THE EFFECTS OF A MUSHROOM-EGG WHITE BLEND PRODUCT ON ENDOTHELIAL HEALTH AND CARDIOVASCULAR BIOMARKERS

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ABSTRACT

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This study investigated the effects of mushroom-egg white blend (MEWB) product consumption on endothelial health and cardiovascular biomarkers. In a randomized, single-blind, crossover trial, 40 overweight and obese adults consumed an egg whites only (EWO) control product and a MEWB treatment product daily for 6 weeks each with a 4-week washout period in between. An EndoPAT measured endothelial function, a dual energy x-ray absorptiometry (DXA) machine measured body composition, and a fasting blood draw was collected to assess cardiovascular and lipid biomarkers. EWO consumption significantly decreased visceral adipose tissue volume from baseline to final ($p = 0.043$). MEWB consumption did not significantly change endothelial function or cardiovascular biomarkers. However, the treatment group showed improved levels of total cholesterol, low-density lipoprotein cholesterol cholesterol, and triglycerides from baseline to final. These findings suggest that incorporation of a MEWB product may improve lipid biomarkers, therefore reducing the rate of cardiovascular disease.

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CHAPTER I

INTRODUCTION

The prevalence of obesity in the United States has dramatically increased over the last 30 years and has appropriately been declared a public health crisis (Wang & Beydoun, 2007). The Centers for Disease Control and Prevention (CDC, n.d.b) defined overweight and obese as conditions characterized by weight that is higher than what is considered healthy for a given height. Overweight and obese individuals are at an increased risk for developing several comorbidities with adverse health impacts, including cardiovascular disease (CVD), type 2 diabetes mellitus, cancer, hypertension, and dyslipidemia.

It is well established that a strong relationship exists between obesity and CVD incidence and prevalence (Powell-Wiley et al., 2021). Obesity is often associated with hypertension, dyslipidemia, and insulin resistance, all of which have shown to increase the risk of developing CVD (Powell-Wiley et al., 2021). According to data collected by the National Health and Nutrition Examination Survey, individuals in the US with central obesity had an increased risk of cardiovascular-related mortality compared to individuals with the same body mass index (BMI) but without central obesity (Powell-Wiley et al., 2021). The Global Burden of Disease determined that high BMI accounted for 4 million deaths worldwide in 2015, more than two-thirds of which were related to CVD (Powell-Wiley et al., 2021). CVD is the leading cause of death in the US and worldwide (World Health Organization [WHO], 2021). It is also the most costly of all diseases. By 2035, the number of adults in the US with CVD are projected to rise to 131.2 million with costs expected to reach $1.1 trillion (Benjamin et al., 2018).

Many cardiovascular conditions are related to atherosclerosis, a progressive disease in which plaque builds up in the walls of arteries (Mudau et al., 2012). Endothelial health has a

major role in the development of atherosclerosis. The endothelium plays a key role in regulating the precise balance between the vasodilatory and vasoconstrictory state, known as vascular homeostasis (Mudau et al., 2012). In cases of endothelial dysfunction, the vasoconstrictory state tends to dominate the vasodilatory state and promotes pro-oxidant, pro-inflammatory, pro-adhesion, and pro-thrombotic effects (Mudau et al., 2012).

Long-term management and effective treatments for CVD are imperative for improving overall health and preventing debilitating health complications (National Heart, Lung, and Blood Institute, n.d.a). CVDs are generally treated with therapeutic lifestyle interventions, including healthy eating and physical activity. Individuals with CVD are primarily recommended to adopt a heart-healthy diet consisting of fruits, non-starchy vegetables, whole grains, and legumes while being low in saturated fat, sodium, and refined carbohydrates (Pallazola et al., 2019). An endothelium-protective diet would minimize contributors to endothelial dysfunction (elevated blood sugar, high blood pressure, increased low-density lipoprotein [LDL] cholesterol, and increased triglyceride [TG] levels) and optimize cardio-protective effects (increased high-density lipoprotein [HDL] cholesterol, support vasodilation, and reduce oxidative stress; Casas et al., 2018). A diet rich in plant foods has been associated with reduced risk of CVD as plants are abundant in bioactive phytochemicals that have the ability to modulate immune response, inflammation, and antioxidant activity (Pandey & Rizvi, 2009).

Mushrooms are a commonly consumed plant food known to be rich in several bioactive compounds, including dietary fiber and polyphenols. Many studies have demonstrated that mushroom intake may reduce the risk of CVD development through their immune-enhancing, antioxidant, anti-inflammatory, anti-atherogenic, cholesterol-lowering, and hypoglycemic effects (Kim et al., 2019; Yadav & Negi, 2021). β-glucans are the main polysaccharides present in

mushrooms and may have protective effects on the cardiovascular system. β-glucans are believed to have the ability to lower cholesterol levels by binding to cholesterol, inhibiting cholesterol synthesis, and increasing LDL cholesterol catabolism (Cerletti et al., 2021). Mushrooms are also rich in several types of polyphenols. Evidence suggests that polyphenols may prevent several steps in the development of atherosclerosis, including endothelial dysfunction, inflammatory process by monocytes, and LDL oxidation (Cheng et al., 2017).

Many studies have demonstrated that mushroom intake may reduce the risk of CVD development. One study found that consumption of mushrooms resulted in decreased levels of total cholesterol [TC], TG, and plasma glucose in diabetic rats (Wang et al., 2015). Another study found that consumption of *Agaricus bisporus*, also known as the white button mushroom, resulted in significantly improved levels of TC, LDL cholesterol, and HDL cholesterol in hypercholesteroemiac rats (Jeong et al., 2009). These studies all suggest that the lipid-lowering effect of mushrooms may be the result of a combination of mechanisms involving polyphenols and dietary fiber. However, no clinical trial has investigated the effects of whole mushrooms on endothelial function and cardiovascular biomarkers in individuals with overweight or obesity.

