post-Counseling	Group	Counseling	Interaction
Baseline							
Q1	49 ± 26	52 ± 29	37 ± 30	37 ± 30	0.069	0.735	0.646
Q2	49 ± 25	50 ± 29	35 ± 29	35 ± 28	**0.041**	0.892	0.892
Q3	20 ± 14	23 ± 17	26 ± 23	22.16 ± 18	0.583	0.994	0.215
Q4	50 ± 14	53 ± 23	49 ± 25	42 ± 22	0.247	0.571	0.098
Q5	51 ± 19	57 ± 22	56 ± 24	60 ± 22	0.414	0.213	0.658
Post-Meal Primer (0)							
Q1	47 ± 24	46 ± 26	34 ± 24	32 ± 26	**0.023**	0.816	0.789
Q2	47 ± 24	48 ± 25	35 ± 26	29 ± 24	**0.010**	0.678	0.24
Q3	25 ± 14	30 ± 15	30 ± 21	35 ± 21	0.216	0.089	0.951
Q4	49 ± 18	51 ± 22	47 ± 22	39 ± 25	0.160	0.57	0.054
Q5	45 ± 17	52 ± 22	48 ± 24	54 ± 23	0.687	0.082	0.884
Post-Meal Primer (60)							
Q1	54 ± 19	48 ± 24	44 ± 24	38 ± 21	0.077	0.075	0.9
Q2	53 ± 18	53 ± 21	42 ± 25	39 ± 20	**0.016**	0.565	0.663
Q3	21 ± 12	28 ± 16	27 ± 16	31 ± 18	0.237	**0.044**	0.526
Q4	57 ± 13	51 ± 20	52 ± 17	44 ± 19	0.152	**0.015**	0.582
Q5	48 ± 18	53 ± 21	59 ± 20	58 ± 19	0.103	0.446	0.276
Post-Buffet (0)							
Q1	14 ± 14	11 ± 10	8 ± 9	12 ± 8	0.388	0.768	0.193
Q2	12 ± 12	11 ± 11	6 ± 6	8 ± 7	**0.033**	0.87	0.468
Q3	68 ± 15	67 ± 20	72 ± 14	72 ± 18	0.271	0.871	0.782
Q4	17 ± 14	13 ± 10	14 ± 15	14 ± 14	0.762	0.333	0.551
Q5	34 ± 21	37 ± 23	41 ± 24	42 ± 22	0.271	0.561	0.831
Post-Buffet (60)							
Q1	15 ± 15	15 ± 14	11 ± 8	11 ± 9	0.158	0.795	0.952
Q2	12 ± 9	14 ± 15	10 ± 9	10 ± 9	0.29	0.563	0.689
Q3	62 ± 19	63 ± 19	57 ± 22	56 ± 21	0.218	0.929	0.78
Q4	20 ± 14	19 ± 17	21 ± 17	19 ± 16	0.854	0.373	0.845
Q5	38 ± 19	43 ± 22	40 ± 18	48 ± 17	0.409	**0.03**	0.585
Post-Buffet (120)							
Q1	22 ± 16	24 ± 14	22 ± 18	15 ± 13	0.265	0.345	**0.044**
Q2	20 ± 15	20 ± 14	21 ± 14	15 ± 10	0.425	0.222	0.235
Q3	54 ± 20	56 ± 17	52 ± 22	49 ± 20	0.336	0.938	0.429
Q4	20 ± 19	24 ± 14	30 ± 18	28 ± 18	0.624	0.211	0.603
Q5	43 ± 21	44 ± 20	46 ± 18	50 ± 18	0.528	0.372	0.74
Post-Buffet (180)							
Q1	35 ± 20	36 ± 19	33 ± 24	25 ± 20	0.2	0.249	0.116
Q2	35 ± 20	36 ± 20	34 ± 25	25 ± 21	0.254	0.12	0.105
Q3	43 ± 15	48 ± 18	41 ± 20	40 ± 20	0.205	0.499	0.324
Q4	40 ± 13	36 ± 18	40 ± 19	33 ± 21	0.645	0.053	0.693
Q5	47 ± 21	47 ± 23	52 ± 17	56 ± 18	0.149	0.511	0.561

Data: Mean ± SD

Units for all scores: mm

Q1: How strong is your desire to eat right now?

Q2: How hungry do you feel right now?

Q3: How full do you feel right now?

Q4: How much do you think you could eat right now?

Q5: How thirsty do you feel right now?

Table 7: Dietary Logs and Recall Data Derived in Week 0, Week 5, and Week 10 of Dietary Counseling

	Pill A			Pill B			ANOVA P-Values		
	Week 0	**Week 5**	**Week 10**	**Week 0**	**Week 5**	**Week 10**	**Group**	**Counseling**	**Interaction**
Total Kcal	2181 ± 533	1586 ± 386	1644 ± 484	1820 ± 492	1698 ± 672	1544 ± 444	0.236	**<0.001**	**0.041**
Protein (g)	85 ± 24	74 ± 29	82 ± 40	80 ± 25	74 ± 31	78 ± 36	0.656	0.299	0.886
CHO (g)	246 ± 73	167 ± 61	173 ± 78	197 ± 59	186 ± 79	160 ± 62	0.316	**<0.001**	**0.019**
Fat (g)	91 ± 28	68 ± 23	70 ± 24	77 ± 26	72 ± 39	65 ± 31	0.425	**0.005**	0.251
Protein (%)	16 ± 3	19 ± 8	20 ± 8	18 ± 5	18 ± 6	20 ± 7	0.73	**0.017**	0.201
CHO (%)	45 ± 9	42 ± 10	42 ± 13	43 ± 7	44 ± 9	41 ± 11	0.999	0.244	0.423
Fat (%)	37 ± 7	38 ± 10	39 ± 11	38 ± 7	37 ± 9	38 ± 11	0.805	0.817	0.705

