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EFFECT OF A DRIED PLUM NUTRITIONAL INTERVENTION ON GLUCOSE REGULATION IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Estrogen deficiency during the postmenopausal time period leads to significant metabolic changes, putting women at risk for metabolic syndrome (MetS), type 2 diabetes mellitus (T2DM), and cardiovascular diseases (CVD). As such, finding treatments which enhance healthy metabolism and insulin sensitivity in postmenopausal women is a pressing health concern. Recently, dried plums (DP) have been investigated as a potential nutraceutical treatment for metabolic abnormalities, such as insulin resistance, impaired glucose and lipid handling, and inflammation. This randomized control trial assessed the effects of a 12-month DP nutritional intervention on blood glucose, insulin resistance, and body fat distribution. Participants were postmenopausal women with osteopenia (n=124) who were randomized to consume either 50g DP/day (50g DP; n=46), 100g DP/day (100g DP; n=35), or no DP/day (control; n=46). At baseline, the women were 62.3±0.5 years in age and had an average age of menopause of 50.7±0.4 years. Seventy-two percent of participants had no previous hormone therapy use. There were no significant differences between the intervention groups or control group at baseline. The aims of this study were to assess the relationship between a DP nutritional intervention, glucose metabolism, and body fat distribution. Fasting blood glucose increased by 3.5 mg/dL in the 50g DP group from baseline to 6-months (p=0.037), contrary to our hypothesis. Additionally, the absolute change over 12-months in fasting blood glucose was significantly different between 50g DP and 100g DP (p=0.009), with 50g DP group increasing by 4.1% and 100g DP group decreasing by 3.2%. Total android mass increased significantly in the entire cohort from baseline to 6-months (p=0.006). The 50g DP group had significantly higher visceral adiposity measurements (mass: p=0.036); volume: p=0.036; area: p=0.033), BMI (p=0.025), and

total body fat (p=0.019) compared to the control throughout the entire study. All measures of central adiposity were found to be significantly and positively correlated with 12-month fasting blood glucose, fasting blood insulin, and HOMA-IR (all $p<0.001$). Such findings indicate that while dried plum consumption does not result in a dose-dependent reduction in fasting blood glucose since the 50g DP group experienced an increase in fasting glucose, consuming a larger dosage of dried plums (i.e., 100g DP) may be beneficial to reduce fasting glucose in postmenopausal women. Despite an increase in glucose concentration throughout the intervention in the 50g DP group, glucose concentrations remained below a pre-diabetic threshold, and it is unlikely that postmenopausal women will be at an increased risk of MetS, T2DM, or CVD as a result of consuming a moderate amount of dried plums. As such, the 100g DP intervention may be a better supplement dosage than 50g in postmenopausal women as it demonstrated no adverse effects on glucose metabolism or insulin resistance.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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Chapter 1

Introduction

Dried Plum and Menopause-related Metabolic Abnormalities

Menopause marks an important physiological change in a woman's life. The decrease in ovarian secretion of estrogen has widespread consequences beyond reproductive health. Estrogen deficiency during the postmenopausal time period leads to significant metabolic changes [1]. Indeed, metabolic disturbances including dyslipidemia, altered body fat distribution, and indicators of insulin resistance have all been shown to be associated with the menopause transition [2-5]. These changes have important implications in the risk of metabolic diseases for postmenopausal women [2-5].

Estrogen deficiency-related metabolic changes share many common features with the abnormal metabolic profile known as metabolic syndrome (MetS). Diagnosis of MetS requires the presence of at least three of the following five features: increased waist circumference, high triglycerides (TG), low high-density lipoprotein (HDL), hypertension, and hyperglycemia [6]. Menopause is associated with increased visceral body fat, low HDL, and hyperglycemia [2-5], and as such these commonalities imply that menopause may lead to MetS. MetS is a detrimental syndrome because it significantly increases the risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [6]. Because CVD is the number one cause of death for women [4] and T2DM can lead to life threatening consequences, such as diabetic coma as well as increased risk of CVD [7], it is important to find therapies for postmenopausal women to improve the metabolic abnormalities that place them at risk for MetS and associated chronic diseases.

Menopause is associated with changes in body fat distribution, with postmenopausal women tending to store fat in the abdomen compared with premenopausal women [2, 4]. Specifically, visceral fat mass increases with menopause [2]. An increase in visceral fat mass is significant to health as visceral fat is related to pathological metabolic conditions, such as dysfunctional adipocytes, impaired insulin function, and excess lipids and inflammatory molecules exported into the blood [5, 8]. A study in postmenopausal women reported a strong positive relationship between visceral fat mass and insulin resistance [5]. Additionally, the increase in total fat mass that occurs as a function of age is also related to insulin resistance in postmenopausal women [5].

Dietary and lifestyle interventions have been observed to prevent progression to T2DM in patients with pre-diabetes, that is fasting glucose levels between 99 and 125 mg/dL [9, 10]. Modifications to diet and lifestyle are advantageous over pharmacological interventions as they are less expensive, do not cause side effects, and provide whole body health benefits beyond diabetes prevention. One such dietary intervention that is being investigated as a treatment for metabolic abnormalities is the consumption of plum products including fresh plums, plum concentrate, and dried plums.

In rat models, plum-derived products have shown promising impacts on indicators of metabolic health. In obese Zucker rats, plum juice consumption over 11 weeks prevented body weight gain [11]. Further, markers of MetS including blood glucose, insulin, and leptin were all reduced [11]. Additionally, plum juice improved dyslipidemia, as indicated by lowered plasma total cholesterol (TC) and TG, and reduced oxidation of low-density lipoprotein (LDL), as indicated by reduced thiobarbituric acid reactive substances in the rats [11]. In insulin-resistant Wistar fatty rats, plum concentrate (0.25% concentration in water) administered for 2 weeks was

shown to improve markers of insulin sensitivity, namely blood glucose and TG [12]. Following ovariectomy, female rats consuming a 25% dried plum diet did not experience the rise in LDL cholesterol typically observed in ovariectomy-induced estrogen deficiency [13].

Despite the promising research in animal models, research investigating the health benefits of dried plum in humans is limited. However, in healthy young men, 250 grams (g) of dried plum lowered insulin secretion after 22 days of consumption [13]. Another study in men reported lower LDL cholesterol after 4 weeks of dried plum consumption at a dose of 100g per day, compared to a 360mL per day grape juice intervention [13]. In postmenopausal women, markers of inflammation, namely lipid hydroperoxide and C-reactive protein, were reduced following consumption of 100g dried plum per day for 12 months, which may lower risk of CVD and T2DM [14].

Dried plums are commonly recognized for their health benefits related to gastrointestinal motility due to dietary fiber content [13]. However, dried plums provide more nutritional value than fiber alone; a key nutritional component of dried plums that impacts carbohydrate and lipid metabolism is dietary phenolics [11, 12]. Dried plums have a high antioxidant capacity due to their high levels of phenolic compounds, namely chlorogenic acid and neochlorogenic acid [15]. Dietary phenolics have been shown to lower various markers of metabolic health such as blood glucose, insulin resistance, oxidation, and inflammation in a variety of experimental models including rats, men, and postmenopausal women [11, 12, 14, 16].

Phenolics in plums improve carbohydrate and lipid metabolism by stimulating peroxisome proliferator-activated receptor (PPAR) expression which improves insulin sensitivity [11]. In the liver, phenolics stimulate PPARα leading to a decrease in TG in the blood by promoting hepatic uptake of TG via hepatic lipoprotein lipase (LPL), hepatic uptake of non-

esterified fatty acids, and subsequent fatty acid oxidation in the hepatocytes [12, 17]. Another anti-dyslipidemic effect of PPARα activation is an increase in plasma HDL [17]. Current literature indicates PPARα activation prevents development of T2DM in individuals with prediabetes [17]. In adipose tissue, phenolics upregulate PPARγ which leads to increased secretion of adiponectin from adipocytes, increased flux of lipids from the liver and skeletal muscle to adipose tissue, and associated improved insulin sensitivity [12, 17]. In vascular cells, PPARγ promotes insulin sensitivity when stimulated by phenolics [11]. The net effect of PPAR activation by phenolics is improved markers of metabolic health, specifically insulin sensitivity. Beyond PPAR activation, phenolics, namely chlorogenic acid, impact carbohydrate metabolism by inhibiting both intestinal absorption of glucose and hepatic glycogenolysis [13].

The phenolic profile of dried plums also imparts a high antioxidant capacity. Despite the loss of anthocyanins, a class of dietary phenolics [16], in the process of heating fresh plums to create dried plums, the antioxidant activity of dried plums is higher than that of fresh plums [15]. Phenolics protect against oxidative damage by promoting antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase [11]. Investigators have shown that the phenolics in dried plums protect against LDL oxidation [13], an inflammatory response which can lead to atherosclerosis [14]. Phenolics in dried plum have also been shown to lower lipid hydroperoxide, a marker of oxidative stress, in postmenopausal women [14].

Phenolics act as anti-inflammatory agents by inhibiting the production of inflammatory molecules [16]. As mentioned above, phenolics are PPAR agonists. PPARs produce anti-inflammatory effects by both inhibiting inflammatory cytokine production and promoting the expression of anti-inflammatory genes [17]. In the study of obese Zucker rats mentioned above, plum juice reduced levels of inflammatory cytokines in rat hearts [11]. In postmenopausal

women, dried plum intervention has been shown to lower C-reactive protein (CRP), a marker of inflammation [14].

Dried plum phenolics improve markers of metabolic health through multiple mechanisms, including PPAR upregulation, antioxidant activity, and anti-inflammatory activity [11-14]. Improving insulin sensitivity is a key feature of dried plum phenolic compounds, as insulin resistance interacts with multiple risk factors for T2DM and CVD [6, 7]. Insulin resistance is the factor that ties abnormal adipocyte function to clinical effects, including hyperglycemia and dyslipidemia [6, 18]. Because menopause is associated with many of the same metabolic abnormalities observed in insulin resistance, dried plum could be a viable intervention for improving risk factors of T2DM and CVD in postmenopausal women.

Purpose of this Study

The purpose of this study was to quantify the effect of a 12-month dried plum nutritional intervention in postmenopausal women with osteopenia/osteoporosis aged 55-75 consuming either 50g (6 dried plums/day) or 100g (12 dried plums/day) of dried plum daily on metabolic disease risk factors including fasting blood glucose, insulin, HOMA-IR, and indicators of central adiposity.

Aim and Hypothesis 1: Metabolic Markers

Aim: To determine the effects of a 12-month intervention of consuming dried plums on fasting blood glucose concentration and HOMA-IR in postmenopausal women with

osteopenia/osteoporosis consuming 50g dried plums/day, 100g dried plums/day, or no plums (control group).

Hypothesis 1A: Following the 12-month intervention, postmenopausal women consuming either 50g or 100g dried plums/day will have a greater reduction (from baseline values) in fasting blood glucose, compared to the control group.

Hypothesis 1B: Following the 12-month intervention, HOMA-IR will be lower in postmenopausal women consuming either 50g or 100g dried plums/day compared to the control group.

Aim and Hypothesis 2: Body Fat Distribution

Aim: To determine the effects of a 12-month intervention of consuming dried plums on DXA measures of central adiposity (visceral adipose tissue mass, visceral adipose tissue volume, visceral adipose tissue area, android regional fat mass, android to gynoid percent fat ratio) in postmenopausal women with osteopenia/osteoporosis consuming 50g dried plums/day, 100g dried plums/day, or no plums (control group).

Hypothesis 2A: Following the 12-month intervention, central adiposity, as estimated by visceral adipose tissue mass, visceral adipose tissue volume, visceral adipose tissue area, and android regional fat mass, will be lower in postmenopausal women consuming either 50g or 100g dried plums/day compared to the control group.

Hypothesis 2B: Following the 12-month intervention, android to gynoid percent fat ratio will be lower in postmenopausal women consuming either 50g or 100g dried plums/day compared to the control group.

Aim and Hypothesis 3: Adiposity and Glucose

Aim: To determine the effects of central adiposity on 12-month fasting blood glucose in postmenopausal women participating in a 12-month intervention of consuming 50g dried plums/day, 100g dried plums/day, or no plums (control group).

Hypothesis 3A: Baseline central adiposity will be predictive of 12-month fasting blood glucose, independent of treatment group.

Hypothesis 3B: Fasting blood glucose and HOMA-IR will be positively correlated with central adiposity.

Rationale for this Study

T2DM and CVD are major health concerns for postmenopausal women [4, 7]. Changes in body fat distribution, which reflect metabolic alterations that occur in conjunction with menopause place women at increased risk for MetS, which significantly increases risk of T2DM and CVD [1-5]. It is therefore important to investigate potential treatments that target metabolic abnormalities of MetS.

The current research on dried plum as a potential nutraceutical therapy for metabolic abnormalities is extremely limited. Much of the existing literature describes studies on rat models. Furthermore, of the studies using human models, even fewer investigate a postmenopausal population of women. Further, many investigators use an intervention of a plum product, but often not specifically dried plum. Fresh plum, plum juice, and plum concentrate are common interventions found in the existing literature. While similar in nutrient profile to dried

plums, fresh plums have key differences in the phenolic composition, most notably the presence of anthocyanins, a class of phenolics which are absent in dried plums [15].