Hypothesis and Specific Aims

Hypothesis

The combination of mushroom and egg white in comparison to egg white alone will improve endothelial function and have beneficial effects on biomarkers of cardiovascular health in an overweight and obese population.

Specific Aims

Aim 1. To determine the effects of daily consumption of a MEWB treatment, in comparison to an EWO treatment, on endothelial function in overweight and obese individuals.

Aim 2. To determine the effects of daily consumption of a MEWB treatment, in comparison to an EWO treatment, on plasma biomarkers of cardiovascular health (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, sICAM-1, sVCAM-1, sP-Selectin) in overweight and obese indiviudals.

CHAPTER II

REVIEW OF LITERATURE

Obesity

The prevalence of overweight and obesity has dramatically increased over the last 30 years and has appropriately been declared a global public health crisis (Wang & Beydoun, 2007). In 2016, The WHO estimated that about 1.9 billion adults worldwide were overweight and over 650 million were obese (Cercato & Fonseca, 2019). Based on current trends, the prevalence of obesity in adults will continue to increase in the US. It is predicted that by the year 2030, nearly half (48.9%) of U.S. adults will be obese (Ward et al., 2019).

The CDC (n.d.b) described overweight and obese as conditions characterized by weight that is higher than what is considered healthy for a given height. There are no symptoms of overweight and obesity, but children and adults are annually screened for high BMI (CDC, n.d.b). BMI is a widely used screening tool that utilizes an individual's height and weight to distinguish between overweight and obesity (Wang & Beydoun, 2007). An individual with a BMI of 25.0 to 29.9 classifies as overweight, and an individual with a BMI > 30.0 classifies as obese (CDC, n.d.b). Even though BMI is unable to directly measure fat stores, an elevated BMI is correlated with abnormal fat distribution and excess fat accumulation, both known to have harmful effects on vital aspects of physiology (Mitchell et al., 2011). This could reduce the life expectancy of overweight and obese individuals and put them at a major risk developing a variety of comorbid conditions, including but not limited to CVD, type 2 diabetes, cancer, gastrointestinal issues, respiratory problems, and psychological issues (Cercato & Fonseca 2019; Fruh, 2017).

Oftentimes, when an individual is diagnosed as overweight or obese, they are encouraged to adopt healthful behaviors that promote gradual weight loss. Common methods include calorie reduction, increased physical activity, and eating a well-balanced diet composed of fruits, vegetables, lean protein sources and whole-grain carbohydrates (NHLBI, n.d.b). Some overweight and obese individuals that struggle with losing weight solely through lifestyle modifications may benefit from weight management programs, weight-loss medicines, and weight-loss surgery (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], 2018).

Impact of Obesity on Cardiovascular Health

It is well established that a strong relationship exists between obesity and CVD incidence and prevalence (Powell-Wiley et al., 2021). Excess adiposity associated with obesity encourages changes in heart function directly by affecting the vasculature and indirectly through obesity-related comorbidities (Powell-Wiley et al., 2021). Obesity is often associated with hypertension, dyslipidemia, hyperglycemia, insulin resistance, and inflammation, all of which have shown to increase the risk of developing CVD by promoting and accelerating the atherosclerotic process (Powell-Wiley et al., 2021). The prevalence of CVD morbidity and mortality is also known to be greater in individuals who are overweight, particularly those with central distribution of adipose tissues (Powell-Wiley et al., 2021). According to data collected by the National Health and Nutrition Examination Survey, individuals in the US with central obesity had an increased risk of cardiovascular-related mortality compared to individuals with the same BMI but without central obesity (Powell-Wiley et al., 2021). Powell-Wiley et al. (2021) also found that individuals with obesity tend to experience CVD-related events at an earlier age and consequently live with CVD for a longer amount of time and have a shorter life span than

individuals with normal weight. The Global Burden of Disease determined that high BMI accounted for 4 million deaths worldwide in 2015, more than two-thirds of which were related to CVD (Powell-Wiley et al., 2021). The Framingham Heart Study also revealed that each BMI increment by 1 kg/m^2 is associated with increasing the risk of heart failure (HF) by 5% in men and 7% in women (Koliaki et al., 2018).

Cardiovascular Disease

CVD is a general term that encompasses a number of conditions involving the heart and blood vessels, including coronary artery disease, cerebrovascular disease, peripheral arterial disease, HF, and stroke (American Heart Association [AHA], n.d.c). Blood vessels are responsible for circulating blood throughout the whole body. Blocked or weakened blood vessels are unable to supply adequate blood to vital organs such as the heart, brain, and kidneys (Mudau et al., 2012). Many cardiovascular conditions are related to atherosclerosis, a progressive disease in which plaque builds up in the walls of arteries, blood vessels that supply oxygenated blood from the heart to the body. Over time, this leads to narrowing and reduced blood flow within the arteries, ultimately decreasing the amount of oxygen reaching the body (Mudau et al., 2012). The most common type of CVD in the US is coronary artery disease, which involves the narrowing and blockage of coronary arteries, blood vessels that supply oxygenated blood to the heart (CDC, n.d.a). Non-modifable, predisposing risk factors of CVD include age, sex, race, ethnicity, and genetics. On the other hand, modifiable risk factors that can be changed to reduce one's risk of CVD include diet, physical activity, smoking, body weight, and levels of blood pressure, blood sugar, and cholesterol (Dahlöf, 2010).