Data: Mean ± SD

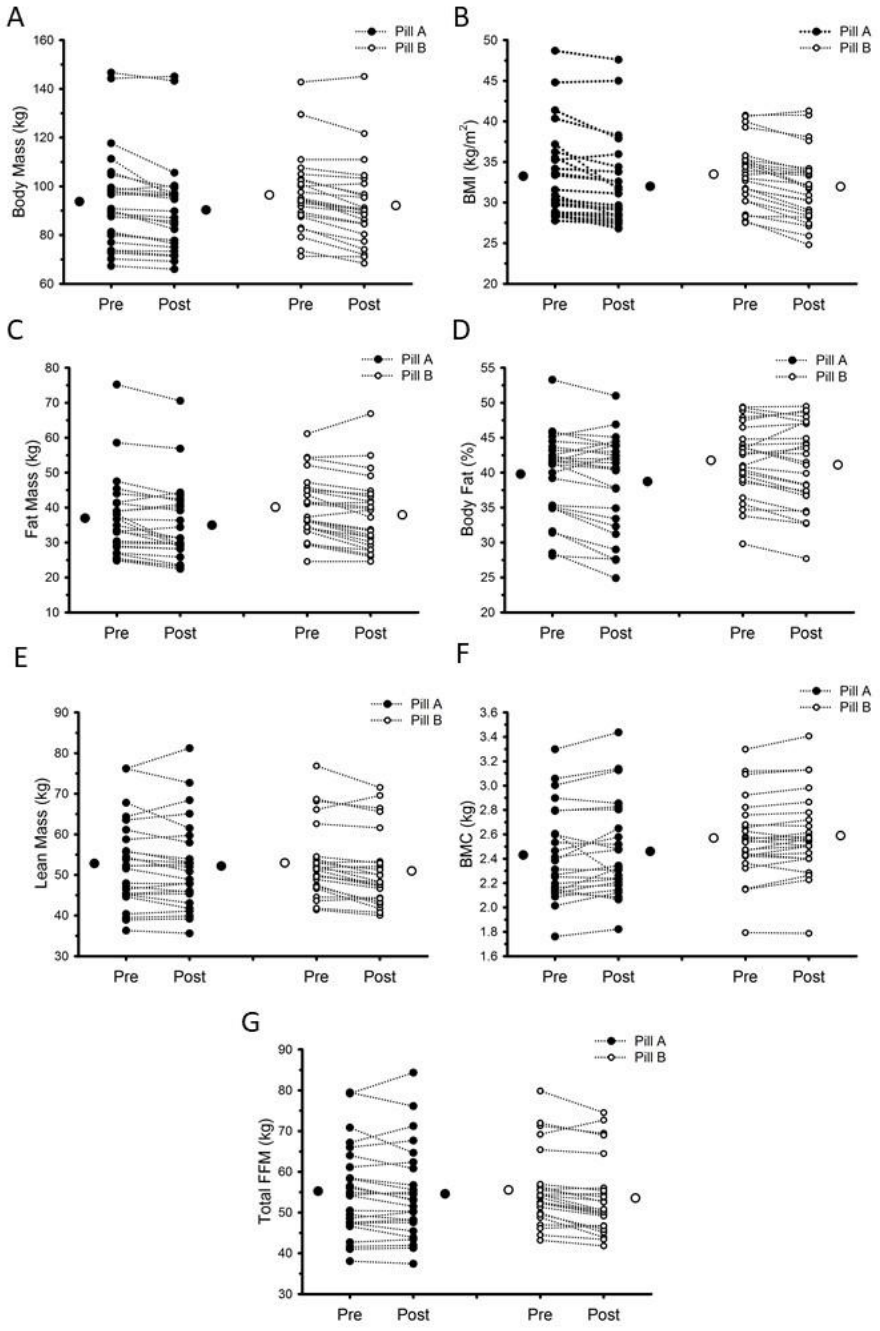

Figure 2: Dietary counseling favorably modifies body mass and composition; SGLT2 inhibition does not influence any of these adaptations except for lean mass. A. Body mass. B. Body mass index. C. Fat mass. D.% Body Fat. E. Lean Body Mass. F. Bone Mineral Content. G. Total Fat Free Mass. In all graphs Pre and Post refer to data collected before and after 12-weeks of dietary counseling. Large dark filled circles and large open white circles represent mean responses for placebo and SGLT2 inhibition, respectively. Circles connected via dotted lines represent individual participants pre and post intervention. See Table 3 for results of statistical analysis.