Evaluating the effectiveness of dried plum at promoting metabolic health could lead to clinical use of dried plum as a treatment for dyslipidemia and hyperglycemia secondary to menopause. Nutraceutical therapies offer patients an alternative to pharmaceutical therapy, with advantages including whole body benefits beyond the intended metabolic and body composition targets. Dried plum is recognized for its impact on bone health, which is important for postmenopausal women as they are at an increased risk for osteoporosis [19]. Additionally, using alternative strategies to drug therapy can avoid unwanted side effects. For these combined reasons, dried plum has potential to become a recognized "superfood" for promoting health and wellbeing in postmenopausal women.

Expected Findings

It was expected that dried plum dietary interventions would lower fasting blood glucose. Dietary phenolics consumed during dried plum intervention are associated with improved insulin sensitivity and therefore lower fasting blood glucose [11]. In the current study, we expected that the 100g dried plums/day group would have the lowest fasting blood glucose, while the control group (no dried plums) would have the highest fasting blood glucose after the 12-month dietary intervention.

Central body fat was also expected to be reduced by dried plum dietary interventions. Dietary phenolics consumed during dried plum interventions are associated with preventing body weight gain [11]. We expected that the 100g dried plum/day group would have the lowest

central body fat, while the control group (no dried plums) would have the highest fasting blood glucose after the 12-month dietary intervention.

Finally, a positive correlation between fasting blood glucose and central body fat was expected when dried plums are consumed. Similarly, a positive correlation between HOMA-IR and central body fat was also expected. Body fat mass is associated with insulin sensitivity in postmenopausal women [1]. Visceral fat mass, in particular, is associated with insulin sensitivity [5].

Statistical Plan

Specific Aim 1

i. Hypothesis 1A: A one-way repeated-measures ANOVA will be conducted to compare 12-month treatment effect on fasting blood glucose. A one-way ANOVA will be conducted to compared treatment effect on pre-to-post change in fasting blood glucose.

ii. Hypothesis 1B: A one-way repeated-measures ANOVA will be conducted to compare 12-month treatment effect on HOMA-IR. A one-way ANOVA will be conducted to compared treatment effect on pre-to-post change in HOMA-IR.

Specific Aim 2

i. Hypothesis 2A: A one-way repeated-measures ANOVA will be conducted to compare 12-month treatment effect on central adiposity variables (visceral adipose tissue mass, visceral adipose tissue volume, visceral adipose tissue area, android regional fat mass). A

one-way ANOVA will be conducted to compared treatment effect on pre-to-post change in central adiposity variables.

ii. Hypothesis 2B: A one-way repeated-measures ANOVA will be conducted to compare 12-month treatment effect on android/gynoid percent fat ratio.

Specific Aim 3

i. Hypothesis 3A: Linear regression will be conducted to determine whether baseline central adiposity is a significant predictor of 12-month fasting blood glucose.

ii. Hypothesis 3B: Pearson's correlation will be conducted to test the relationship between central adiposity, HOMA-IR, and 12-month fasting blood glucose.

Chapter 2

Literature Review

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease in which muscle, adipose, and liver cells become resistant to the hormone insulin, leading to elevated blood glucose (i.e., hyperglycemia), and can ultimately lead to health complications if left untreated. T2DM presents a major public health concern as it is widespread and leads to serious health conditions [7, 20]. According to the 2017 National Diabetes Statistics report, over 100 million Americans have diabetes [21]. T2DM can lead to serious, acute complications such as diabetic ketoacidosis and diabetic coma, as well as chronic cardiovascular diseases (CVD), including coronary heart disease, stroke, peripheral artery disease, cardiomyopathy, and congestive heart failure [20].

T2DM is clinically observed as hyperglycemia and differs from other types of diabetes in that the etiology of hyperglycemia is insulin resistance [20]. Insulin is a signaling hormone produced by the beta cells of pancreas in response to a rise in blood glucose. In a condition of adequate insulin sensitivity, hyperglycemia (such as that which occurs after a meal) is corrected by the action of insulin stimulating peripheral cells, such as liver, skeletal muscle, and adipose tissue, to remove glucose from the blood through transmembrane glucose transporter type 4 (GLUT4) proteins [22, 23]. However, in cases of insulin resistance, insulin signaling does not stimulate GLUT4 translocation into the peripheral cell membrane, or stimulation does not result in successful glucose removal from the blood, ultimately creating a situation of uncontrolled hyperglycemia [23]. At the beginning of disease progression, pancreatic insulin production is uninhibited and even elevated to meet the rising need for insulin by increasingly resistant

peripheral cells [20]. Over time, the pancreas becomes unable to continuously meet the high demand for insulin to maintain glucose homeostasis. Criteria for diagnosis of T2DM include a fasting plasma glucose of ≥126 mg/dL or a random plasma glucose of ≥200 mg/dL [20]. During the early stages of insulin resistance, the clinical criteria for T2DM are not met, meaning patients can be unknowingly incurring health consequences such as vascular damage from chronic hyperglycemia [20]. Indeed, hallmark symptoms of T2DM, including polyuria, polydipsia, polyphagia, and weight loss, do not present until the late stages of disease progression [20]. As insulin resistance alone can lead to detrimental consequences and is present in approximately 20-25% of the nondiabetic population, it constitutes a considerable public health concern independent of T2DM [3].

A cluster of abnormalities, known as metabolic syndrome (MetS), often precedes onset of T2DM [17] and diagnosis of MetS requires at least three of the following five abnormalities: increased waist circumference, high triglycerides (TG), low high-density lipoproteins (HDL), hypertension, and hyperglycemia [6], as seen in Table 1. Each of these factors are related to insulin resistance, either as a cause or consequence. Obesity, particularly the accumulation of visceral fat within the abdominal cavity, often crudely quantified using waist circumference, can lead to insulin resistance via chronic inflammation and dyslipidemia, which includes high TG and low HDL [8, 11, 18]. Further, insulin resistance leads to low-density lipoprotein (LDL) oxidation, impaired antioxidant activity, and vascular damage and dysfunction, which may lead to hypertension and CVD [11]. Additionally, abnormalities in the levels of insulin (hyperinsulinemia), glucose (hyperglycemia), cholesterol (high TG and low HDL), and leptin are associated with insulin resistance [8, 11]. The combination of these related pathological

conditions is known as MetS and is associated with a 5x greater risk for T2DM and 3x greater risk for CVD [6].

Table 1. Diagnostic criteria for MetS set by the National Cholesterol Education program- Third Adult Treatment Panel.

Risk Factor	Cut off Value
Waist Circumference	>40" (male), >35" (female)
Triglycerides	≥150 mg/dL
High-density lipoprotein	<40 mg/dL (male), <50 mg/dL (female)
Blood pressure	>130/85 mmHg
Fasting plasma glucose	≥110 mg/dL

Table derived from [8].

Many Americans are at risk for diabetes or already have prediabetes, defined as impaired fasting glucose: 100-125 mg/dL [20, 21]. Prevention of diabetes is a major public health priority due to its widespread incidence and serious clinical complications. The Diabetes Prevention Program Research Group found that lifestyle changes, including healthy eating and exercise habits, to be the most effective strategy for preventing the development of diabetes in high-risk individuals [9, 10, 24]. Postmenopausal women constitute one such population at an elevated risk for insulin resistance, T2DM, and CVD [2, 3, 5, 25]. Therefore, the potential for lifestyle interventions such as dietary changes to prevent insulin resistance and diabetes in postmenopausal women requires investigation.

Metabolic Consequences of Menopause

Metabolic changes associated with the menopause transition create a metabolic profile similar to that of MetS, which may indicate that women have an increased risk for developing insulin resistance, T2DM, and CVD during the postmenopausal time period. Menopause is characterized by a decrease in ovarian secretion of estrogen, in which the resulting estrogen deficiency has pervasive consequences beyond reproductive health [4]. Pathological metabolic conditions including altered body fat distribution, dyslipidemia, and inflammation are associated with both insulin resistance and the menopause transition [2-5], suggesting a possible relationship between menopause and insulin resistance.

Menopause is associated with changes in body fat distribution, such that postmenopausal women tend to store more fat in the abdomen compared to premenopausal women [2, 4]. Multiple investigators using dual-energy X-ray absorptiometry (DXA) have found a significant increase in central adiposity in postmenopausal women compared to premenopausal women [2], which not only supports the understanding that menopause is associated with a change in fat distribution to favor abdominal adiposity, but also indicates that DXA is a useful measurement technique for assessing fat distribution in postmenopausal women. Studies in which computed tomography and magnetic resonance imaging were used to assess the specifics of abdominal fat distribution indicated that intra-abdominal, or visceral fat, in particular increases with menopause [2]. An increase in visceral fat mass poses a health concern as visceral fat is related to pathological metabolic conditions, such as increased waist circumference, dysfunctional adipocytes, impaired insulin function, and excess lipids and inflammatory molecules exported into the blood [5, 8]. Indeed, it has been found in postmenopausal women that altered body fat distribution was associated with dyslipidemia [1]. Further, total fat mass, which increases during

the menopause transition as a function of age, is positively associated with insulin resistance indicators in postmenopausal women [1, 5]. As such, visceral fat mass is closely associated with MetS and insulin resistance. Two key pathways by which visceral fat relates to metabolic disease are impaired lipid metabolism and chronic inflammation.

Dyslipidemia, a result of impaired lipid metabolism, has been frequently observed in postmenopausal women; specifically, elevated total cholesterol (TC), LDL, and TG, as well as decreased HDL are associated with menopause [3]. A study comparing postmenopausal and perimenopausal women to premenopausal women showed a significant increase in TC and LDL, and a significant decrease in HDL in perimenopausal and postmenopausal women, which are factors of MetS [3]. Further, body fat percent, which increases during the menopause transition as a function of age, is positively correlated with dyslipidemia [1]. Expanded visceral fat mass contributes to impaired lipid metabolism, as it causes an increase in lipids exported into the blood. The resulting dyslipidemia can lead to ectopic lipid deposition, or the accumulation of lipids in the liver and muscle tissue [5]. Ectopic lipid deposition is a pathological consequence of impaired lipid metabolism which predicts insulin resistance in postmenopausal women [5]. In addition to altering blood lipid levels, visceral fat also causes systemic inflammation.

Chronic low-grade inflammation is associated with MetS, obesity, insulin resistance, T2DM, and CVD [8, 17]. Excess adipose tissue, particularly visceral fat, is associated with increased plasma concentrations of inflammatory cytokines including C-reactive protein (CRP), tumor necrosis factor (TNF-α), and interleukin-6 (IL-6) [8]. It has been well established that these inflammatory cytokines predict T2DM [26]. TNF-α has been observed to directly impact the cellular insulin signaling pathway in rodents, resulting in insulin resistance [8]. IL-6 secretion also impairs insulin function and may have significant impacts on the liver, including

stimulating CRP production and increasing hepatic lipid export into the blood, altering blood lipid levels [8]. Elevated CRP plasma concentration is clinically relevant as CRP functions in inflammatory cytokine release and oxidative stress [3], and is positively associated with various disease states including CVD and T2DM [14]. Such findings indicate that alterations in inflammatory cytokines related to visceral fat mass are detrimental to health. In a study comparing postmenopausal and perimenopausal women to premenopausal women, researchers found an increase in TNF-α and IL-1 (a cytokine in the same family as IL-6) in postmenopausal women, and an increase in CRP in both perimenopausal and postmenopausal women, indicating increasing inflammation during the menopause transition [3]. Further, in postmenopausal women, body fat percent is positively correlated with the inflammatory cytokines CRP and IL-6 [1]. Therefore, inflammation caused by the increase visceral fat represents a major causal factor in the development of insulin resistance, MetS, and T2DM in postmenopausal women.

Estrogen deficiency resulting from menopause causes a complex cascade of interrelated physiological effects, many of which are pathological. Figure 1 describes the mechanisms by which menopause-related estrogen deficiency can lead to the metabolic abnormalities of MetS (identified by blue boxes within the green dashed region) via an increase in visceral fat mass which leads to dyslipidemia and inflammation. These consequences then increase insulin resistance which results in worsened dyslipidemia, hypertension, and hyperglycemia. MetS may then progress to diseases including T2DM and CVD.

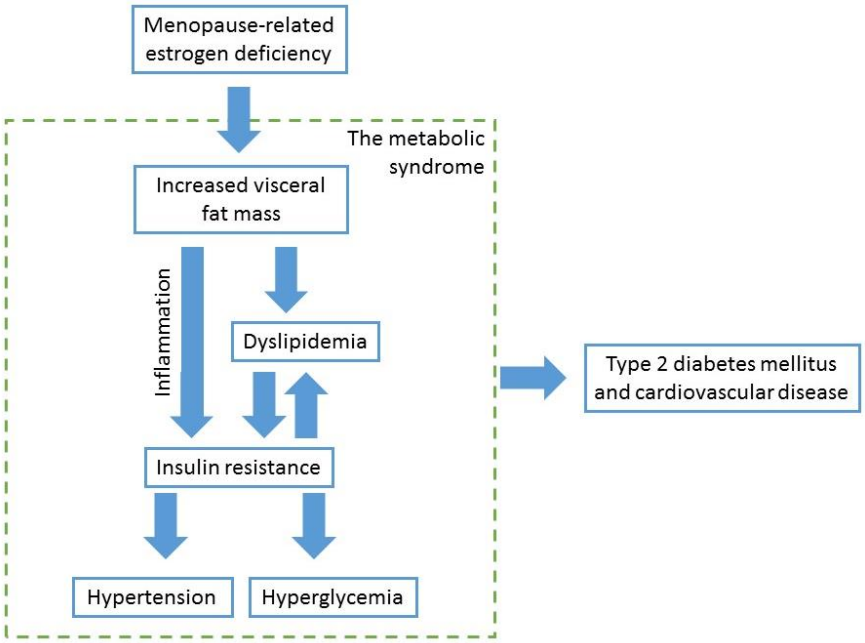

Figure 1. Mechanism by which menopause-related estrogen deficiency leads to metabolic syndrome, a risk factor for cardiovascular disease and type 2 diabetes mellitus.