Impact and Economic Burden

CVD is the leading cause of death in the US and worldwide (WHO, 2021). In 2019,

around 18 million people worldwide died from CVDs, accounting for approximately 32% of all deaths. Eighty-five percent of these deaths were due to heart attack and stroke (WHO, 2021). In the US, about 659,000 people die from CVD each year (Virani et al., 2021). From 2016 to 2017, the annual direct and indirect cost of CVD in the US was estimated at almost $363 billion (Virani et al., 2021). These costs accounted for 14% of total health expenditures during this year, which is more than any major diagnostic group. This annual number continues to increase and is projected to trend upward (Virani et al., 2021). By 2035, the number of adults in the US with CVD are projected to rise to 131.2 million with costs expected to reach $1.1 trillion (Benjamin et al., 2018). This can be further divided into direct medical costs estimated to reach $748.7 billion and indirect costs estimated to reach $368 billion (Benjamin et al., 2018). Direct medical costs refer to medical expenses associated with CVD while indirect costs refer to costs related to lost productivity in the workplace and at home (Benjamin et al., 2018). Compared to an employee without CVD, an employee with CVD can cost an employer, on average, almost 60 hours and over $1,100 more in lost productivity each year (Benjamin et al., 2018). In a study investigating the association between favorable cardiovascular health status and health care expenditures in a large and ethnically diverse population of one employer, annual employer healthcare expenditures were on average $2,021 less for employees with an optimal cardiovascular health profile and $940 less for employees with a moderate cardiovascular health profile (Osondu et al., 2017).

Diagnosis

Most people are unaware they have CVD, so it is often undetected or not diagnosed until a person experiences symptoms of an arrythmia (palpitations/fluttering in the chest), a heart attack (chest and/or neck pain, indigestion, dizziness, nausea, or vomiting), or HF (shortness of

breath, swelling, or fatigue; AHA, n.d.c). Due to the complexity of the disease, a CVD diagnosis usually involves a comprehensive examination of symptoms, family medical history, past medical history, anthropometric measurements, blood pressure, and diagnostic tests such as blood tests and imaging studies (AHA, n.d.a; CDC, n.d.a). A detailed assessment of symptoms and medical history can help detect non-modifiable and modifiable risk factors of CVD. Non-invasive screening tests such as calculating BMI and measuring blood pressure provide more information for estimating an individual's level of cardiovascular risk (AHA, n.d.a). Blood pressure is actually considered one of the most important CVD screening measurements because it rarely produces clinical symptoms and will remain undetected unless it is measured. If left untreated, high blood pressure (hypertension) increases heart disease and stroke risk by causing the heart to work harder and eventually become stiff and incapable of functioning properly over time (AHA, n.d.b). Blood tests are also collected to assess risk for CVD. A common blood test used for screening cardiovascular risk is a lipid profile, which measures TC, LDL cholesterol, HDL cholesterol, and TG (CDC, n.d.c). Imaging tests may also be ordered to provide a closer look into the anatomy and function of the heart. Common imaging tests are electrocardiograms, chest x-rays, stress tests, coronary angiograms, and cardiac catheterizations are also used to diagnose CVD (CDC, n.d.a).

Risk Factors

Identifying and monitoring risk factors for CVD is useful for preventing the development and onset of cardiovascular diseases. Due to the high mortality rate and large indirect and direct costs associated with CVD, several studies are investigating the main risk factors contributing to CVD in the hopes of improving cardiovascular health. The AHA tracks the seven leading behaviors and risk factors that may put someone at a higher risk for heart disease (Virani et al.,

2021). This list includes an unhealthy diet, physical inactivity, excess body weight, smoking, and elevated levels of cholesterol, blood sugar, and blood pressure (Sanchez, 2018). Ninety-nine percent of the adult U.S. population is diagnosed with at least one of these seven cardiovascular risk factors (Goetzel et al., 2017). These seven risk factors were used to compose Life's Simple 7, health habits and behaviors that are measured to monitor the progress toward improving the cardiovascular health of all Americans (Sanchez, 2018). Life's Simple 7 promotes the following lifestyle changes to counteract the leading risk factors and achieve optimal cardiovascular health: manage blood pressure, control cholesterol, reduce blood sugar, get active, eat better, lose weight, and stop smoking. Individuals who met at least five of these metrics had a 78% reduced risk for heart-related death compared to individuals who did not meet any metrics (Ford et al., 2012).

CVD can be described as a continuum that begins with the presence of cardiovascular risk factors and progresses into CVDs that target and damage vital organs (Dahlöf, 2010). Cardiovascular risk factors usually cluster in individuals and interact with each other, increasing one's cardiovascular risk (Dahlöf, 2010). Approaching CVD through this lens brings to light the importance of targeting CVD at any point in the continuum to impede the pathophysiological progression (Dahlöf, 2010). It also makes a total cardiovascular risk assessment essential for effective prevention and treatment rather than targeting individual risk factors in isolation (Dahlöf, 2010). Emberson et al. (2004) determined the effectiveness of different risk reduction strategies for preventing CVD and found that overall risk assessment was more effective compared to detecting and targeting one specific risk factor. This was likely because an overall risk assessment was able to detect multiple risk factors and therefore multiple interventions could be implemented to provide greater health benefits (Emberson et al., 2004).

Endothelial Health and Dysfunction

Endothelial health has a major role in the development of atherosclerosis, a common underlying cause of several CVDs. Endothelial cells form a continuous, semi-permeable lining known as the endothelium in every blood vessel in our body (Fitridge & Thompson, 2011). The cells provide a protective barrier and facilitate the passage of blood gasses and macromolecules between body tissues and circulating blood (Verma et al., 2003). Endothelial cells play integral roles in the development of new blood vessels, cellular adhesion, the regulation of exchanges between the bloodstream and surrounding tissues, and the modulation of vascular permeability and tone to adapt to blood flow (Kwaifa et al., 2020). Due to the extensive roles endothelial cells have within the vascular system, maintaining endothelial health is imperative for proper tissue function (Axtell et al., 2010).