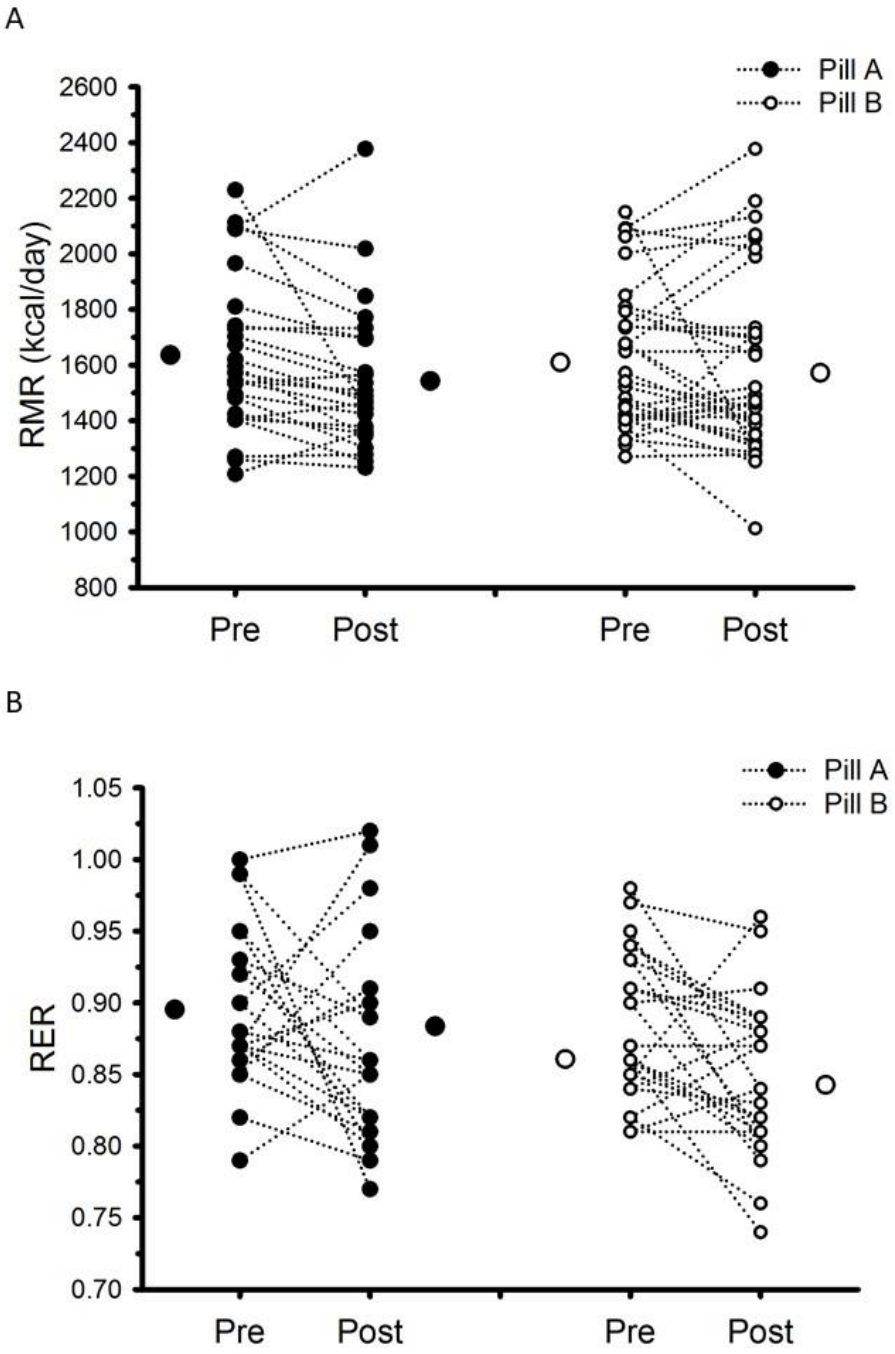

Figure 3: Dietary counseling decreases RMR and RER; SGLT2 inhibition does not influence any of these adaptations except for lean mass. A. RMR. B. RER. In all graphs Pre and Post refer to data collected before and after 12-weeks of dietary counseling. Large dark filled circles and large open white circles represent mean responses for placebo and SGLT2 inhibition, respectively. Circles connected via dotted lines represent individual participants pre and post intervention. See Table 4 for results of statistical analysis.