Dried Plum Phenolic Compounds

The Diabetes Prevention Program Research Group found promoting lifestyle changes, including healthy eating and exercise habits to be the most effective strategy for preventing the development of diabetes in high-risk individuals [9, 10, 24]. One possible dietary intervention to prevent insulin resistance and T2DM in post-menopausal women is the addition of phenolic compounds to the diet.

Phenolics are compounds produced by plants as a byproduct of metabolism. In the plant, phenolics function in growth and protection from radiation damage [16]. The term phenolic covers a broad range of molecules that possess the common the ability to neutralize reactive oxygen species (ROS) [27]. Due to the deleterious effects of ROS accumulation, including damage to crucial compounds such as DNA, proteins, and lipids, neutralization of these potent

oxidizers is important for cell health [27]. The antioxidant capacity of phenolics proves beneficial to human health when such compounds are consumed in the diet [28]. Some of the classes of phenolics include phenolic acids, flavonoids, stilbenes, coumarins, lignins, and tannins; the relationship of which is demonstrated in Figure 2 [16].

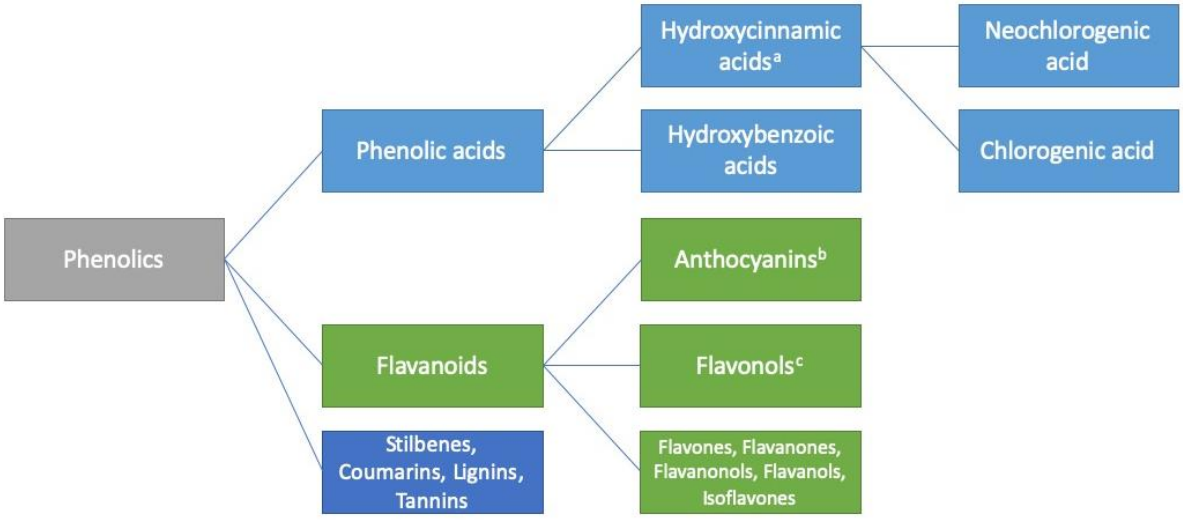

Figure 2. Phenolic compounds classifications.

[a] Primary phenolics in dried plums. [b] Absent in dried plums. [c] Small quantities in dried plums.

Foods that provide a high amount of phenolics include fruits, such as blueberries and plums [13]. Dried plums are particularly high in certain phenolic acids, namely neochlorogenic acid and chlorogenic acid [13]. Of note is the difference in phenolic profile between fresh plums and dried plums. Though fresh plums are high in anthocyanins, the drying process destroys these compounds, leaving nearly zero anthocyanins in dried plums [15]. Because of the drying process, the estimates in Table 2 suggest 100g of dried plum provides a more concentrated dosage of phenolics per unit mass, and do not indicate a synthesis of new phenolics [13]. In a

separate study conducted by Piga et al. [15], the drying process was found to decrease the total phenolic content, but increase the total antioxidant capacity of the plum.

Table 2. Phenolic composition of fresh and dried plums (per 100g).

Phenolic Compound	Fresh plum	Dried plum
Neochlorogenic acid	81mg	131mg
Chlorogenic acid	14.4mg	44mg
Anthocyanins	7.6mg	---

Table derived from [24].

Chlorogenic acid (5-O-caffeoylquinic acid), a key phenolic compound in dried plums, is known for its antioxidant capacity and has additionally been studied as an anti-inflammatory, antilipidemic, antidiabetic, and antihypertensive compound, which suggests it is a promising compound for combating MetS [28]. Investigators conducting rat studies have shown that chlorogenic acid favorably impacts body weight, visceral fat mass, plasma insulin, plasma free fatty acids, TG, TC, LDL, HDL, fasting plasma glucose, insulin sensitivity, hypertension, and ectopic lipid deposition [28]. Researchers studying a population of overweight men found that chlorogenic acid improved insulin sensitivity [29]. A clinical study in adults with MetS suggested that the supplement Kepar, which contains chlorogenic acid, reduced body weight, waist circumference, fasting glucose, and TC [30]. Chlorogenic acid may also improve glucose metabolism by inhibiting the intestinal absorption of glucose [16, 28]. The closely related isomer neochlorogenic acid (3-O-caffeoylquinic acid), which also contributes significantly to the phenolic profile of dried plums, was shown to have similar antioxidant activity to chlorogenic acid [27]. It is important to note that when ingesting chlorogenic acid in the context of dried plums, digestion and absorption of the whole food matrix affects the profile of phenolic

compounds that enter the body [28]. When unabsorbed chlorogenic acid reaches the colon, the gut microbiome metabolizes the compounds to other phenolic metabolites, which may be absorbed and propagate benefits to metabolic health independently of chlorogenic and neochlorogenic acid [28].

Phenolic Mechanistic Theories

While dietary phenolics have been shown in animal studies to lower blood glucose and decrease insulin resistance, thereby decreasing the risk of developing T2DM [11], the mechanism by which phenolics improve insulin sensitivity is a developing field of research. Studies recently have investigated phenolics as peroxisome-proliferator-activated receptor (PPAR) agonists, antioxidants, and anti-inflammatory agents, described below.

Glucose and Lipid Metabolism

Insulin resistance reflects abnormal metabolism of carbohydrates and lipids in tissues such as the liver, skeletal muscle, and adipose. One mode by which phenolics improve insulin sensitivity is by modulating lipid metabolism via PPAR activation. PPAR is a transcription factor in the cell nucleus that promotes the expression of insulin sensitizing genes, and functions to regulate metabolism through monitoring lipid levels from the diet, de-novo lipogenesis, and lipolysis [17]. PPARs are classified into three different subcategories, PPARα, PPARβ, and PPARγ. For the purpose of this review, the actions of PPARα and PPARγ in metabolically active tissues, specifically liver and adipose, will be discussed.

In the liver, PPARα improves dyslipidemia and insulin sensitivity by promoting the action of lipoprotein lipase, an enzyme that hydrolyzes TG carried by LDL and other lipoproteins to allow fatty acids to enter the hepatocyte, thereby decreasing the high plasma TG concentrations characteristic of dyslipidemia [12, 28]. Once TG enter the hepatocyte, PPARα promotes the breakdown of fatty acids for energy production, through a process called β-oxidation [28]. The net energetic effect of PPARα activation is the dissipation of stored lipid energy within hepatocytes, or the utilization of the energy in lipids [17]. The PPARα-stimulated removal of lipids improves dyslipidemia which can subsequently correct abnormal cellular lipid and carbohydrate utilization characteristic of insulin resistance. This has been demonstrated by investigators using PPARα agonist interventions to improve in dyslipidemia in MetS [17]. Specifically, trials such as the Helsinki Heart Study [31], Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial [32], Benzofibrate Infarction Prevention [33], Fenofibrate Intervention and Event Lowering in Diabetes [34], and Action to Control Cardiovascular Risk in Diabetes [35] have demonstrated that use of fibrates, synthetic PPARα agonists, favorably impacted dyslipidemia [17]. Additionally, through mechanisms involving the pancreas and insulin clearance, PPARα activation has been shown to improve glucose handling in prediabetics, preventing progression to T2DM [17].

In adipose tissue, PPARγ promotes export of adiponectin to the circulation and controls fatty acid flux [17]. Adiponectin is a cytokine associated with improved insulin sensitivity and negatively correlated with T2DM and abdominal adiposity [8, 12]. Adiponectin exerts anti-lipogenic and anti-glucogenic effects on the liver, which may be a mechanism for improving insulin sensitivity [8]. Additionally, PPARγ shifts the flux of fatty acids away from the liver and skeletal muscle, preventing lipotoxicity and allowing the tissues to transition from lipid energy

utilization to glucose energy utilization thereby increasing glucose utilization and uptake, and improving glucose homeostasis and insulin sensitivity [17]. The pancreatic beta cells are also protected from lipotoxicity by this mechanism [17]. The net energetic effect of PPARγ activation is the sequestering of lipid energy in adipose, or the removal and storage of lipids in fat tissue to prevent both accumulation of lipids in the blood, and excessive lipid utilization in the liver and skeletal muscle [17]. PPARγ agonists have been shown to improve insulin sensitivity and glucose handling, but also increase body weight as a result of the increasing store of lipids in adipose [17]. However, one important deleterious effect resulting from pharmacologic PPARγ activation is impaired bone turnover due to increased formation of osteoclasts and decreased formation of osteoblasts, which is relevant to this population as postmenopausal women are at an increased risk for osteopenia/osteoporosis [17, 19].

The differential metabolic effects of PPARα, a promoter of lipid energy dissipation in the liver, and PPARγ, a promoter of lipid energy sequestering in adipose tissue, may appear to conflict, but in fact the actions of the two operate in a balance which improves lipid and glucose metabolism [17]. The ability of phenolic compounds to stimulate both PPARα and PPARγ could therefore protect against the development of insulin resistance MetS, T2DM, and CVD.

Antioxidant

Phenolic compounds are most commonly recognized for their antioxidant properties. Antioxidant capacity refers to the ability of phenolics, chlorogenic acid isomers in particular, to neutralize ROS to prevent cellular damage [27]. Phenolics reduce free radical ROS by donating

a hydrogen atom to the unstable radical [27, 36], thereby preventing damage to cellular structures like DNA or lipid membranes, as well as alleviating oxidative stress.

Oxidative stress, which results from ROS activity, is associated with multiple facets of MetS including obesity and hyperglycemia [28]. Obesity leads to oxidative stress in adipose tissue which increases production of inflammatory cytokines [37]. Additionally, hyperglycemia creates oxidative stress via auto-oxidation of glucose which yields ROS [38]. This oxidative stress from ROS, obesity, and hyperglycemia causes LDL oxidation (the beginning stage of atherosclerotic plaque formation), and abnormal levels of plasma insulin and plasma glucose due to insulin resistance and decreased beta cell insulin secretion [11, 12, 14, 37, 38].

The antioxidant capacity of phenolics has been demonstration both in vitro and in vivo [36]. Xu et al. demonstrated the antioxidant effects of chlorogenic acid, through chemical assays that monitored the radical scavenging, or neutralizing, ability of different chlorogenic acid isomers [27]. Wistar rats with hyperglycemia-induced oxidative stress were found to have lower levels of lipid hydroperoxide, an indicator of oxidative stress, following a 45 day treatment of chlorogenic acid administered orally [38]. Because oxidative stress is associated with multiple facets of MetS [28] and is observed in T2DM [38], the antioxidant capacity of phenolics could play a role in preventing or correcting insulin resistance, MetS, T2DM, and CVD. Consumption of dietary phenolics may therefore reduce these problems through preventing oxidative stress.

Anti-Inflammatory

Another consequence of PPAR activation is a downregulation in the expression of pro-inflammatory genes and an upregulation in the expression of anti-inflammatory genes [17].

PPARα and PPARγ each combat inflammation, likely in an independent manner [17]. In adipose, PPARγ directly inhibits production of pro-inflammatory cytokines, while simultaneously indirectly preventing inflammation by increasing plasma adiponectin and thereby reducing TNF-α secretion by macrophages in the adipose [17]. Beyond the effects in adipose-related macrophages, PPAR expression in the vascular walls also combats inflammation [17]. The antioxidant activity of phenolics mentioned above may also reduce inflammation [28]. By disrupting inflammatory cytokines production, PPAR activation via phenolic compounds can improve insulin sensitivity. Therefore, the anti-inflammatory properties of phenolic compounds represents another pathway by which dietary phenolics can combat the deleterious effects, including insulin resistance, MetS, T2DM, and CVD, of adipose-related chronic inflammation [28].