The endothelium plays a key role in regulating the precise balance between the vasodilatory and vasoconstrictory state, known as vascular homeostasis (Mudau et al., 2012). A healthy endothelium is able to maintain vascular homeostasis by continuously responding to physical and chemical stimuli produced by a variety of factors that regulate cellular adhesion, thromboresistance, and vascular tone (Verma et al., 2003). In the vasodilatory state, the blood vessels widen as the muscles within the blood vessels relax, allowing for an increase in blood flow. This state is associated with anti-oxidant, anti-inflammatory, anti-adhesion, and anti-thrombotic effects (Mudau et al., 2012). On the other hand, the vasoconstrictory state is associated with the narrowing of blood vessels, causing a decrease in blood flow and an increase in blood pressure. This narrowing promotes pro-oxidant, pro-inflammatory, pro-adhesion, and pro-thrombotic effects (Mudau et al., 2012). In cases of endothelial dysfunction, the vasoconstrictory state tends to dominate the vasodilatory state and progressively leads to

detrimental pathophysiological changes. This process is considered to be the first step in the development of CVD in cases of obesity (Lobato et al., 2012). This is because chronic exposure to obesity-related conditions, such as elevated blood pressure and dyslipidemia, disrupt the vasodilating properties of the endothelium and consequently induce the progression of endothelial dysfunction (Lobato et al., 2012).

Cellular Adhesion Molecules

Cell adhesion is the ability of a cell to interact and stick to neighboring cells or an extracellular matrix (Khalili & Ahmad, 2015). Cellular adhesion molecules (CAMs) are found on the surfaces of cells and are involved in this interaction between cells, meaning they are responsible for cells attaching to each other (DeLisser, 2006). CAMs are integral in the assembly of endothelial cells into functional vascular networks within the endothelium. However, a substantial upregulation of CAMs and other proteins involved in cell-to-cell interactions is often a modification seen in endothelial dysfunction (Mudau et al., 2012).

Soluble intracellular adhesion molecule-1 (sICAM-1) and soluble vascular adhesion molecules (sVCAM-1), members of the immunoglobulin adhesion molecule family, both contribute to CVD by facilitating the interaction between the endothelium and monocytes, white blood cells that differentiate into foam-ladened macrophages and eventually form atherosclerotic plaque (Hope & Meredith, 2003). Soluble platelet selectin (sP-Selectin) is another adhesion molecule strongly associated with atherosclerosis. It is a member of the selectin family, which are only found on vascular-related cells that mediate the interactions between the endothelium and circulating leukocytes at sites of inflammation (Alehagen & Lindahl, 2015). sP-selectin has the specific function of mediating the interaction between endothelial cells and platelets with leukocytes (Mulhem et al., 2020). High concentrations of it can be found in those with impaired

cardiac function compared with those with a normal cardiac function (Alehagen & Lindahl, 2015). In individuals with obesity, increased circulation of CAMs have shown to contribute to the development of endothelial dysfunction and atherosclerosis (Mulhem et al., 2020). Mulhem et al. (2020) found that metabolically healthy obese indivudals had higher serum concentrations of sICAM-1 and sP-selectin compared to metabolically healthy lean individuals. They deduced that obesity-related increased cardiovascular risk could be mediated by significantly increased levels of CAMs (Mulhem et al., 2020).

The enhanced expression of sICAM-1, sVCAM-1, sP-selectin, and endothelial leukocyte adhesion molecule-1 (E-selectin) in endothelial cells is a characteristic of endothelial cell activation, which is considered a precursor of endothelial dysfunction (Collins et al., 2000; DeLisser, 2006). Endothelial cell activation is typically induced through the body's inflammatory response and ultimately leads to prothrombotic and proinflammatory activity in the blood vessels (DeLisser, 2006). This process creates an environment of decreased anti-oxidant and anti-inflammatory activity and enhanced endothelial permeability, all of which contribute to atherosclerosis initiation (Hope & Meredith, 2003). sP-selectin and E-selectin mediate the first step of cellular adhesion, which is the rolling of leukocytes along the endothelial surface (DeLisser, 2006). This is followed by sICAM-1 and sVCAM-1 facilitating the firm attachment of monocytes to endothelial cells and the transendothelial migration of monocytes into the subendothelial space (Hope & Meredith, 2003; Mulhem et al., 2020). The monocytes differentiate into macrophages and express receptors that facilitate the uptake of lipids (Mudau et al., 2012). Lipid accumulation causes the macrophages to turn into foam cells, which initiate an early atherosclerotic lesion that can eventually form atherosclerotic plaque (Mudau et al., 2012).

Dyslipidemia

Dyslipidemia plays a significant role in disrupting endothelial function. Dyslipidemia is defined as the imbalance of one or more kinds of lipids: TC, LDL cholesterol, HDL cholesterol, and TG, and it is closely linked with the development of CVD (Pappan & Rehman, 2021). Although endothelial dysfunction and dyslipidemia individually serve as risk factors for atherosclerosis, some studies suggest that the presence of one factor may trigger the initiation of the other (Hurtubise et al., 2016). Hyperlipidemia, an excess level of lipids in the blood, can lead to the production of excessive levels of reactive oxygen species (ROS), which are known to damage the endothelium and can ultimately progress to atherogenesis (Hurtubise et al., 2016). Elevated lipids also serve as irritants, which initiate the formation of atherosclerotic plaque by disrupting the endothelial layer. This leads to the accumulation and trapping of LDL cholesterol in the subendothelial space. The LDL cholesterol is subject to oxidation by ROS released from the endothelium. This series of events result in the increased expression of CAMs and is unlike the poor adhesion seen in healthy vasculature. After a series of inflammatory responses, fatty streaks can develop, calcify, and lead to the hardening of plaque in the arteries (Linton et al., 2019).

Current Treatments and Therapies

CVDs are generally treated with lifestyle interventions, including healthy eating and physical activity. Weight loss has shown to improve major contributors of CVD, including endothelial dysfunction (Powell-Wiley et al., 2021). Individuals with CVD are primarily recommended to adopt a heart-healthy diet, consisting of fruits, non-starchy vegetables, whole grains, and legumes while also being low in saturated fat, sodium, and refined carbohydrates (Pallazola et al., 2019). The CDC also recommends that individuals with CVD stop smoking,

maintain a healthy weight, and treat other conditions that could contribute to the exacerbation of CVD, including hypertension, high cholesterol, and diabetes. If lifestyle interventions alone are not enough, medications and medical procedures may be recommended depending on the type and severity of CVD (NHLBI, n.d.a). Medications are prescribed to manage CVD risk factors and treat underlying causes of CVD (NHLBI, n.d.a). They work to reduce blood pressure or widen the arteries, which targets the underlying mechanisms that contribute to endothelial dysfunction (Su, 2015). Common cardiovascular drugs used to improve endothelial function include angiotensin-converting enzyme inhibitors, antioxidant agents, beta blockers, and statins (NHLBI, n.d.a; Su, 2015). In more advanced cases, interventional procedures or surgery are necessary. Common procedures used to treat blocked arteries include coronary angioplasty, coronary artery bypass graft, and transmyocardial revascularization (NHLBI, n.d.a).