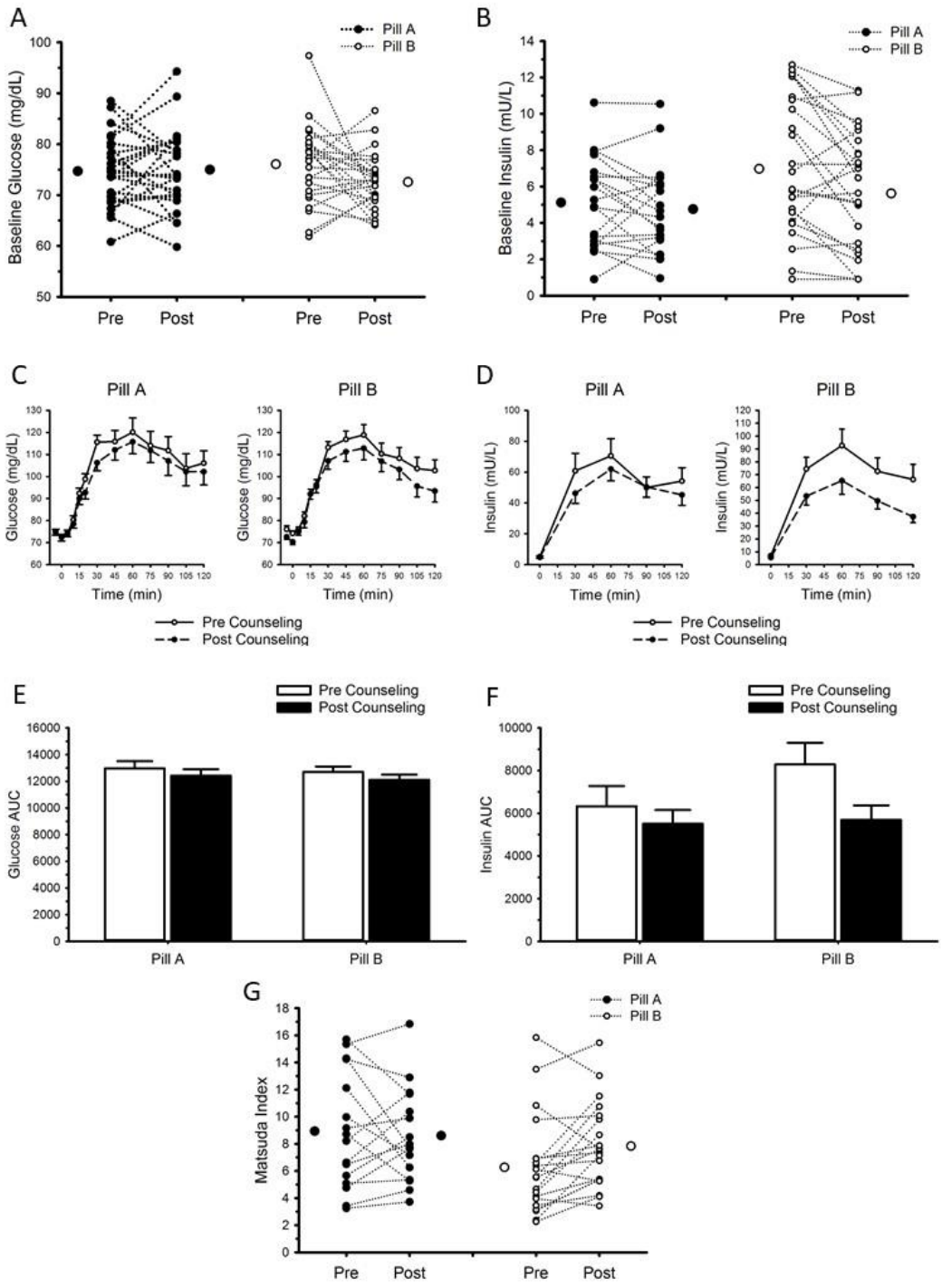

Figure 4: Dietary counseling favorably modifies baseline circulating insulin and insulin AUC; SGLT2 inhibition does not influence these adaptations. A. Baseline blood glucose concentration. B. Baseline blood insulin concentration. C. Circulating glucose concentrations during OGTT (post 75 g of glucose ingestion). D. Circulating insulin concentrations during OGTT (post 75 g of glucose ingestion). E. OGTT Glucose AUC. F. OGTT Insulin AUC. G. Matsuda Index. See Table 4 for data and statistical analysis