Plum Interventions

The current state of the literature includes studies in both rat and human models using a variety of plum product interventions including plum juice, plum juice concentrate, fresh plums, and dried plums. The specific intervention methods and relevant key findings are discussed below.

Rat Models

A study conducted by Noratto et al. [11], assessed the effect of plum juice administered ad libitum on obese Zucker rats for 11 weeks. The control group consisted of obese Zucker rats that consumed a glucose-matched sugar water ad libitum in place of plum juice. Investigators

found that plum juice consumption inhibited multiple factors associated with MetS: weight gain, plasma glucose increase, plasma insulin increase, dyslipidemia, hepatic lipid deposition; plum juice conversely increased levels of mRNA for PPARγ in heart tissue, as compared with control rats.

Further support for the benefits of plum juice products was found by Utsunomiya et al. [12] who assessed the effect of diluted plum-ekisu, or plum juice concentrate, administered as 0.25% concentration on Wistar fatty rats for 2 weeks. Investigators found that plum-ekisu prevented an increase in TC, TG, glucose-insulin index; treatment also led to an increase in adiponectin and PPARγ mRNA in liver tissue, as compared to the control Wistar fatty rats that were provided water in place of plum-ekisu [12]. Additionally, in an oral glucose tolerance test and intraperitoneal insulin tolerance test, plum-ekisu treated rats displayed better insulin sensitivity than control rats. Together, Noratto et al. [11] and Utsunomiya et al. [12] provide compelling evidence for the utility of plum juice products in treating MetS.

Beyond plum drink interventions, dried plum has also been investigated in rat trials. A study conducted by Lucas et al. [39] assessed the effect of dried plums on serum cholesterol in ovariectomized female Sprague-Dawley rats. This rat population models the condition of menopause-related estrogen deficiency. The intervention consisted of either 5% (low dose) or 25% (high dose) of the normal diet substituted with powdered prunes consumed ad libitum for 45 days, while the control consumed no prunes in the casein-based diet. Ovariectomized rats consuming a high dose dried plum experienced no significant increase in TC; the low dose dried plum group experienced an attenuated increase in TC, as compared to the control group which modeled menopause-related estrogen deficiency. These results suggest dried plum in the diet

may be beneficial in treating hypercholesterolemia during the postmenopausal time period in women.

Human Clinical Trials

Further evidence of the benefits of plum products has been observed in human clinical trials. In humans, plum products have been shown to improve antioxidant capacity, lipid levels, inflammation, and hypertension. González-Flores et al. [40] administered 390g fresh whole plum/day for five days to 6 young, 6 middle-age, and 6 elderly participants and assessed antioxidant activity via colorimetric assay kit. The investigators observed in all age groups a significant increase in antioxidant activity after plum intervention relative to baseline. Though these findings are favorable regarding antioxidant outcomes, they do not necessarily indicate that dried plum will have the same outcome as the phenolic profile of dried plums differs from fresh plums [15].

Dried plums in specific have been shown to favorably impact lipid levels, improving dyslipidemia. A study conducted by Tinker et al. [41] assessed the effect of a 100g dried plum/day dietary intervention in mildly hypercholesterolemic men on plasma LDL after four weeks. Investigators found that dried plum intervention significantly decreased LDL from the control value obtained after 4 weeks of grape juice intervention [41]. This investigation, however, did not assess dried plum effects in the context of menopause-related estrogen deficiency.

Favorable effects of dried plum have also been demonstrated in postmenopausal women. Chai et al. [14] administered a 100g dried plum/day intervention to postmenopausal women for a

period of 12 months. Dried plum was intended to be the control in this study, with the intervention of interest being dried apple consumption. Dried plum was shown to reduce TC and LDL, but not to a statistically significant extent. Dried plum did significantly lower serum CRP as early as 3 months, indicating decreased inflammation. Lipid hydroperoxide, an indicator of oxidative stress, also was reduced, indicating an antioxidant effect of dried plums in postmenopausal women.

Ahmed et al. [42] found further evidence of anti-dyslipidemic effects of dried plums, in addition to anti-hypertensive effects. They assessed the effect of 8 weeks of consuming soaked dried plums, either a single dose of three plums or a double dose of six plums, in pre-hypertensive patients on blood pressure and serum lipids. The single dose alone reduced blood pressure. Both dried plum doses also reduced TC and LDL significantly. These findings indicate that dried plums can improve multiple disorders characteristic of MetS, but the generalizability of this finding to postmenopausal women may not be appropriate.

Conclusion

Postmenopausal women are at risk for serious health consequences due to decreased estrogen secretion, including MetS and T2DM, a key feature of which is insulin resistance. It is therefore important to explore potential clinical interventions to prevent this disease progression following the menopause transition. Dietary phenolics, which are abundant in dried plums, show potential as a protective nutraceutical therapy to prevent insulin resistance in postmenopausal women. Plum products and dietary phenolics in general have already been shown to have positive impacts on metabolic markers in both rat models and clinical studies. However, to our

knowledge no study has investigated fasting blood glucose and insulin resistance outcomes following a dried plum intervention in postmenopausal women with osteopenia. Dried plums show promise in improving bone mineral density in postmenopausal women and the potential for a secondary benefit to glucose metabolism would further support the benefits of consuming dried plums for this population.

Chapter 3

Methods

Study Design

This study was a randomized controlled trial of a dried plum nutritional intervention in postmenopausal women with osteopenia or osteoporosis (n=124). The trial period was 12 months and the intervention groups were 50g dried plums/day (50g DP), 100g dried plums/day (100g DP), and a control group of no dried plums/day (control). Outcome variables were assessed at baseline, 6-months, and 12-months. Various bone health markers were assessed for the primary goals of the study. For this secondary analysis, outcome variables of interest included weight, BMI, fat mass, lean mass, percent body fat, android fat mass, android total mass, android percent fat, gynoid fat mass, gynoid total mass, gynoid percent fat, android/gynoid mass ratio, visceral adipose mass, visceral adipose volume, visceral adipose area, fasting blood glucose, cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), insulin, homeostatic model assessment of insulin resistance (HOMA-IR). Exploration of correlations between central adiposity measurements and blood glucose was also assessed. This study was approved by the Institutional Review Board at the Pennsylvania State University, University Park, PA.

For this thesis, I not only analyzed body composition and blood chemistry outcomes of the parent dried plum study, but also ran insulin assays specific to this project. I prepared serum samples from the study for immunoassay analysis of insulin concentration and operated the IMMULITE 1000 analyzer, as described below.

Intervention

Initial screening period was followed by a 1-2 week Baseline Period, after which participants were randomized into one of three intervention groups: 1) control group who received 1200 mg calcium and 800 IU vitamin D_3 daily, 2) 50g/day plus 1200 mg calcium and 800 IU vitamin D_3 daily (roughly 6 dried plums), or 3) 100g/day plus 1200 mg calcium and 800 IU vitamin D_3 daily (roughly 12 dried plums). Dried plums were consumed in divided doses three times per day. Dried plums were provided to the two DP groups monthly for the study duration. A 2-week run-in period for the consumption of 50g and 100g of dried plums was followed by all participants to minimize gastro-intestinal issues during initiation of therapy (details below). If necessary, some participants required extra time to adjust their gastro-intestinal system to the increased consumption of dried plums, as an additional run-in time of up to two weeks. Compliance was monitored by urinary total phenolic levels and daily records of dried plum and supplement consumption.

Participants

Women were included if they were postmenopausal; aged 55 to 75 years; not severely obese (BMI <35 kg/m^2); healthy (determined by a screening questionnaire, complete metabolic panel); willing to include dried plums in their daily diet; not taking any natural dietary supplement containing phenolics, ate < 1 cup/day of blueberries or apples for at least 2 months prior to study entry; non-smoking; ambulatory; and low BMD as measured by dual energy X-ray absorptiometry (DXA). Eligible BMD values (T-scores) for DXA measures of the lumbar spine, total hip and/or femoral neck corresponded to T-scores between 0 and -3.0.

Exclusion Criteria: Women who regularly consumed dried plums, dried apples, prune juice, or heavy consumers of blueberries (1 cup or more/day); vitamin D deficiency (<20 ng/mL); history of vertebral fracture or fragility fracture of the wrist, humerus, hip or pelvis after age 50 yr); untreated hyper- or hypothyroidism; current hyper- or hypoparathyroidism; significantly impaired renal function; current hypo- or hypercalcemia; history of spinal stenosis; history of heart attack, stroke, thromboembolism, kidney disease, malabsorption syndrome, seizure disorders; positive for HIV, Hep-C or Hep-B surface antigen and malignancy. Use of the following agents affecting bone metabolism were also exclusion criteria: intravenous bisphosphonates at any time; fluoride (for osteoporosis) within the past 24 months; denosumab at any time; bisphosphonates, parathyroid hormone or strontium within the past 12 months; calcitonin; selective estrogen receptor modulators within the past 12 months; systemic oral or transdermal estrogen within the past 3 months; systemic glucocorticosteroids (≥ 5 mg prednisone equivalent per day for more than 10 days); or tibolone within the past 3 months. Finally, participants who would not consume the study therapy or would not stop taking natural product supplements of their own selection were excluded.

Anthropometrics and Demographics

Participants completed demographic, reproductive, and medical history questionnaires which include questions regarding use of supplements, prescription medications, and NSAIDs, history of weight, purposeful physical activity, and dietary practices [42]. Responses were used to determine if eligibility requirements were met. Physical exams including height, weight, and BMI were conducted prior to study entry (screening) to ensure that participants were in good

health and assessment of medical history and blood screening tests were used to ensure that participants have no contraindications to the consumption of dried plums. Total body mass was measured to the nearest 0.1 kg on a physician's scale (Seca, Model 770, Hamburg, Germany). Height was measured by stadiometer at the beginning of the study (baseline). Body mass index (BMI) was calculated as weight/height squared (kg/m^2). Following, the screening/baseline appointments, body weight was monitored at monthly study visits; the baseline, 6-month, and 12-month body weights will be presented herein.

Body Fat Distribution Analysis

DXA was utilized at screening/baseline, and the 6- and 12-month visits for assessment of body composition, specifically fat mass, lean mass, percent body fat, android fat mass, android total mass, android percent fat, gynoid fat mass, gynoid total mass, gynoid percent fat, android/gynoid mass ratio, estimated visceral adipose mass, estimated visceral adipose volume, estimated visceral adipose area. Central adiposity variables included visceral adipose tissue mass, visceral adipose tissue volume, visceral adipose tissue area, android fat mass, android total mass, android percent fat. An International Society of Clinical Densitometry (ISCD) certified technician performed and analyzed all DXA scans on a Hologic QDR4500W DXA scanner (Hologic, Bedford, MA).

Blood Chemistry

At baseline, 6-month, and 12-month visits, a morning blood draw following an overnight fast was collected for measurement and analysis. A comprehensive metabolic panel, including

fasting blood glucose, and a standard lipid panel, including total cholesterol, HDL, and LDL were analyzed by Quest Diagnostics (Secaucus, NJ). Fasting blood insulin was analyzed by Immulite solid-phase, enzyme-labeled chemiluminescent immunometric assay. This assay analyzes insulin concentration in plasma by catalyzing the formation of a sandwich complex between sample insulin and anti-insulin antibody during incubation. Addition of chemiluminescent substrate indicates the concentration of insulin by photometric analysis of the intensity of luminescence. Fasting blood glucose and insulin were used to calculate HOMA-IR, as (insulin mIU/mL * glucose mmol/L)/22.5 [43].

Blood Sampling and Storage

Blood samples were collected once between 0800 and 1000h, every six months. Volunteers were fasted and refrained from exercise for 12 hours prior to blood sampling. Antecubital blood samples were drawn using a 21-gauge 19 mm needle and blood collection tubes (Vacutainer, Franklin Lakes, NJ). Samples clotted for 30 minutes at room temperature (20-24ºC) and then were centrifuged for 15 minutes at 4ºC. The serum was aliquoted into 2-mL polyethylene storage tubes and frozen at -80ºC until analysis.

Statistical Analysis

Outlier detection (>3 SD of the mean) and examination of variable distributions for normality were conducted prior to statistical analysis to identify whether the data met the assumptions required by specific statistical techniques. One-way repeated measures analysis of variance (ANOVA) were conducted on normally distributed data to compare 12-month treatment

effects on anthropometric, central adiposity, and blood chemistry measures, with Bonferroni post-hoc comparison of time points and groups. Friedman's related samples two-way ANOVA was conducted on non-normally distributed data. One-way ANOVA were conducted to compare treatment effect on pre-to-post change in anthropometric, central adiposity, and blood chemistry measures. Pearson's correlation was conducted to test the relationship between central adiposity, HOMA-IR, and 12-month fasting blood glucose. A stepwise linear regression was conducted to determine whether central adiposity (baseline and 12-month values) significantly predicted 12-month fasting blood glucose. All statistical analysis was performed in IBM SPSS Statistics for Windows, (Version 25.0. Armonk, NY: IBM Corp.). Data were expressed as mean±standard error of the mean (SEM).