Impact of Diet on Obesity and Endothelial Health

Obesity is a complex health issue resulting from behavioral, environmental, and biological factors (Cercato & Fonesca, 2019). However, in most cases, weight gain is a consequence of excess calorie consumption combined with inadequate physical activity. The environment in the US promotes the combination of these deleterious lifestyle habits, which can explain why the US environment is referred to as “obesogenic” (Mitchell et al., 2011; Wang & Beydoun, 2007). Although diet contributes to obesity, research supports the idea that diet modifications can also be used to prevent and treat obesity. For example, the ASPIRE trial found that small changes to diet and physical activity lead to significantly more weight loss, weight loss maintenance, and abdominal fat loss in overweight and obese sedentary adults (Mitchell et al., 2011). Not only is the Western diet considered obesogenic, it can also be described as an “atherogenic” diet. CVD development is associated with unhealthy dietary patterns such as

excessive intake of sodium and processed foods, added sugars, unhealthy fats, low intake of fruit and vegetables, whole grains, fiber, and healthy fats (Casas et al., 2018). A diet high in dietary fat, specifically saturated fat and trans fat, has been shown to rapidly induce adhesion molecules and contribute to atherogenesis (Collins et al., 2000).

Considering both excessive weight gain and CVD are a result of poor quality nutrition, therapeutic dietary interventions are often utilized to treat CVD and obesity. Since obesity and CVD are closely linked with endothelial health, healthful diet modifications can also serve as a powerful tool for protecting and maintaining the endothelium. This specific diet would serve to minimize contributors to endothelial dysfunction, including elevated blood sugar, high blood pressure, increased LDL cholesterol levels, and increased triglyceride levels (Mudau et al., 2012). An endothelium-protective diet would also support endothelial health by contributing to increased HDL cholesterol levels, support vasodilation, and reduce oxidative stress (Casas et al., 2018). A diet rich in plants has been associated with reduced risk of chronic diseases, including CVD. This could be due to the abundance of bioactive phytochemicals within plant-based foods that are able to modulate physiological processes, including immune response, inflammation, and antioxidant activity (Pandey & Rizvi, 2009).

Mediterranean Diet

One notable and highly researched therapeutic diet that encompasses these recommendations is known as the Mediterranean diet. The Mediterranean-style diet consists of high intake of fruits, vegetables, nuts, grains, cereals, and olive oil; moderate intake of lean animal protein sources such as fish and poultry; and low intake of red meat, processed meats, dairy products, and sweets (Estruch et al., 2018). Adherence to this diet has been shown to reduce cardiovascular risk. In 2004, Esposito et al. (2004) investigated how a

Mediterranean-style diet intervention rich in fruits, vegetables, whole grains, nuts, and olive oil affects endothelial function compared to a control diet of 50-60% of carbohydrates, 15-20% protein, and <30% of total fat. After 2 years, they discovered that patients following the Mediterranean-style diet had improved endothelial function values compared to values remaining stable in patients consuming the control diet (Esposito et al., 2004). Another study conducted by Estruch et al. (2018) investigated the efficacy of two types of Mediterranean diets, one supplemented with mixed nuts and one supplemented with extra-virgin olive oil, compared to a low-fat control diet. This study found that among individuals with high cardiovascular risk, energy-unrestricted Mediterranean diets supplemented with either extra virgin olive oil or mixed nuts reduced the incidence of major cardiovascular events (stroke, myocardial infarction, and stroke; Estruch et al., 2018). They suggested that a synergy may exist among the nutrient-rich foods contained in the Mediterranean diet that promote favorable changes in intermediate pathways of cardiometabolic risk (lipids biomarkers, insulin sensitivity, oxidation resistance, and inflammation; Estruch et al., 2018). These findings support the notion that a Mediterranean diet contributes to cardiovascular risk reduction.

Mushrooms: Nutrient Profile, Bioactive Compounds, and Disease Prevention

Mushrooms, known for their unique texture and distinct flavor, have been a staple component of many cuisines for centuries. Although they have been recognized for their culinary attributes since early history, they have recently become a prevalent topic of research for their nutritional and therapeutic properties (Kim et al., 2019). Mushrooms are a highly nutritive food. The fruiting bodies of mushrooms are approximately 90% moisture. The remaining dry matter contains digestible and non-digestible carbohydrates, all essential amino acids, and polyunsaturated fatty acids. These properties make mushrooms a significant source of fiber,

protein, and healthy fats (Yadav & Negi, 2021). Mushrooms are also rich in vitamins, especially vitamin D, vitamin C, and B vitamins (Yadav & Negi, 2021). They also contain a variety of minerals, including potassium, phosphorus, magnesium, iron, and zinc (Yadav & Negi, 2021).

Studies suggest that dietary fiber found in mushrooms may be one mechanism by which mushrooms contribute to decreased CVD risk (Kim et al., 2019). β-glucans are the main polysaccharides present in mushrooms and may have protective effects on the cardiovascular system (Cerletti et al., 2021). Currently, there are a limited number of studies investigating the cardiovascular effects of β-glucans from mushrooms, but there are many studies that have investigated β-glucans from cereals such as oats and barley. β-glucans from cereals are recognized in the body as non-digestible dietary fibers that positively affect the cardiovascular system by lowering cholesterol, TG, and apolipoprotein B levels in adult subjects (Cerletti et al., 2021). β-glucans are believed to have the ability to lower cholesterol levels by binding to cholesterol and by functioning as a prebiotic (Cerletti et al., 2021; Kim et al., 2019). By undergoing microbial fermentation, β-glucans produce short-chain fatty acids that can inhibit cholesterol synthesis and increase LDL cholesterol catabolism, resulting in decreased levels of cholesterol in the blood (Cerletti et al., 2021).