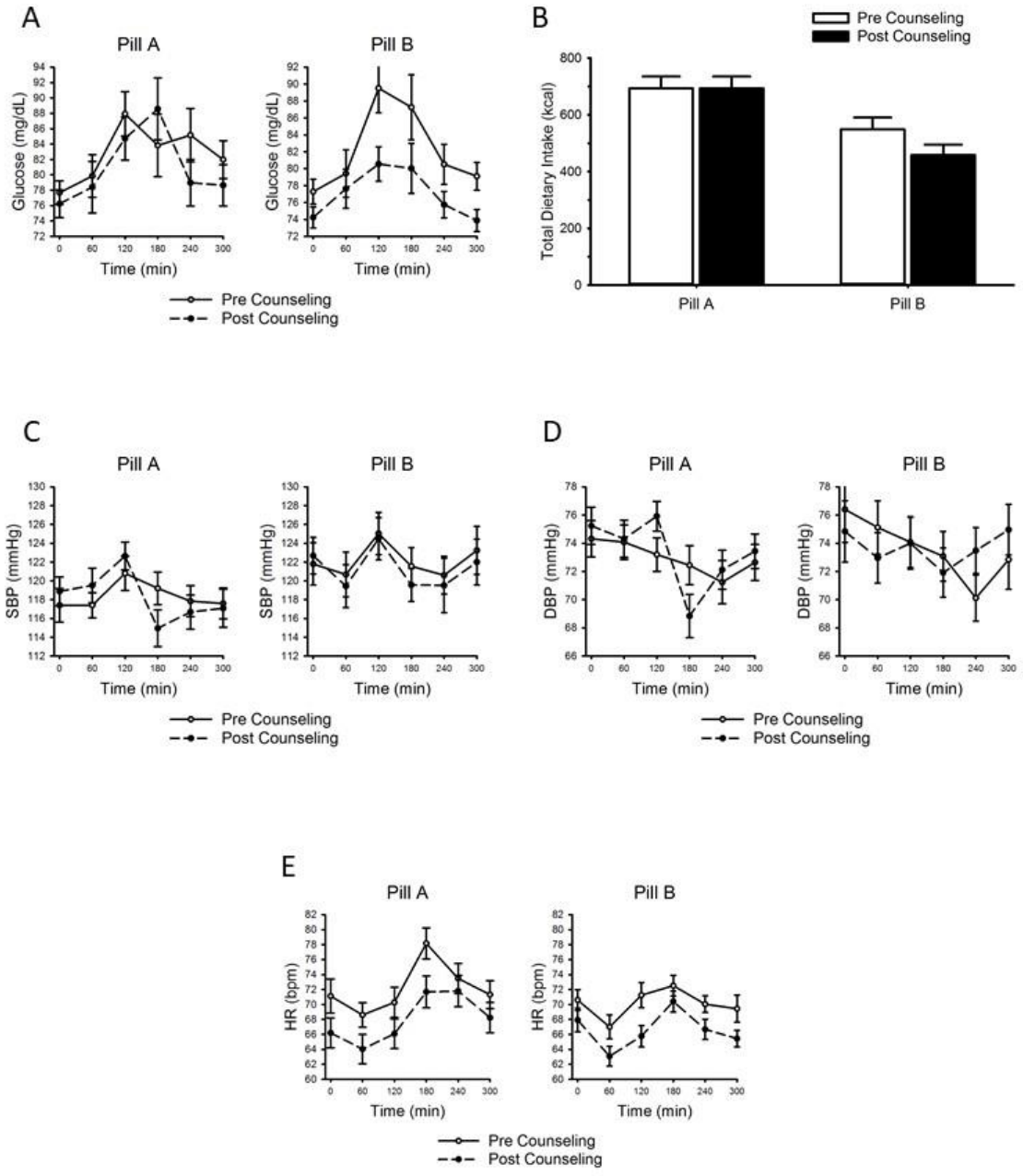

Figure 5: Dietary counseling favorably modifies glucose regulation and heart rate and decreases fat intake; SGLT2 inhibition does not influence these adaptions or outcomes. A. Circulating glucose concentration during hunger and appetite assessment. B. Food consumption in kcal for 20 minutes all-you-can-eat breakfast buffet. C. Systolic blood pressure D. Diastolic blood pressure. E. Heart rate responses See Table 5 for data and statistical analysis.

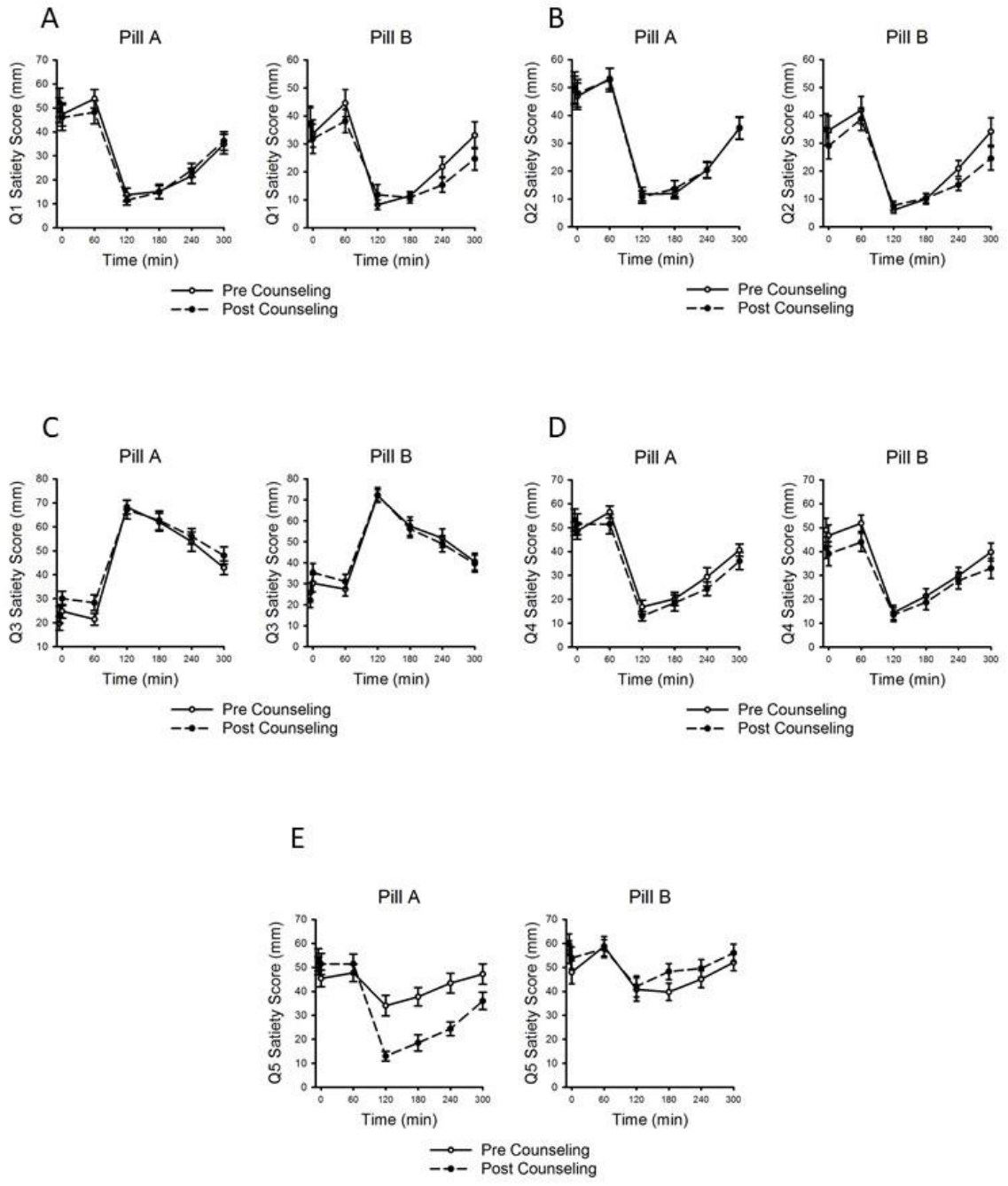

Figure 6: Dietary counseling has no effect on appetite or satiation; SGLT2 has no influence on these outcomes. A. Question 1. B. Question 2. C. Question 3. D. Question 4. E. Question 5. All panels are time course during hunger and appetite assessment. Specific content of each question is found in the Methods section. See Table 6 for data and statistical analysis.