Chapter 4

Manuscript

Abstract

Estrogen deficiency during the postmenopausal time period leads to significant metabolic changes, putting women at risk for metabolic syndrome (MetS), type 2 diabetes mellitus (T2DM), and cardiovascular diseases (CVD). As such, finding treatments which enhance healthy metabolism and insulin sensitivity in postmenopausal women is a pressing health concern. Dried plums (DP) have been investigated recently as a potential nutraceutical treatment for metabolic abnormalities such as insulin resistance, impaired glucose and lipid handling, and inflammation. This randomized control trial assessed the effects of a 12-month DP nutritional intervention on blood glucose, insulin resistance, and body fat distribution. Participants were postmenopausal women with osteopenia (n=124) who were randomized to consume either 50g DP/day (50g DP; n=46), 100g DP/day (100g DP; n=35), or no DP/day (control; n=46); all women received calcium and vitamin D. The cohort had an average age at baseline of 62.3±0.5 years and an average age of menopause of 50.7±0.4 years. Seventy-two percent of participants had no previous hormone therapy use. There were no significant differences between the intervention groups or control group at baseline. The aims of this study were to assess the relationship between a DP nutritional intervention, glucose metabolism, and body fat distribution. Fasting blood glucose increased by 3.5 mg/dL in the 50g DP group from baseline to 6-months (p=0.037), contrary to our hypothesis. Additionally, the absolute change over 12-months in fasting blood glucose was significantly different between 50g DP and 100g DP (p=0.009), with 50g DP group increasing by 4.1% and 100g DP group decreasing by 3.2%.

Total android mass increased significantly in the entire cohort from baseline to 6-months ($p=0.006$). The 50g DP group had significantly higher visceral adiposity measurements (mass: $p=0.036$); volume: $p=0.036$; area: $p=0.033$), BMI ($p=0.025$), and total body fat ($p=0.019$) compared to the control throughout the entire study. All measures of central adiposity were found to be significantly and positively correlated with 12-month fasting blood glucose, fasting blood insulin, and HOMA-IR (all $p<0.001$). Such findings indicate that while dried plum consumption does not result in a dose-dependent reduction in fasting blood glucose, as the 50g DP group experienced an increase in fasting glucose, consuming a larger dosage of dried plums (i.e., 100g DP) may be beneficial to reduce fasting glucose in postmenopausal women. Despite an increase in glucose concentration throughout the intervention in the 50g DP group, which resulted in glucose concentrations below a pre-diabetic threshold, it is unlikely that postmenopausal women will be at an increased risk of MetS, T2DM, or CVD as a result of consuming a moderate amount of dried plums. As such, the 100g DP intervention may be a better supplement dosage than 50g in postmenopausal women as it demonstrated no adverse effects on glucose metabolism or insulin resistance.

Introduction

Menopause marks an important physiological change in a woman's life. The decrease in ovarian secretion of estrogen has widespread consequences beyond reproductive health. Estrogen deficiency during the postmenopausal time period leads to significant metabolic changes [1]. Indeed, metabolic disturbances including dyslipidemia, altered body fat distribution, and indicators of insulin resistance have all been shown to be associated with the

menopause transition [2-5]. These changes have important implications in the risk of metabolic diseases for postmenopausal women [2-5].

Estrogen deficiency-related metabolic changes share many common features with the abnormal metabolic profile known as metabolic syndrome (MetS). MetS is a cluster of metabolic abnormalities related to insulin resistance which significantly increases the risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). Menopause is associated with metabolic changes consistent with MetS and these commonalities imply that menopause may lead to MetS. Because CVD is the number one cause of death for women [4] and T2DM can lead to life threatening consequences, such as diabetic coma and increased risk of CVD [7], it is important to find therapies for postmenopausal women to improve metabolic abnormalities to reduce the risk of developing MetS and associated chronic diseases.

Dietary and lifestyle interventions have been observed to prevent progression to T2DM in patients with pre-diabetes [9, 10]. Modifications to diet and lifestyle are advantageous over pharmacological interventions as they are less expensive, do not cause side effects, and provide whole body health benefits beyond diabetes prevention. One such dietary intervention that is being investigated as a treatment for metabolic abnormalities is the consumption of plum products including fresh plums, plum concentrate, and dried plums.

Dried plums are commonly recognized for their health benefits related to gastrointestinal motility due to dietary fiber content [13]. However, dried plums provide more nutritional value than fiber alone; a key nutritional component of dried plums that impacts carbohydrate and lipid metabolism is dietary phenolics [11, 12]. Dried plums have a high antioxidant capacity due to their high levels of phenolic compounds, namely chlorogenic acid and neochlorogenic acid [15].

Dietary phenolics have been shown to improve various markers of metabolic health such as blood glucose, insulin resistance, oxidation, and inflammation in a variety of models including rats, men, and postmenopausal women [11, 12, 14, 16].

Dried plum phenolics improve markers of metabolic health through multiple mechanisms, including PPAR transcription factor upregulation, antioxidant activity, and anti-inflammatory activity [11-14]. Improving insulin sensitivity is a key feature of dried plum phenolic compounds, as insulin resistance interacts with multiple risk factors for T2DM and CVD [6, 7]. Insulin resistance is the factor that ties abnormal adipocyte function to clinical effects, including hyperglycemia and dyslipidemia [6, 18]. Because menopause is associated with many of the same metabolic abnormalities observed in insulin resistance, dried plum could be a viable intervention for improving risk factors of T2DM and CVD in postmenopausal women.

The purpose of this study was to assess the effect of a 12 month dried plum nutritional intervention on body fat distribution, glucose metabolism, and insulin resistance in postmenopausal women. Postmenopausal with osteopenia/osteoporosis women were randomly assigned to consume 50g dried plum/day (50g DP), 100g dried plum/day (100g DP), or no dried plums (control). Aim 1 was to determine the effect of a 12-month intervention of consuming dried plums on fasting blood glucose concentration and HOMA-IR in postmenopausal women. Aim 2 was to determine the effects of a 12-month intervention of consuming dried plums on DXA measures of central adiposity (visceral adipose tissue mass, visceral adipose tissue volume, visceral adipose tissue area, android regional fat mass, android to gynoid percent fat ratio) in

postmenopausal women. Aim 3 was to determine the association between central adiposity and 12-month fasting blood glucose and HOMA-IR in postmenopausal women.

Methods

Study Design

This study was a randomized controlled trial of a dried plum nutritional intervention in postmenopausal women with osteopenia or osteoporosis (n=124). The intervention period was 12 months and the intervention groups were 50g dried plums/day (50g DP), 100g dried plums/day (100g DP), and a control group of no dried plums/day (control). Outcome variables were assessed at baseline, 6 months, and 12 months. Various bone health markers were assessed for the primary goals of the study. For this secondary analysis, outcome variables of interest included weight, BMI, fat mass, lean mass, percent body fat, android fat mass, android total mass, android percent fat, gynoid fat mass, gynoid total mass, gynoid percent fat, android/gynoid mass ratio, visceral adipose mass, visceral adipose volume, visceral adipose area, fasting blood glucose, cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), insulin, homeostatic model assessment of insulin resistance (HOMA-IR). Exploration of correlations between central adiposity measurements and blood glucose was also assessed. This study was approved by the Institutional Review Board at the Pennsylvania State University, University Park, PA.

Intervention

Initial screening period was followed by a 1-2 week baseline period, after which participants were randomized into one of three intervention groups: 1) control group who received 1200 mg calcium and 800 IU vitamin D_3 daily, 2) 50g/day plus 1200 mg calcium and 800 IU vitamin D_3 daily (roughly 6 dried plums), or 3) 100g/day plus 1200 mg calcium and 800 IU vitamin D_3 daily (roughly 12 dried plums). Dried plums were consumed in divided doses three times per day. Dried plums were provided to the two DP groups monthly for the study duration. A 2-week run-in period for the consumption of 50g and 100g of dried plums was followed by all participants to minimize gastro-intestinal issues during initiation of the intervention (details below). If necessary, some participants were provided with extra time to adjust their gastro-intestinal system to the increased consumption of dried plums, of up to two weeks. Compliance was monitored by urinary total phenolic levels and daily records of dried plum and supplement consumption.

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Women were included if they were postmenopausal; aged 55 to 75 years; not severely obese (BMI <35 kg/m^2); healthy (determined by a screening questionnaire, complete metabolic panel); willing to include dried plums in their daily diet; not taking any natural dietary supplement containing phenolics, ate < 1 cup/day of blueberries or apples for at least 2 months prior to study entry; non-smoking; ambulatory; and low BMD as measured by dual energy X-ray absorptiometry (DXA). Eligible BMD values (T-scores) for DXA measures of the lumbar spine, total hip and/or femoral neck corresponded to T-scores between 0 and -3.0.

Exclusion criteria included the following, women who regularly consumed dried plums, dried apples, prune juice, or heavy consumers of blueberries (1 cup or more/day); vitamin D deficiency (<20 ng/mL); history of vertebral fracture or fragility fracture of the wrist, humerus, hip or pelvis after age 50 yr); untreated hyper- or hypothyroidism; current hyper- or hypoparathyroidism; significantly impaired renal function; current hypo- or hypercalcemia; history of spinal stenosis; history of heart attack, stroke, thromboembolism, kidney disease, malabsorption syndrome, seizure disorders; positive for HIV, Hep-C or Hep-B surface antigen and malignancy. Use of the following agents affecting bone metabolism were also exclusion factors: intravenous bisphosphonates at any time; fluoride (for osteoporosis) within the past 24 months; denosumab at any time; bisphosphonates, parathyroid hormone or strontium within the past 12 months; calcitonin; selective estrogen receptor modulators within the past 12 months; systemic oral or transdermal estrogen within the past 3 months; systemic glucocorticosteroids (≥ 5 mg prednisone equivalent per day for more than 10 days); or tibolone within the past 3 months. Participants who would not consume the study therapy or would not stop taking natural product supplements of their own selection were excluded.

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health and assessment of medical history and blood screening tests were used to ensure that participants have no contraindications to the consumption of dried plums. Total body mass was measured to the nearest 0.1 kg on a physician's scale (Seca, Model 770, Hamburg, Germany). Height was measured by stadiometer at the beginning of the study (baseline). Body mass index (BMI) was calculated as weight/height squared (kg/m^2). Following, the screening/baseline appointments, body weight was monitored at monthly study visits; the baseline, 6-month, and 12-month body weights will be presented herein.

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DXA was utilized at screening/baseline, and the 6- and 12-month visits for assessment of body composition, specifically fat mass, lean mass, percent body fat, android fat mass, android total mass, android percent fat, gynoid fat mass, gynoid total mass, gynoid percent fat, android/gynoid mass ratio, estimated visceral adipose mass, estimated visceral adipose volume, estimated visceral adipose area. Central adiposity variables included visceral adipose tissue mass, visceral adipose tissue volume, visceral adipose tissue area, android fat mass, android total mass, android percent fat. An International Society of Clinical Densitometry (ISCD) certified technician performed and analyzed all DXA scans on a Hologic QDR4500W DXA scanner (Hologic, Bedford, MA).

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fasting blood glucose, and a standard lipid panel, including total cholesterol, HDL, and LDL were analyzed by Quest Diagnostics (Secaucus, NJ). Fasting blood insulin was analyzed by Immulite solid-phase, enzyme-labeled chemiluminescent immunometric assay. This assay analyzes insulin concentration in plasma by catalyzing the formation of a sandwich complex between sample insulin and anti-insulin antibody during incubation. Addition of chemiluminescent substrate indicates the concentration of insulin by photometric analysis of the intensity of luminescence. Fasting blood glucose and insulin were used to calculate HOMA-IR, as (insulin mIU/mL * glucose mmol/L)/22.5 [43].

Blood Sampling and Storage

Blood samples were collected once between 0800 and 1000h, at baseline and every six months. Volunteers were fasted and refrained from exercise for 12 hours prior to blood sampling. Antecubital blood samples were drawn using a 21-gauge 19 mm needle and blood collection tubes (Vacutainer, Franklin Lakes, NJ). Samples clotted for 30 minutes at room temperature (20-24ºC) and then were centrifuged for 15 minutes at 4ºC. The serum was aliquoted into 2-mL polyethylene storage tubes and frozen at -80ºC until analysis.

Statistical Analysis

Outlier detection (>3 SD of mean) and examination of variable distributions for normality were conducted prior to statistical analysis to identify whether the data met the assumptions required by specific statistical techniques. One-way repeated measures analysis of variance (ANOVA) were conducted on normally distributed data to compare 12-month treatment effects

on anthropometric, central adiposity, and blood chemistry measures, with Bonferroni post-hoc comparison of time points and groups. Friedman's related samples two-way ANOVA was conducted on non-normally distributed data. One-way ANOVA were conducted to compare treatment effect on pre-to-post change in anthropometric, central adiposity, and blood chemistry measures. Pearson's correlation was conducted to test the relationship between central adiposity, HOMA-IR, and 12-month fasting blood glucose. A stepwise linear regression was conducted to determine whether central adiposity (baseline and 12-month values) significantly predicted 12-month fasting blood glucose. All statistical analysis was performed in IBM SPSS Statistics for Windows, (Version 25.0. Armonk, NY: IBM Corp.). Data were expressed as mean±standard error of the mean (SEM).