Multiple studies have also investigated the therapeutic effects of the diverse bioactive compounds contained in mushrooms on cardiovascular biomarkers. In addition to vitamins, minerals, and polysaccharides, mushrooms also contain polyphenols, common micronutrient antioxidants found in many plant foods. Evidence suggests that polyphenols may prevent several steps in the development of atherosclerosis, including endothelial dysfunction, inflammatory process by monocytes, and LDL oxidation (Cheng et al., 2017). These compounds found specifically in mushrooms have exhibited immune-enhancing, antioxidant, anti-inflammatory,

anti-atherogenic, cholesterol-lowering, and hypoglycemic activities; all of which are associated with improved CVD risk (Kim et al. 2019; Yadav & Negi, 2021).

Many studies have demonstrated that mushroom intake can reduce the risk of CVD development. One study found that consumption of mushrooms resulted in decreased levels of TC, TG, and plasma glucose in diabetic rats (Wang et al., 2015). Another study found that consumption of *Agaricus bisporus*, also known as the white button mushroom, resulted in significantly improved levels of TC, LDL cholesterol, and HDL cholesterol in hypercholesterolemic rats (Jeong et al., 2009). Another study by Kim et al. (2019) found that consumption of portobello mushrooms and shiitake mushrooms is able to prevent the development of high-fat diet induced atherosclerosis in mice. Mice that were fed a high-fat plus shiitake mushroom diet had decreased aortic lesion area in comparison to the mice fed a high-fat control diet (Kim et al., 2019). These studies suggest that the lipid-lowering effect of mushrooms may be the result of a combination of mechanisms involving polyphenols and dietary fiber.

Mushrooms and Endothelial Health

Endothelial dysfunction promotes a pro-oxidant and pro-inflammatory state. As mentioned, mushrooms contain a diverse range of bioactive molecules that promote an anti-oxidant and anti-inflammatory environment, which may be protective against endothelial dysfunction. A cell study conducted by Keith R. Martin (2010) tested the effects of common and specialty mushrooms on adhesion molecule expression. Martin (2010) noted that most studies that have analyzed the protective properties of mushrooms have been done on exotic and specialty species of mushrooms. Martin (2010) specifically included white button mushrooms in his study as they are the most frequently consumed mushroom in the US and could be just as effective for preventing CVD development. Martin (2010) presumed that preincubation of

human aortic endothelial cells with dimethylsulfoxide extract, a bioactive component of whole mushrooms, could inhibit the binding of monocytes to the cytokine-stimulated endothelium through the use of an *in vitro* model of atherogenesis. He found that white button mushrooms consistently reduced the expression of VCAM-1, ICAM-1, and E-selectin while all mushrooms significantly reduced the binding of monocytes to the endothelium (Martin, 2010).

Egg Whites: Nutrient Profile and Effects on Cardiovascular and Endothelial Health

The link between egg consumption and CVD risk has been a topic of controversy for many years. Past recommendations for preventing CVD involved limiting dietary cholesterol intake, but this has since been removed due to inconsistent evidence supporting dietary cholesterol as a nutrient of concern for overconsumption (Drouin-Chartier et al., 2020). This recommendation often targeted eggs, specifically the yolk, due to egg yolk being a major source of dietary cholesterol. Although this is true, whole eggs are also an accessible and affordable source of high quality proteins, iron, vitamins, unsaturated fatty acids, and phospholipids (Drouin-Chartier et al., 2020). Focusing on the egg white, this portion of the egg is highly concentrated in proteins. A total of 150 distinct proteins have been identified in egg whites (Réhault-Godbert et al., 2019). Ovalbumin, which is assumed to provide essential amino acids for chicken embryo development, makes up 50% of the total egg white proteins. This makes ovalbumin a significant source of amino acids for human nutrition (Réhault-Godbert et al., 2019). Egg whites also contain significant amounts of B vitamins, especially high amounts of B2 (Riboflavin), B3 (Niacin), and B5 (Pantothenic acid; Réhault-Godbert et al., 2019). Findings from a 2021 large cohort study by Zhuang et al. showed that replacing whole egg consumption with egg whites was associated with lower all-cause mortality and mortality related to CVD. Another study that sought to investigate the effect of egg consumption on CVD tested different

portions and preparations of the egg in rats (Chairuk et al., 2021). In this study, Chairuk et al. (2021) found that the rats that consumed egg whites had decreased plasma cholesterol levels and body fat accumulation while whole egg and only egg yolk increased blood pressure. Currently, the majority of trials investigating the relationship between eggs and cardiovascular or endothelial health look at the whole egg, so there is not a lot of research looking at the specific effects of egg white on cardiovascular and endothelial health.

In summary, evidence suggests that including mushrooms in the diet may greatly benefit cardiovascular and endothelial health. Mushrooms have a unique, "meaty" flavor that allows them to serve as an animal protein substitute. Mushrooms also have a cardio-protective nutrient profile. They are naturally low in unhealthy fats and sodium but are rich in fiber, amino acids, polyunsaturated fats, and polyphenols. The study investigated whether the consumption of mushroom combined with egg whites in comparison to egg whites alone would improve endothelial function and levels of cardiovascular biomarkers in individuals classified as overweight or obese.

CHAPTER III

METHODOLOGY

Study Design and Subject Recruitment

A randomized, single-binded, crossover study design was used with an evaluation at the baseline and final points of the starting and alternate treatment courses (four evaluations total). A total of 40 individuals with self-reported difficulties with weight management were recruited to participate in the study. Participants were recruited from Texas Woman's University (TWU) via email, local Denton organizations, and through social media platforms such as Facebook (see Appendix A). All interested participants were assessed for qualification of the inclusion and exclusion criteria through a phone screening. During the phone screening, the participants completed a detailed questionnaire that inquired about demographics, smoking history, medical history, medication and supplement intake, dietary history (including food allergies), and physical activity history (see Appendix B). Once a participant was determined eligible to participate in the study, an appointment was scheduled at the TWU Human Research Laboratory located on the Denton campus.