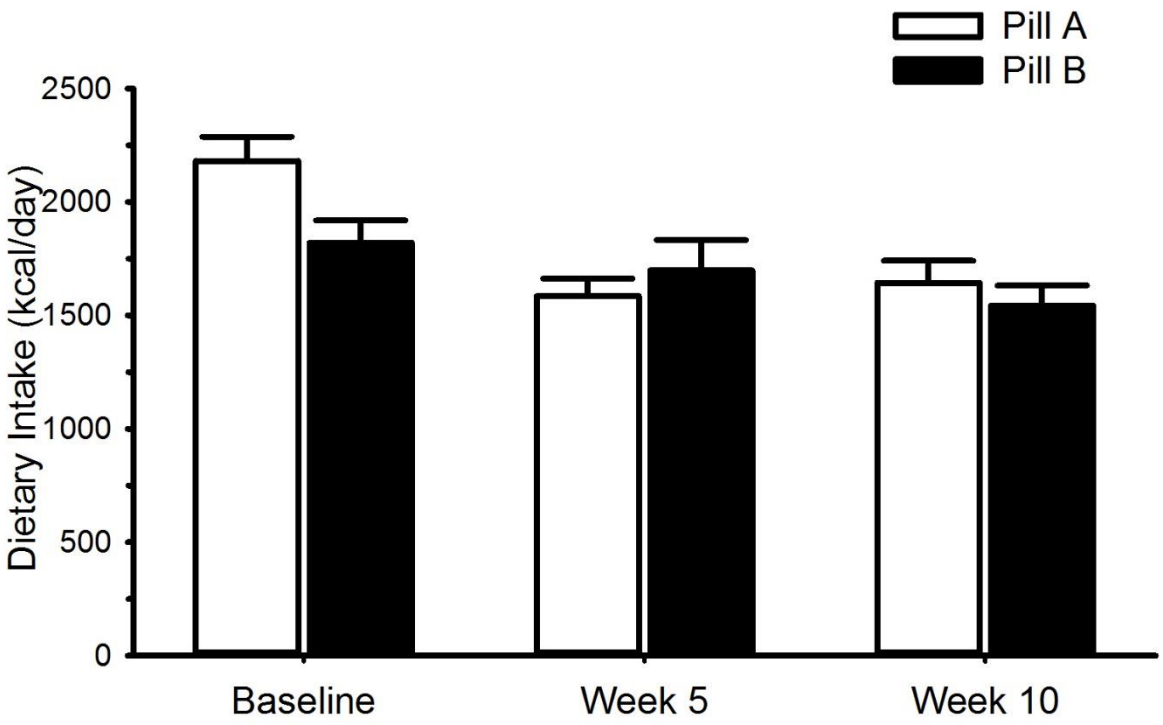

Figure 7: Dietary counseling decreases total caloric consumption, specifically by decreasing carbohydrate and fat intake; SGLT2 inhibition does not influence these outcomes. See Table 6 for data and statistical analysis.

5. DISCUSSION

The purpose of the current study was to determine if SGLT2 inhibition would augment the effects of 12-weeks of dietary counseling. Twelve weeks of dietary counseling favorably modified body mass, fat mass, and heart rate; SGLT2 inhibition did not influence any of these outcomes. The novel observation of the current study is SGLT2 inhibition neither augmented nor attenuated the beneficial physiological outcomes; however, Pill A attenuated the loss of lean mass or Pill B exacerbated the loss of lean mass.

Body Composition

There is overwhelming evidence that obesity is a major determinant in the development of Type 2 Diabetes (King et al., 1990; Lindström et al., 2003; Manson et al., 1991). Excess adipose tissue is associated with ectopic lipid accumulation resulting in lipotoxicity, the damaging effects of excess fat accumulation on glucose metabolism (DeFronzo, 2010; Unger, 2003). Therefore, by promoting glucosuria and weight loss, SGLT2 inhibition can theoretically reverse the lipotoxic effects on insulin sensitivity. In the current study, SGLT2 inhibition did not influence the favorable modification of body composition with dietary counseling. However, Pill B exacerbated the loss of lean mass.

Patients with T2D have elevated renal glucose reabsorption compared to their healthy counterparts (Gerich, 2010; Liang et al., 2012; Meyer et al., 1998; Meyer et al., 2004; Mogensen, 1971; Rave et al., 2006; Ruhnau et al., 1997; Tabatabai et al., 2009; Wilding et al., 2014). Thus, SGLT2 inhibition may have a greater magnitude of change on patients with T2D than in healthy adults. A person without T2D already has normal glucose excretion. Therefore, glucosuria will theoretically enhance weight loss by increasing urinary excretion of calories as glucose.

Unfortunately, this investigation did not measure the amount of glucose excreted with and without SGLT2 inhibition. Future research needs to quantitatively compare the magnitude of influence on renal glucose excretion between patients with T2D and healthy adults.