Results

Basic Demographics

A total of 124 participants were included in this analysis. Subject intervention groups and demographics are displayed in Table 1. There were no significant differences among the groups with respect to age at start of study, age of menarche, age of menopause, or the number of women with previous hormone therapy use, history of smoking, or hysterectomy ($p>0.050$).

Table 3. Summary of Subject Demographics.

Variable	Control *(n=46)*	50g DP *(n=43)*	100g DP *(n=35)*	p-value
Age (yr)	61.8±0.72	62.9±0.72	62.5±1.0	0.594
Age of Menarche (yr)	13.0±0.23	12.9±0.19	13.0±0.19	0.904
Age of Menopause (yr)	50.6±0.62	50.6±0.79	51.0±0.66	0.880
Previous Hormone Therapy use (n, %)	13 (28.3)	14 (32.6)	8 (22.9)	0.639
History of smoking (n, %)	9 (19.6)	7 (16.3)	7 (20.0)	0.742
Hysterectomy (n, %)	4 (8.7)	8 (18.6)	2 (5.7)	0.158

Values represent mean±SEM.

Anthropometrics

Participant anthropometric measurements including weight, BMI, fat mass, lean mass, and percent body fat were assessed via DXA at baseline, 6-months, and 12-months, and results are displayed in Table 4. There was a significant difference between groups for BMI ($p=0.024$) and percent body fat ($p=0.022$) at baseline, 6-months, and 12-months. The 50g DP group had significantly higher BMI than the control group at baseline, 6-months, and 12-months ($p=0.025$). Additionally, the 50g DP group had significantly higher percent body fat than the control group

at baseline, 6-months, and 12-months (p=0.019). No other significant differences over time or between groups were observed in any anthropometric measure recorded. There were no significant absolute changes from baseline to 12-months in any of the anthropometric variables, as displayed in Table 5.

Table 4. Anthropometrics across 12-months.

Variable	Control *(n=46)*			50g DP *(n=43)*			100g DP *(n=35)*			p-value		
										Time	Group	Group *Time
Time (month)	0	6	12	0	6	12	0	6	12			
Height (cm)	163.3 ±0.9	-	-	161.9 ±0.8	-	-	163.0 ±1.0	-	-	-	-	-
Weight (kg)	65.2 ±1.6	65.5 ±1.6	65.7 ±1.6	69.8 ±1.7	69.9 ±1.7	69.7 ±1.6	66.8 ±1.6	67.2 ±1.8	66.7 ±1.7	0.36	0.15	0.51
BMI (kg/m^2)	24.5± 0.57	24.6 ±0.58	24.7 ±0.59	27.7 ±1.3	26.7 ±0.7	26.6 ±0.7	25.1 ±0.51	25.3 ±0.58	25.1 ±0.56	0.46	**0.02**	0.31
Fat Mass (kg)	25.5 ±1.2	25.7 ±1.2	26.1 ±1.2	29.4 ±1.2	29.8 ±1.3	29.6 ±1.2	26.7 ±1.0	27.1 ±1.1	26.8 ±1.1	0.09	0.06	0.48
Lean Mass (kg)	36.7 ±0.6	36.9 ±0.6	36.8 ±0.6	37.2 ±0.57	36.9 ±0.6	36.9 ±0.6	37.1 ±0.8	37.1 ±0.9	36.9 ±0.8	0.23	0.96	0.38
Percent Body Fat (%)	38.9 ±1.0	39.0 ±1.0	39.2 ±1.0	42.2 ±0.8*	42.6 ±0.9*	42.6 ±0.9*	40.1 ±0.8	40.5 ±0.8	40.3 ±0.9	0.08	**0.02**	0.72

Values represent mean±SEM. *Significantly different from control (p<0.05).

Table 5. Percent Change in Anthropometrics across 12-months.

Variable	Control *(n=46)*	50g DP *(n=43)*	100g DP *(n=35)*	p-value
Change in Weight (%)	0.73±0.39	-0.08±0.58	-0.19±0.54	0.800
Change in BMI (%)	0.62±0.41	-1.49±1.45	0.06±0.54	0.896
Change in Fat Mass (%)	2.55±1.07	0.89±1.02	0.15±0.92	0.534
Change in Lean Mass (%)	0.13±0.42	-0.78±0.51	-0.67±52	0.347
Change in Percent Body Fat (%)	1.13±0.63	0.94±0.61	0.33±0.62	0.660

Values represent mean±SEM.

Regional Body Composition

A significant change over time was observed in android total mass (p=0.022). Android total mass significantly increased, regardless of intervention group, from baseline to 6-months (p=0.006). Significant differences among groups were observed in all visceral adiposity estimates (visceral adipose mass: p=0.036; visceral adipose volume: p=0.036; visceral adipose area: p=0.034). The 50g DP group had significantly greater visceral adiposity (mass: p=0.036; volume: p=0.036; area: p=0.033) than the control group at all time points. No other significant differences over time or between groups were observed for any regional body composition measure recorded. None of the regional body composition measurements showed a significant absolute change from baseline to 12 months, as displayed in Table 7.

Table 6. Regional body composition across 12-months.

Variable	Control *(n=46)*			50g DP *(n=43)*			100g DP *(n=35)*			p-value		
										Time	Group	Group *Time
Time (month)	0	6	12	0	6	12	0	6	12			
Android fat mass (kg)	1.8 ±0.1	1.8 ±0.1	1.8 ±0.1	2.1 ±0.1	2.2 ±0.1	2.2 ± 0.1	1.9 ±0.1	1.9 ±0.1	1.9 ±0.1	0.07	0.12	0.43
Android total mass (kg)**	4.6 ±0.2	4.7 ±0.2	4.7 ±0.2	5.1 ±0.2	5.1 ±0.2	5.1 ±0.2	4.8 ±0.2	4.8 ±0.2	4.8 ±0.2	**0.02**	0.17	0.31
Android percent fat (%)	36.9 ±1.4	36.2 ±1.4	37.1 ±1.4	40.0 ±1.3	40.2 ±1.4	40.9 ±1.3	38.2 ±1.3	38.4 ±1.3	38.3 ±1.4	0.11	0.14	0.18
Gynoid fat mass (kg)	4.6 ±0.2	4.6 ±0.2	4.6 ±0.1	4.9 ±0.2	5.0 ±0.2	5.0 ±0.2	4.8 ±0.2	4.9 ±0.2	4.7 ±0.2	0.28	0.21	0.51
Gynoid total mass (kg)	10.6 ±0.2	10.6 ±0.2	10.5 ±0.2	11.0 ±0.3	11.0 ±0.3	10.9 ±0.3	10.9± 0.3	10.9± 0.3	10.8 ±0.3	0.09	0.50	0.99
Gynoid percent fat (%)	42.8 ±0.7	42.6 ±0.7	43.1 ±0.6	44.6 ±0.7	45.0 ±0.8	45.1 ±0.7	43.9± 0.7	44.4± 0.8	43.8 ±0.8	0.22	0.11	0.08
Android/ Gynoid ratio	0.85 ±0.0	0.84 ±0.0	0.85 ±0.0	0.89 ±0.0	0.89 ±0.0	0.90 ±0.0	0.87 ±0.0	0.87 ±0.0	0.87 ±0.0	0.08	0.39	0.93
Visceral adipose mass (kg)*	0.5 ±0.03	0.5 ±0.04	0.5 ±0.04	0.6± 0.05*	0.7± 0.05*	0.7± 0.05*	0.5± 0.04	0.6± 0.04	0.6± 0.04	0.07	**0.04**	0.15
Visceral adipose volume (cm^3)*	546 ±38	547 ±39	566 ±42	695 ±50*	708 ±52*	724 ±51*	585 ±46	613 ±46	587 ±45	0.07	**0.04**	0.15
Visceral adipose area (cm^2)	105 ±7	104 ±7	109 ±8	133 ±10*	136 ±10*	139 ±10*	112 ±9	118 ±9	113 ±9	0.07	**0.03**	0.11

Values represent mean±SEM. *Significantly different from control ($p<0.05$).

Table 7. Percent Change in Regional Body Composition.

Variable	Control *(n=46)*	50g DP *(n=43)*	100g DP *(n=35)*	p-value
Android fat mass (%)	3.55±1.49	3.40±1.47	-0.19±0.54	0.199
Android total mass (%)	2.60±0.69	0.90±0.83	-0.06±0.54	**0.050**
Android percent fat (%)	0.86±1.09	2.40±1.07	0.01±1.14	0.302
Gynoid fat mass (%)	0.87±0.99	0.91±1.08	-0.83±0.90	0.495
Gynoid total mass (%)	-0.15±0.49	-0.51±0.65	-0.58±0.59	0.851
Gynoid percent fat (%)	0.97±0.69	1.34±0.62	-0.19±0.58	0.365
Android/Gynoid ratio (%)	-0.09±1.04	1.08±0.80	0.16±0.85	0.630
Est. visercal adipose mass (%)	3.70±2.13	4.76±2.00	1.21±2.55	0.458
Est. visceral adipose volume (%)	3.72±2.13	4.77±2.00	1.20±2.55	0.461
Est. visceral adipose area (%)	3.73±2.14	4.81±2.00	1.20±2.55	0.455

Values represent mean±SEM.

Blood Measures

Fasting blood samples were analyzed for glucose, total cholesterol, HDL, LDL, and insulin. HOMA-IR was calculated from the results for glucose and insulin. Blood chemistry was analyzed at baseline, 6-months, and 12-months (Table 8). At baseline, there were no significant differences among the groups for blood chemistry measures. There was a significant group*time interaction (p=0.020) for fasting glucose, such that in the 50g DP group, glucose significantly increased from baseline to 12-months (p=0.037), with a non-significant increase from 6-months to 12-months (p=0.054), as displayed in Figure 1a. Additionally, the absolute change in glucose from baseline to 12-months was significantly different between the 100g DP and 50g DP groups (p=0.014), as the 50g DP group increased by 3.5±1.3 mg/dL and the 100g DP group decreased by -2.4±1.6 mg/dL (Figure 4). Similarly, the percent change in glucose from baseline to 12-months was significantly different between 100g DP and 50g DP groups

(p=0.009), with the 50g DP group increasing 4.1±1.5% and the 100g DP group decreasing 3.2±2.0% (Figure 5).

Table 8. Blood Measures across 12-months.

Variable	Control *(n=46)*			50g DP *(n=43)*			100g DP *(n=35)*			p-value		
										Time	Group	Group *Time
Time (month)	0	6	12	0	6	12	0	6	12			
Glucose (mg/dL)	92.0 ±1.2	91.3 ±1.1	91.9 ±1.1	92.8 ±1.8	93.5 ±1.6	96.3* ±1.7	98.8 ±4.3	93.3 ±1.8	93.8 ±2.0	-	-	**0.020**
Cholesterol (mg/dL)	205.1 ±4.1	207.2 ±4.7	212.1 ±4.7	207.3 ±5.7	199.0 ±5.4	206.5 ±6.0	197.4 ±6.6	201.9 ±6.6	198.9 ±6.9	0.326	0.490	0.105
HDL (mg/dL)	67.5 ±2.1	68.7 ±2.0	68.6 ±2.1	67.1 ±2.7	65.9 ±2.4	67.0 ±2.4	66.4 ±2.7	66.0 ±2.8	65.2 ±2.6	0.979	0.761	0.401
LDL (mg/dL)	119.3 ±3.7	119.8 ±4.5	124.2 ±4.2	117.4 ±4.7	113.9 ±5.0	117.9 ±5.0	113.6 ±6.2	117.0 ±6.2	118.9 ±6.4	0.137	0.706	0.685
Insulin (uIU/mL)	6.6 ±0.6	7.5 ±0.7	8.0 ±1.0	9.8 ±1.1	9.3 ±1.2	9.8 ±1.5	7.6 ±0.9	7.5 ±0.8	7.2 ±0.8	0.667	0.153	0.280
HOMA-IR	1.5 ±0.2	2.7 ±0.8	1.9 ±0.3	2.3 ±0.3	3.2 ±0.8	2.5 ±0.5	1.9 ±0.3	1.8 ±0.2	2.3 ±0.5	0.196	0.369	0.466

Values represent mean±SEM. *Significant difference in 50g DP from BL to 12-months (p=0.037).

Table 9. Percent Change in Blood Measures.

Variable	Control *(n=46)*	50g DP *(n=43)*	100g DP *(n=35)*	p-value
Glucose (%)	0.85±1.39	4.08±1.46	-3.15±2.00*	**0.009**
Cholesterol (%)	3.38±1.97	0.94±1.93	1.43±1.91	0.877
HDL (%)	2.92±1.91	1.15±1.72	-1.83±1.47	0.484
LDL (%)	4.64±3.00	1.63±2.72	8.00±4.21	0.962
Insulin (%)	12.0±7.02	2.83±6.91	2.95±7.95	0.543
HOMA-IR (%)	12.7±7.38	7.69±7.65	2.90±10.51	0.408

Values represent mean±SEM. *Significantly different from 50g DP group (p=0.006).