Participants were randomly assigned to start the first treatment arm with either the control product or the treatment product. Those assigned to start with the control product consumed two 50 gram (100 grams total) patties made of EWO while those assigned to start with the treatment product consumed two 50 gram (100 grams total) patties made of a MEWB. The mushrooms used were a blend of white button mushrooms and cremini mushrooms. Both the control product and treatment product were flash frozen right after they were prepared to minimize crystallization and maintain flavor and texture as tested by Du et al. (2021) in their consumer acceptability study. The participants were instructed to consume their assigned product as their

first meal of the day for 6 weeks. This was followed by a 4-week washout period in which no product was consumed. After those 4 weeks, participants proceeded with the second arm consuming the alternate product, either the control product or the treatment product, for 6 weeks. The study protocols were approved by the Institutional Review Board at TWU before any clinical work was initiated (see Appendix C).

Inclusion and Exclusion Criteria

Inclusion criteria included adult men and women between 20 and 45 years of age with a BMI between 25 to 35 kg/m^2 and were otherwise healthy. Participants needed a history of participating in physical activity less than twice per week, less than 20 minutes per session, and had a sedentary occupation for at least 1 year prior to study enrollment. Individuals who met the inclusion criteria were considered for the study regardless of ethnicity and race. Exclusion criteria included any form of pre-existing disease (e.g. cancer, heart disease, diabetes, liver, or renal disorders, anemia, pregnancy and lactation). Participants were also excluded if they were taking extra large doses of antioxidants or fish oil supplements, had abnormal hemoglobin, WBC or platelets, hypo/hyperthyroidism, hyperlipidemia, abnormal liver enzymes, abnormal kidney function, smoking, and heavy alcohol consumption (>2 drinks per day for men, >1 drink per day for women). Other exclusion criteria included individuals who refrained from egg or egg-based products, had egg allergies and/or were unable to tolerate mushrooms.

Baseline and Final Measurements for Treatment Arms

Participants who qualified were invited to participate in the 16-week study, including a 4-week wash out period in between treatment arms. A total of four study visits (1-week and 6-week time point visits that correspond with the starting treatment; 11-week and 16-week time point visits that correspond with the cross-over treatment) were held at the TWU Human

Research Laboratory in the Denton campus. At the first study visit, each participant was provided with a written consent form (see Appendix D), which informed participants of every aspect of the study. At the first visit, participants were randomly assigned to start the first treatment arm with either the EWO product (control group) or the MEWB product (treatment group). Participants were asked to fast for at least 8 hours prior to each visit. At the beginning of each study visit, anthropometric measurements were obtained, including height, weight, waist circumference, and blood pressure. These measurements were taken three times and the average was recorded. Afterwards, endothelial function was measured using an EndoPAT. Body composition measurements were obtained at each visit using a dual-energy x-ray absorptiometry (DXA) machine. A fasting blood draw was collected prior to consumption of the product and then repeated blood draws were taken at timed intervals. At the beginning of each treatment arm, each participant met with the registered dietitian to receive personalized nutrition recommendations. Participants also submitted a diet record of the 3 days leading up to the baseline and final visits of each treatment arm, but this was not evaluated as part of this study. At the end of the baseline visits of each treatment arm (1-week and 11-week time points), participants were provided with a 3-week supply of their assigned treatment. They were instructed and scheduled to return to the clinic after 3 weeks to receive another 3-week supply of their assigned treatment.

Treatment Compliance

Participant compliance to their assigned treatment was measured in two ways. A calendar food log was provided to each participant at the beginning of each treatment arm. These food logs were used by the participant throughout the duration of the study to record daily consumption of the assigned treatment. Treatment compliance was also measured by noting the

number of products the participant had remaining in their provided cooler bag when they returned to the clinic to replenish their treatment product stock.

EndoPAT and Endothelial Health Analysis

Endothelial vasodilator function was measured by a trained professional at each visit with the EndoPAT, a Food and Drug Administration (FDA)-cleared, non-invasive diagnostic test used to measure endothelial dysfunction. The trademarked Peripheral Arterial Tone (PAT) technology is used to measure arterial tone changes in peripheral arterial beds through the fingertip. This test was conducted prior to the participant consuming their assigned treatment. Participants were instructed to lie supine in a dimly lit, quiet room with minimal to no distractions. The trained professional instructed the patient on the procedures then prepared the blood pressure cuff, PAT probe, and EndoPAT software to conduct the study. The system calculated a Reactive Hyperemia Index (RHI) value after the test was completed, which correlates with the measurement of endothelial vasodilator function. A lower RHI value (RHI < 1.67) can be predictive of the individual having a greater degree of cardiovascular risk and endothelial dysfunction (Axtell et al., 2010).

Blood Collection and Storage

Participants were instructed to fast for at least 8 hours prior to their visit. A certified phlebotomist collected venous blood samples at each study visit (at baseline and final of each treatment arm). Blood specimen were collected in a vacutainer containing EDTA to allow for separation of plasma. All blood samples were centrifuged at 3,000 rpm for 15 minutes and plasma aliquoted within 2 hours of collection. Aliquoted samples were appropriately labeled then stored at -80 °C in an ultra-low temperature freezer until analysis.

Plasma Endothelial Biomarker Analysis

The MILLIPLEX MAP Human Cardiovascular Disease Magnetic Bead Panel 2 was used to analyze sICAM-1, sVCAM-1, and sP-Selectin. Analysis of plasma TC, HDL cholesterol, and TG were each done by using a colorimetric assay based on procedures by Stanbio. TC, HDL, and TG were used in the Friedewald formula to calculate for LDL cholesterol.