Dietary-induced weight loss is typically accompanied by a decline in skeletal muscle mass (Bopp et al., 2008; Cava et al., 2017). The current investigation found that Pill B exacerbated the loss of lean mass during dietary counseling for weight loss. It is expected that SGLT2 inhibition may enhance loss of lean mass through urinary glucose excretion. When glucose is excreted, the energy demanding tissues turn to fat and protein as alternate sources of fuel; hence increasing protein degradation (Cava et al., 2017). Additionally, with less circulating glucose and in caloric deficit, the rate of glycogenolysis increases causing a decrease in muscle glucose storage (Berg et al, 2002). Although the muscle glycogen stores only make up about 1-2% of muscle mass, a 70kg person can store roughly 400 grams of glycogen (Wasserman, 2009). In fact, the average lean body mass in the current study was 52.83 kg. If glycogen accounts for 1-2% of lean mass, participants potentially lost between 0.5-1 kg of lean mass due to glycogen depletion. If SGLT2 inhibition increases lean mass loss, it may be important for those taking SGLT2 inhibitors to increse protein consumption. Protein consumption is known to attenuate lean mass loss during caloric restriction (Cava et al., 2017; Pasiakos et al., 2013; Phillips et al., 2012; Verreijen et al., 2015). In order to remedy the observed loss of lean mass, future studies should investigate high protein dietary weight loss supplemented with SGLT2 inhibitors. Until further investigation, baseline lean mass health status and final goals for weight loss with SGLT2 inhibition require consideration prior to initiation.

A mechanism explaining how SGLT2 inhibition may attenuate lean mass is somewhat elusive. One reason may be due to inhibition of SGLT2 proteins in the glucagon-secreting alpha-

cells of the pancreatic islets. Therefore, SGLT2 inhibition increases glucagon secretion and endogenous (hepatic) glucose production (Bonner et al., 2015). The elevated hepatic glucose production might increase potential for glycogen storage in insulin sensitive adults, thus attenuating lean mass loss. Assuming Pill A is dapagliflozin, these data may have important implications for preserving lean mass, providing a greater reservoir for glucose storage and oxidation, and therefore maintenance of metabolic health, during caloric restriction. However, the liver is included in lean mass calculations. The increase in hepatic glucose production may, in theory, lower liver mass (lean mass). Thus, hepatic glucose production likely plays a limited role, if any, in attenuating lean mass loss. Further research must investigate the mechanism behind SGLT2 inhibition mediated lean mass maintenance.

The higher bone mineral content (BMC) following weight loss was unexpected. All current research points to the expectation that absolute BMC should decrease following weight loss. Additionally, weight loss is often accompanied by lower absolute BMC (Jensen et al. 2001; Taylor et al. 2015). For instance, canagliflozin, another SGLT2 inhibitor, is associated with decreased bone mineral content and increased fracture risk (Blevins et al. 2017). Hence the current finding is surprising. However, it is important to note that although this increase in BMC is statistically significant, the clinical/physiological significance of an increase of the reported magnitude may be questionable. Regardless, these results provide evidence that SGLT2 inhibition via dapagliflozin is not associated with decreased bone health.

RMR

Resting metabolic rate often decreases with weight loss (Busetto et al., 1995; Byrne et al., 2012; Fothergill et al., 2016; Johannsen et al., 2012). The influence of SGLT2 inhibition on energy expenditure is inconsistent across studies. Several investigations found SGLT2 inhibition

shifts macronutrient oxidation from carbohydrates to fat while maintaining energy expenditure in people with T2D despite the calories lost in urine (Abdul-Ghani et al., 2016; Kanazawa et al., 2019). Other studies found a null effect in patients with T2D (Ferrannini et al., 2014) and a decrease in basal metabolic rate in obese and overweight women with Polycystic Ovary Syndrome (Javed et al., 2019). The current investigation discovered SGLT2 inhibition did not influence the dietary counseling induced decline in RMR. Lean mass heavily influences variation in RMR (Rolfe et al., 2017); therefore, we expected Pill B to have a larger decrease in RMR. Despite statistical significance, the magnitude of lean mass lost in participants supplemented with Pill B compared to Pill A may not overcome the large variability observed in RMR. This is supported in the current investigation by the lack of correlation between the magnitudes of change in lean mass and RMR, and a high ANCOVA p-value with RMR as the dependent variable and lean mass as the covariate. Another plausible reason for a decrease in RMR is lower sympathetic stimulation and/or decreased adrenergic receptor responsiveness (Bell et al., 2001; Straznicky et al., 2010). However, quantification of the sympatho-adrenal contribution to RMR is beyond the scope of the current investigation and consequently we are unable to address this potential mechanism directly. Overall, SGLT2 inhibition does not influence dietary counseling for weight loss induced decreases in RMR.