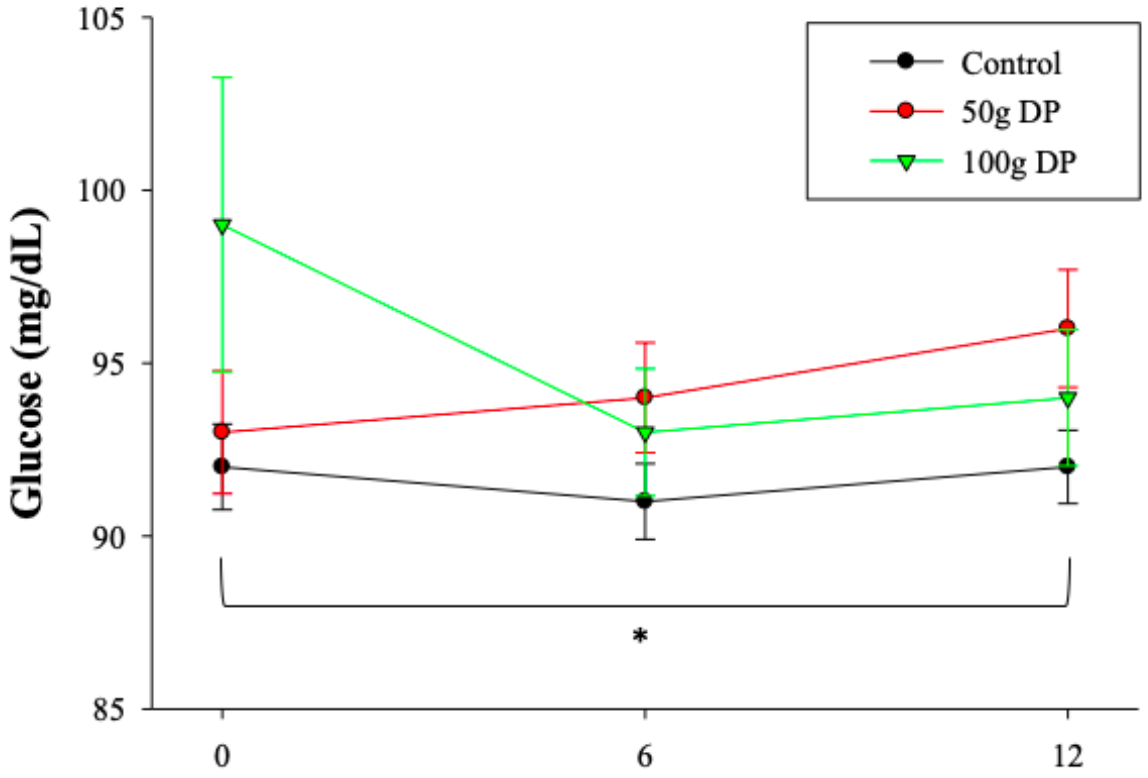

Figure 3. Change in Glucose over 12-months.

Error bars±SEM. *Significant difference in 50g DP from BL to 12-months (p=0.037).

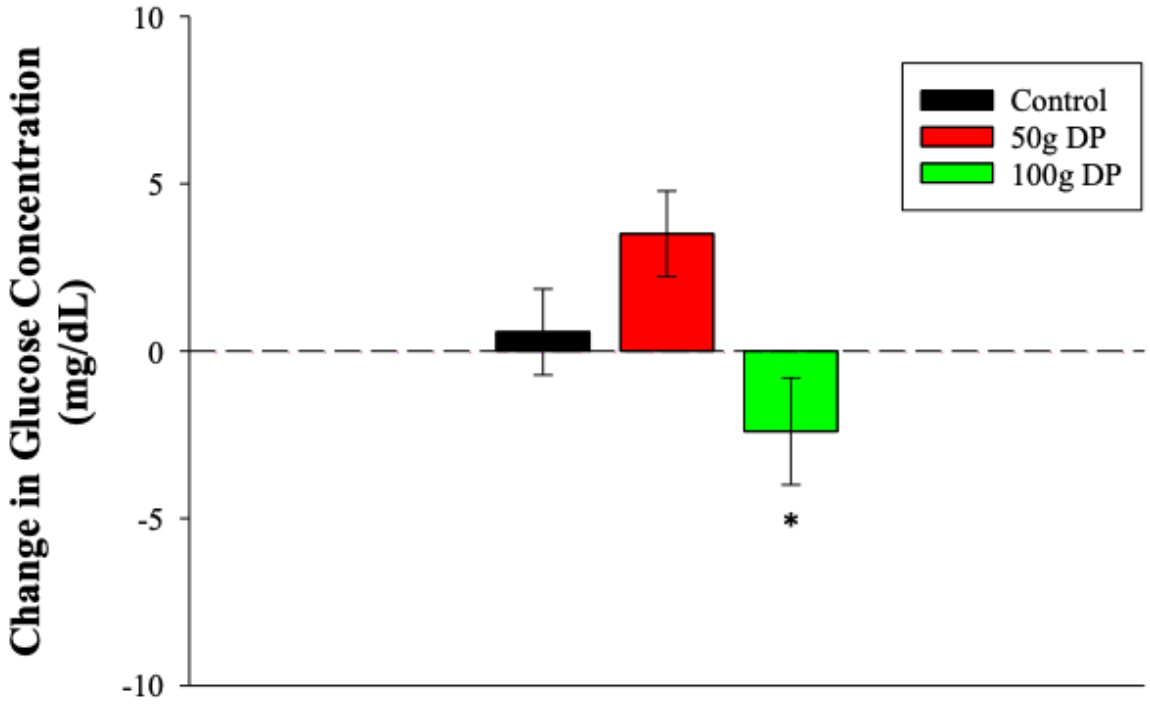

Figure 4. Absolute Change in Glucose Concentrations over 12-months.

Error bars±SEM. *Significant difference between 100g DP and 50g DP (p=0.014).

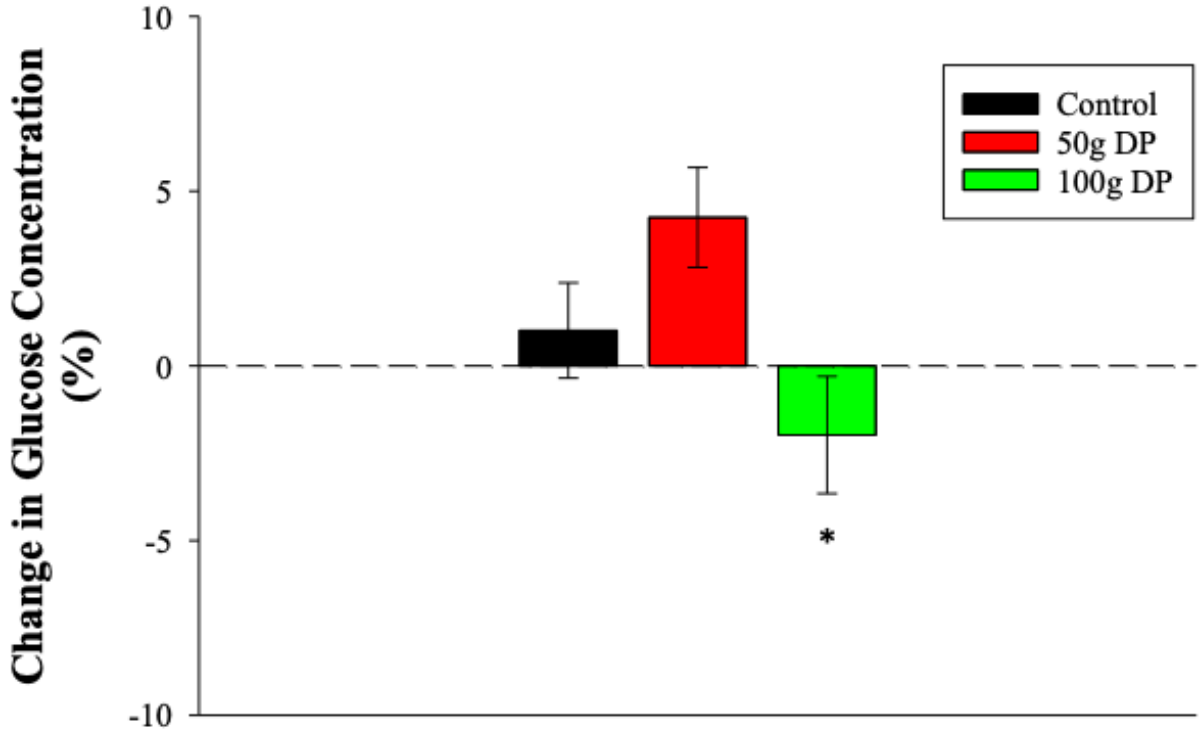

Figure 5. Percent Change in Glucose over 12-months.

Error bars±SEM. *Significant difference between 100g DP and 50g DP (p=0.009).

Relating Blood Glucose, Insulin Resistance, and Central Adiposity

All measures of central adiposity at both baseline and 12-months were found to be significantly positively correlated with 12-month glucose, insulin, and HOMA-IR (all $p<0.001$) (Table 10). In a linear regression model to predict 12-month fasting blood glucose, baseline android total mass was a significant independent variable ($R=0.488$; $F=37.7$; $p<0.001$), accounting for 23.8% of the variability in 12-month glucose levels (Table 11).

Table 10. Correlations between Central Adiposity and Blood Glucose, Insulin, and HOMA-IR.

		12-month values		
	Measures of Central Adiposity	**Glucose (mg/dL)**	**Insulin (uIU/mL)**	**HOMA-IR**
Baseline values	Visceral adipose tissue mass (g)	0.475	0.504	0.444
	Visceral adipose tissue volume (cm^3)	0.475	0.505	0.444
	Visceral adipose tissue area (cm^2)	0.475	0.505	0.444
	Android fat mass (g)	0.461	0.479	0.414
	Android total mass (g)	0.488	0.484	0.418
	Android percent fat (%)	0.376	0.395	0.352
12-month values	Visceral adipose tissue mass (g)	0.445	0.508	0.426
	Visceral adipose tissue volume (cm^3)	0.445	0.508	0.426
	Visceral adipose tissue area (cm^2)	0.446	0.508	0.426
	Android fat mass (g)	0.437	0.487	0.414
	Android total mass (g)	0.446	0.487	0.422
	Android percent fat (%)	0.381	0.414	0.353

All relationships indicate significant positive correlation ($p<0.001$).

Table 11. Linear Regression Model for Prediction of 12-month Glucose Concentration.

Independent Variable	**R**	**R^2**	**S.E.**	**F Statistic**	**P-value**
Baseline Android Total Mass (g)	0.488	0.238	8.2	37.7	<0.001

Discussion

This was a randomized control trial to assess the impact of consuming dried plums on metabolic markers, to include fasting glucose, HOMA-IR, lipids and body composition measures in postmenopausal women with low bone mineral density for a 12-month time period. Our study outcomes highlight the important finding that despite the volume of sugars in the dried plums, the post-treatment effects on fasting glucose observed at the end of the study did not approach the threshold for a pre-diabetes or diabetes diagnosis (>100mg/dL and >126mg/dL, respectively) in either the 50 or 100g study groups [2]. We did observe divergent responses to the dried plum intervention, based on dosage and which did not follow a dose-dependent pattern. Specifically, fasting glucose increased by 3.5 mg/dL (4.1%) in the 50g DP group, while the 100g DP decreased 2.4 mg/dL (3.2%), from baseline to 12 months, resulting in a significantly different percent change in fasting glucose between the 50 and 100g intervention groups ($p=0.009$).

While an increase in fasting glucose was observed over the 12-month intervention in the 50g dried plum group, fasting glucose concentrations for this group did not reach the threshold for pre-diabetes or diabetes diagnosis (>100mg/dL and >126mg/dL, respectively) [2], as at 12 months the 50g DP had an average fasting glucose of 96.3±1.7mg/dL. Such results are consistent with the observations of Sullivan et al. [44] who demonstrated a 2.7 mg/dL increase in mean fasting glucose in response to a 4-week dried fruit intervention (consisting of ¾ cup/day of dried plums, figs, dates, and raisins) in adults with cardiometabolic risk factors. However, these results contrast with a previous randomized parallel group study in which overweight/obese adults consumed 200-kcal/day dried plum for 8 weeks with no demonstrable alterations in fasting glucose concentrations [45]. As such, differences in experimental design, participant

characteristics, and length of intervention may contribute to the divergent findings regarding the effects of dried plum consumption on fasting glucose.

One potential explanation that must be considered for the rise in fasting glucose observed in the 50g DP group may be due to the addition of sugar, particularly fructose, into the diet via the dried plums. However, despite high energy content due to fructose, dried plums do not appear to elevate blood sugar rapidly and have a moderate glycemic index of 54 [13]. In fact, it has been suggested that dried plums may be a reasonable food choice for patients with non-insulin dependent diabetes mellitus or insulin-dependent diabetes mellitus [46].

Another potential explanation for the increase in fasting glucose in the 50g group is that the 50g DP group had significantly higher BMI ($p=0.025$) and percent body fat ($p=0.019$) than the control group at all points throughout the study. A higher BMI and percent fat may indicate an unhealthy metabolic status, thus potentially accounting for the increase in fasting glucose throughout the 12-month intervention. Interestingly, there was a 3.2% decrease in fasting glucose in the 100g DP group. By 6 months, the 100g DP group decreased to values comparable to the 50g DP and control groups. Another noteworthy finding was that despite differences in fasting glucose concentrations among groups, dried plum consumption had no impact on insulin concentrations or HOMA-IR. This finding indicates that the rise in fasting glucose in the 50g DP group was not enough to signify altered hepatic and extrahepatic insulin resistance [47]. These data suggest that the 100g DP dosing may be an ideal supplement dosage in postmenopausal women as it demonstrated no adverse effects on glucose metabolism or insulin resistance.

Investigators of various plum products, including fresh plums and plum juice concentrate, typically report beneficial effects on lipid profiles and inflammatory markers. Noratto et al.

reported that plum juice inhibited dyslipidemia in obese Zucker rats [11], while Utsunomiya et al. reported that plum extract prevented an increase in TC and TG in Wistar fatty rats [12]. The data from the current study report similar findings, that is, we failed to observe any effect of dried plum on any blood lipid measurement. Indeed, there was no increase in blood lipids in any group and it is unclear if the dried plums had a protective effect on lipid metabolism. Since our data indicate that the 50g DP dosing had an effect on fasting glucose but not insulin resistance, the maintenance of blood lipid levels in the 50g DP group may indicate that the relationship between dyslipidemia and hyperglycemia is facilitated by insulin resistance.

DXA measures of central adiposity (visceral adipose tissue mass, visceral adipose tissue volume, visceral adipose tissue area, android regional fat mass, android to gynoid percent fat ratio) were also assessed in this study. Contrary to the hypotheses, neither the 50 nor 100g intervention group had a significant decrease in central adiposity measures. In fact, android total mass increased from baseline to 6-months for the cohort ($p=0.02$), particularly driven by the control group which increased 2.6%. One plausible explanation for the increased android total mass across the entire cohort is that this anthropometric alteration may be a consequence of menopause-related estrogen deficiency. Toth et al. and Ambikairajah et al. [2, 4] previously established that body fat distribution changes with menopause to favor expansion of adipose stores in the abdomen, or in android distribution.

Similar findings were observed for measures that reflect visceral adipose tissue such that the 50g DP group had significantly greater estimated visceral adipose mass ($p=0.036$), estimated visceral adipose volume ($p=0.036$), and estimated visceral adipose area ($p=0.033$) than the control group at baseline, 6-months, and 12-months. The significantly higher estimated visceral adiposity in the 50g DP group compared to the control group throughout the study is to be

expected as the anthropometric results indicate that the 50g DP had a higher BMI and percent body fat at all time points. The lack of other significant anthropometric changes suggests our intervention did not exacerbate the menopause-related shift in body fat distribution.

Measures of central adiposity on 12-month fasting glucose were also assessed in this study. Central adiposity, specifically visceral fat, is proposed to lead to metabolic abnormalities due to dysfunctional adipocytes, inflammatory cytokine release, and excess export of lipids to the blood [10, 16]. Abildgaard et al. [5] found that in postmenopausal women, insulin sensitivity was significantly associated with total fat mass, and visceral fat mass, but not leg fat mass. As expected, all baseline visceral adipose and android composition measures were significantly and positively related to 12-month glucose, insulin, and HOMA-IR values (all $p<0.001$). All 12-month visceral adipose and android composition measures were significantly and positively related to 12-month glucose, insulin, and HOMA-IR values (all $p<0.001$). These results are consistent with other investigations in postmenopausal women which reported a strong positive relationship between visceral fat mass and insulin resistance [5]. Further, when evaluating which central adiposity measures predicted 12-month fasting glucose levels, the best predictor was baseline android total mass ($R=0.488$; $F=37.7$; $p<0.001$) accounting for 23.8% of the variability in 12-month glucose levels. These findings reinforce the notion that central adiposity in postmenopausal women is a risk factor for impaired glucose metabolism, possibly via mechanisms mediated by visceral adipose tissue.

Summary and Conclusion

Dried plums are becoming increasingly appreciated as a potential nutraceutical therapy for various health conditions. Although a common utility of dried plums has been to promote gastric motility, recent research indicates that dried plums may provide benefits to bone health and metabolic health. The presented analysis of concurrent effects of the intervention on blood glucose and insulin resistance provides valuable information regarding potential interactions between the dried plum therapy and risk of MetS. Such side effects, including increased fasting glucose, could determine whether or not dried plums are safe to recommend to postmenopausal women seeking a nonpharmaceutical treatment for their osteopenia.

This investigation demonstrated that the 50g DP treatment significantly increased fasting blood glucose in postmenopausal women between baseline and 12 months ($p=0.037$), although such an increase was not great enough in magnitude to qualify as prediabetic at 12 months. In contrast, the 100g DP experienced a significant reduction in fasting glucose compared to the 50g DP group ($p=0.006$), whereas, insulin resistance and blood lipid levels remained unchanged, regardless of intervention group. As such, the 100g DP intervention may be an ideal supplement dosage in postmenopausal women as it demonstrated no adverse effects on glucose metabolism or insulin resistance.

This analysis also confirmed a relationship between measures of central adiposity and 12-month fasting glucose. There was a significant positive correlation between all measures of central adiposity (visceral adipose mass, visceral adipose volume, visceral adipose area, android total mass, android fat mass, android percent fat) at both baseline and 12-months, and 12-month fasting blood glucose (all $p<0.001$). Further, baseline android total mass was found to be a significant predictor of 12-month fasting blood glucose ($p<0.001$). These relationships confirm

that central adiposity in postmenopausal women is a risk factor for impaired glucose metabolism, possibly via mechanisms mediated by visceral adipose tissue. Despite an increase glucose concentration throughout the intervention in the 50g DP group, which resulted in glucose concentrations below a pre-diabetic threshold, it is unlikely that postmenopausal women will be at an increased risk of MetS, T2DM, or CVD as a result of consuming a moderate amount of dried plums.

These conclusions are limited, however, in that many outcomes were close to, but did not reach, statistical significance, the cohort lacks racial diversity, and the intervention groups varied in baseline BMI and body fat percent. As a number of our outcome variables had significance values close to, but not quite meeting significance ($p<0.05$), it is likely that a larger sample size would have been beneficial to have the power to detect differences. Additionally, these results may not be generalizable to postmenopausal women who are not White, as the cohort consisted of 122 White women, 1 Asian woman, and 1 Hispanic/Latina woman from central Pennsylvania. Finally, analysis was not conducted accounting for potential covariates, baseline BMI and baseline body fat percent, which were significantly different as the 50g DP group had significantly higher BMI ($p=0.025$) and percent body fat ($p=0.019$), compared to the control group. Therefore, additional investigation is warranted, in which a larger sample size and analysis controlling for baseline BMI and baseline body fat percent may address potential impacts on the blood glucose outcome.

Future directions for this research should begin by expanding and diversifying the sample population and controlling for baseline differences in anthropometric measures. As there is heterogeneity in the literature at the present, more studies of the effect of dried plums on blood glucose and insulin resistance should be conducted to attempt to clarify whether dried plums

cause a dose-dependent modest elevation in blood glucose as observed in this data, as this was not an expected outcome. Further, the effect of dried plums on blood glucose and insulin resistance should be investigated in other populations at risk for MetS such as overweight men and premenopausal women to determine whether dried plums have unique effects in a postmenopausal population.

In summary, these results indicate a dried plum nutritional intervention does not have metabolic consequences significant enough to contraindicate their use in postmenopausal women with osteopenia. Despite an increase glucose concentration throughout the intervention in the 50g DP group, which resulted in glucose concentrations below a pre-diabetic threshold, it is unlikely that postmenopausal women will be at an increased risk of MetS, T2DM, or CVD as a result of consuming a moderate amount of dried plums. Additionally, the 100g DP group showed no decline in glucose metabolism throughout the intervention. As such, 100g DP intervention appears most suitable for nutraceutical therapy in postmenopausal women. These results also demonstrate that central adiposity poses a risk for metabolic abnormalities in postmenopausal women, highlighting the relationship between body fat distribution and metabolic health for this population.

Chapter 5

Conclusion

Dried plums are becoming increasingly appreciated as a potential nutraceutical therapy for various health conditions. Although a common utility of dried plums has been to promote gastric motility, recent research indicates that dried plum may provide benefits to bone health and metabolic health. The presented analysis of concurrent effects of the intervention on blood glucose and insulin resistance provides valuable information regarding potential interactions between the dried plum therapy and risk of MetS. Such side effects, including increased fasting glucose, could determine whether or not dried plums are safe to recommend to postmenopausal women seeking a nonpharmaceutical treatment for their osteopenia.

This investigation demonstrated that 50g DP treatment significantly increased fasting blood glucose in postmenopausal women between baseline and 12 months ($p=0.037$), although such an increase was not great enough in magnitude to qualify as prediabetic at 12 months. In contrast, the 100g DP experienced a significant reduction in fasting glucose compared to the 50g DP group ($p=0.006$). Insulin resistance and blood lipid levels remained unchanged, regardless of intervention group. As such, the 100g DP intervention may be an ideal supplement dosage in postmenopausal women as it demonstrated no adverse effects on glucose metabolism or insulin resistance.

This analysis also confirmed a relationship between measures of central adiposity and 12-month fasting glucose. There was a significant positive correlation between all measures of central adiposity (visceral adipose mass, visceral adipose volume, visceral adipose area, android total mass, android fat mass, android percent fat) at both baseline and 12-months, and 12-month

fasting blood glucose (all $p<0.001$). Further, baseline android total mass was found to be a significant predictor of 12-month fasting blood glucose ($p<0.001$). These relationships confirm that central adiposity in postmenopausal women is a risk factor for impaired glucose metabolism, possibly via mechanisms mediated by visceral adipose tissue.

These conclusions are limited, however, in that many outcomes were close to, but did not reach, statistical significance, the cohort lacks racial diversity, and the intervention groups varied in baseline BMI and body fat percent. As a number of our outcome variables had significance values close to, but not quite meeting significance ($p<0.05$), it is likely that a larger sample size would have been beneficial to have the power to detect differences. Additionally, these results may not be generalizable to postmenopausal women who are not White, as the cohort consisted of 122 White women, 1 Asian woman, and 1 Hispanic/Latina woman from central Pennsylvania. Finally, analysis was not conducted accounting for potential covariates, baseline BMI and baseline body fat percent, which were significantly different as the 50g DP group had significantly higher BMI ($p=0.025$) and percent body fat ($p=0.019$), compared to the control group. Therefore, additional investigation is warranted, in which a larger sample size and analysis controlling for baseline BMI and baseline body fat percent may address potential impacts on the blood glucose outcome.

Future directions for this research should begin by expanding and diversifying the sample population and controlling for baseline differences in anthropometric measures. As there is heterogeneity in the literature at the present, more studies of the effect of dried plums on blood glucose and insulin resistance should be conducted to attempt to clarify whether dried plums cause a dose-dependent modest elevation in blood glucose as observed in this data, as this was not an expected outcome. Further, the effect of dried plums on blood glucose and insulin

resistance should be investigated in other populations at risk for MetS such as overweight men and premenopausal women to determine whether dried plums have unique effects in a postmenopausal population.

In summary, these results indicate a dried plum nutritional intervention does not have metabolic consequences significant enough to contraindicate their use in postmenopausal women with osteopenia. Despite an increase glucose concentration throughout the intervention in the 50g DP group, which resulted in glucose concentrations below a pre-diabetic threshold, it is unlikely that postmenopausal women will be at an increased risk of MetS, T2DM, or CVD as a result of consuming a moderate amount of dried plums. Additionally, the 100g DP group showed no decline in glucose metabolism throughout the intervention. As such, 100g DP intervention appears most suitable for nutraceutical therapy in postmenopausal women. These results also demonstrate that central adiposity poses a risk for metabolic abnormalities in postmenopausal women, highlighting the relationship between body fat distribution and metabolic health for this population.

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Education

Pennsylvania State University, Schreyer Honors College
Nutritional Science, Nutritional Physiology and Biochemistry Option
Kinesiology minor, Biology minor
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Thesis

The Effect of a Dried Plum Nutritional Intervention on Glucose Regulation in Postmenopausal Women
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Work and Research Experience

Undergraduate Laboratory Research Assistant 1/2017-Present
Women's Health and Exercise Lab, Penn State Department of Kinesiology, University Park, PA

- Assisted in the BSL2 lab of Dr. Mary Jane De Souza performing wet lab procedures.
- Compiled subject data into nutrition programming for nutrient analysis.
- Developed proficiency in technical writing and scientific literature analysis.

Emergency Medical Technician 9/2018-Present
Centre LifeLink EMS, State College, PA

- Handles all aspects of responding to Basic Life Support 911 calls including patient care and transportation.
- Operates in conjunction with paramedics in Advanced Life Support patients.
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Leadership and Involvement

Fundraising Director - Penn State Project Haiti

- Coordinates with businesses and organizations to plan club fundraisers.
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- Represents the College to high school students and their families as a student panelist
- Meets with current Penn State underclassmen and accepted students at group presentations and one-on-one meetings.
- Developed proficiency in public speaking and interpersonal skills.

Member - Penn State Club Triathlon
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