Insulin Sensitivity

Augmented glucosuria reduces fasting plasma glucose, improves glucose tolerance, and promotes calorie loss; leading to reduced glucose toxicity (decreased chronic hyperglycemia), weight loss, reduced lipotoxicity, improved β-cell function, and insulin sensitivity (Bonner et al., 2015; Chen et al., 2013; González-Ortiz et al., 2018; Idris et al., 2009; Katsiki et al., 2010; Merovci et al., 2015; Tang et al., 2017). In opposition to previous discoveries, the current

investigation found no improvement in insulin sensitivity post dietary counseling, thus suggesting weight loss does not improve insulin sensitivity. However, the influence of SGLT2 inhibition on insulin sensitivity was trending towards significance (P=0.055). Comparing average Matsuda scores, Pill A decreased slightly (Pre: 8.94 mU/L, Post: 8.61 mU/L), while Pill B increased (Pre: 6.27 mU/L, Post: 7.84 mU/L). Neither change was statistically significant (P=0.135). Potentially, a future study with larger sample sizes will find a significant drug interaction on insulin sensitivity. Based on the results of our literature search, this is the first study to find a null result on the influence of SGLT2 inhibition on insulin sensitivity in non-exercising adults without diabetes undergoing dietary counseling. However, SGLT2 inhibition initiates glucagon secretion, stimulating endogenous hepatic glucose release (Bonner et al., 2015) and dampening the potential influence on insulin sensitivity (Newman et al. 2018). Metabolically healthy adults, such as those investigated in this study, adapt to increased endogenous hepatic glucose release by naturally increasing glucosuria, storage, and oxidation (Goodpaster et al., 2017; Wilding, 2014), thus explaining the null effect SGLT2 inhibition has on circulating glucose and insulin sensitivity. It is likely SGLT2 inhibition has greater efficacy in patients with T2D than their healthy counterparts.

Appetite and Satiety

If the weight loss observed with SGLT2 inhibitors is less than hypothesized based on the glucosuria caloric loss, one would assume that SGLT2 inhibitors might increase hunger and appetite (Cefalu, 2014; Ferrannini et al., 2014; Zhang et al., 2014). It is likely that patients increase caloric intake rather than reduce energy expenditure when taking an SGLT2 inhibitor (Ferrannini et al., 2014). Data on whether SGLT2 inhibitors instigate increased appetite and caloric consumption are inconclusive. SGLT2 inhibition does not appear to influence energy

intake (Bertran et al., 2018). The current investigation found no significant changes in appetite and satiety other than lower subjective feeling of "fullness" 60 minutes post meal primer as evidenced by lower scores in question 3 of satiety surveys. Although statistically significant, the improved satiety is likely not clinically significant. Despite lower hunger scores, participants had the same total energy intake pre and post counseling. Therefore, this investigation supports previous evidence indicating SGLT2 inhibition does not influence appetite.

Limitations:

There are several limitations in the current investigation. We chose to focus on minimally active overweight and obese adults, without any diagnosed metabolic disorders. Although free from diabetes, this population is at risk for developing insulin resistance because of a mostly sedentary lifestyle and poor body composition. Our rationale for choosing this population allows investigation into the potential interaction between weight loss and SGLT2 inhibition without complications associated with metabolic dysfunction such as insulin resistance, improper fatty acid oxidation, and intramyocellular lipid accumulation (Andrews et al., 2011; Goodpaster et al., 2017; Kelley et al., 1999; Sitnick et al., 2009). Although it is reasonable to question the effectiveness/relevance of SGLT2 inhibition in a metabolically healthy adult free, at least two independent studies have investigated dapagliflozin (10 mg/day) induced SGLT2 inhibition in healthy, diabetes-free adults (Komoroski et al., 2009; Yang et al., 2013). Furthermore, numerous pharmacotherapy protocols studied healthy, disease-free populations (Bahls et al., 2017; Braun et al., 2008; Carlson, Tou, Parikh, Birmingham, & Butler, 2011; Duff et al., 2017; Kohrt et al., 2010; Ring et al., 2013; Scalzo et al., 2014), including the Diabetes Primary Prevention Trial (Knowler et al., 2002), in which the efficacy of metformin was investigated in adults without diabetes. Thus, we remain convinced with the rationale for our protocol.

Another limitation of our study was the use of 'free-living participants and therefore lack of control over participants' energy intakes. We did not supply calorie-reduced meals to participants; participants were free to cook and eat as they pleased. The rationale for our approach was based on maximizing ecological validity. We recognize that this approach may introduce potential bias and/or variability in our data, thus lowering the chance of finding statistical significance. However we do not believe it influenced our outcomes and/or interpretation of data because this bias was likely randomly distributed and not systematic. Also, while the average weight loss experienced by the study participants was only 3-4 kg, we intentionally chose to focus on modest weight reduction for the following reason. If SGLT2 inhibition alone were to produce a small decrease in body weight, this effect could be totally lost with large decreases in body weight induced by a severely energy-restricted, very-low calorie diet. Based on our findings, there is little evidence to indicate that SGLT2 inhibition contributes to a greater magnitude of the energy deficit when accompanied by 12 weeks of weight loss counseling compared to counseling alone.

Incorporating two more groups: 1). Placebo with no dietary counseling and 2). dapagliflozin with no dietary counseling could allow for further differentiation between dapagliflozin and counseling. However, the time and financial constraints did not make this feasible.

We decided to study glucose regulation using the oral glucose tolerance test instead of the insulin sensitivity via the gold-standard hyperinsulineimic euglycemic clamp technique. Our choice of technique was driven by the lighter burden imposed on research participants.

CONCLUSION:

Dietary counseling significantly influences body composition in overweight/obese adults free of metabolic disorder. Twelve weeks of dietary counseling favorably modified body mass, fat mass, heart rate, and insulin sensitivity. The novel observation of the current study is SGLT2 inhibition neither augmented nor attenuated these beneficial physiological outcomes; however, Pill B exacerbated the loss of lean mass (muscle mass). These data may have important implications in the modification of lean mass, and therefore maintenance of metabolic health, during caloric restriction.

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