Statistical Analysis

G*Power determined that a minimum sample size required to conduct analysis was 48, with alpha = 0.05, power = 0.80, and a moderate effect size. The Wilcoxon Signed-Rank test was used to compare differences between the MEWB treatment and EWO treatment for RHI value (endothelial function), cardiovascular biomarkers, and lipid biomarkers from baseline to final. All continuous variables were calculated with descriptive statistics. Data are presented as mean ±SEM. Statistical significance was achieved with a p-value ≤ 0.05. Statistical analyses were all performed using SPSS version 19 and XLSTAT 2015. A Mann-Whitney test was used to see if there was a significant difference between the participant starting with the MEWB first or the the EWO first, but no significant difference was observed in the order participants were assigned to.

CHAPTER IV

RESULTS

Demographics and Body Composition

A total of 104 obese or overweight men and women were screened to participate in the study. Of those initially screened, a total 40 individuals met the inclusion criteria, agreed to start the study, and were scheduled for an initial visit. Of the 40 participants, 32 were female and 8 were male. Throughout the course of the study, 21 individuals withdrew from the MEWB study and 22 individuals withdrew from the EWO study. Participants withdrew from the study due to lack of interest, taste and palatability of the treatment products, blood collection, gastrointestinal discomfort, schedule conflicts, or COVID-19 restrictions. Data associated with recruitment, compliance, and drop out are provided in Table 1 and Table 2. Demographic data on the study participants is shown in Table 3.

There was no significant difference in BMI between the EWO group and the MEWB group or throughout the duration of either treatment arm. The mass and volume of visceral adipose tissue (VAT), the type of fat stored in the abdominal wall, was measured using a DXA machine. This type of fat is associated with increased risk of metabolic diseases. There was a decrease in VAT mass from baseline to final for the EWO group while it remained stable in the MEWB group. These changes did not reach a level of significance. There was, however, a significant decrease in VAT volume from baseline to final observed in the EWO group (p = 0.043). In the MEWB group, there was an increase in VAT volume from baseline to final, but this change was not considered statistically significant. Percentage of android fat, which is concentrated in the lower abdominal region, decreased from baseline to final in the EWO group but was not considered significant. However, there was a significant decrease in android fat

percentage seen in the MEWB group ($p = 0.046$). Percentage of gynoid fat measures the fat concentrated in the hips, thighs, and buttocks. The gynoid fat percentage remained stable throughout the duration of the study for both treatment groups. Android/gynoid (A/G) fat ratio is also known as the waist-to-hip ratio and can be useful for predicting one's risk for potential health issues. The A/G fat ratio decreased from baseline to final in the EWO group but did not reach a level of significance. On the other hand, the A/G fat ratio significantly increased from baseline to final in the MEWB group ($p = 0.010$). Total body fat percentage remained relatively unchanged throughout the duration of the study for both treatment groups. Neither the EWO group or the MEWB group saw statistically significant changes in fat free mass over time. Anthropometrics and body composition data on the study participants is shown in Table 4.

Endothelial Function and RHI

Endothelial vasodilator function was measured using an EndoPAT device and is indicated by an RHI value. A decreased value is correlated with endothelial dysfunction. There was no significant difference in RHI value between baseline and final for either the MEWB group or the EWO group. The RHI value increased from baseline to final in the EWO group. In comparison, RHI remained stable over the course of study for the MEWB group (see Figure 1).

Endothelial Biomarkers

Plasma levels of sICAM-1 and sVCAM-1 were measured as indicators of endothelial abnormalities. Elevated levels of adhesion molecules are associated with increased risk of atherosclerosis. There was a decrease in levels of sICAM-1 from baseline to final in the EWO treatment group but increased in the MEWB group. However, neither treatment group showed changes that would be considered statistically significant. On the other hand, sVCAM-1

decreased from baseline to final in the MEWB group (p = .457) but increased in the EWO group (p = .544). These changes also did not show statistical significance (see Table 5).

sP-Selectin is part of the selectin family, another type of CAM. In the MEWB group, there was an increase in levels of sP-selectin from baseline to final (p = .109). In comparison, sP-selectin levels decreased in the EWO group (p = 1.023). Once again, the changes did not reach a level of statistical significance (see Table 5).

Lipid Biomarkers

A lipid profile measures the levels of different lipid biomarkers in the blood. These are often measured as they are helpful for detecting an individual's cardiovascular risk. Abnormal levels have been negatively correlated with cardiovascular disease.

TC concentrations decreased from baseline to final in the MEWB treatment group (p = 1.023). However, this change was not considered statistically significant. No significant change from baseline to final was detected in the EWO group (see Figure 2).

HDL cholesterol, often referred to as “good cholesterol” as it helps decrease LDL cholesterol levels, showed no statistically significant changes throughout the study for either treatment group. HDL cholesterol levels were maintained throughout the study in the MEWB treatment group and increased in the EWO treatment group (see Figure 3).

LDL cholesterol, the other type of cholesterol known as “bad cholesterol,” decreased from baseline to final in the MEWB treatment group (p = .631) and increased in the EWO group (p = 1.023). These changes did not reach a level of statistical significance (see Figure 4).

TG levels decreased from baseline to final in the MEWB group, but was not considered significant (p = .893). The TG levels in the EWO group stayed relatively stable from baseline to final (see Figure 5).

CHAPTER V

DISCUSSION

The findings of the present study reveal that consumption of a MEWB product for a 6-week period results in positive effects on endothelial and lipid biomarkers but no effect on endothelial function, compared to an EWO product. A growing area of research has been investigating the health promoting effects of dietary mushrooms. Mushrooms are rich in polyphenols and dietary fiber, both of which have been revealed to have protective effects against CVD. Although there are a few studies that have investigated the effects of edible mushrooms on endothelial function and cardiovascular health, no clinical studies utilizing human participants and consumption of a mushroom-based product has previously been conducted to the best of the researcher's knowledge.

Although obesity is multifactorial, it is often a result of consuming more energy, or calories, than is used by the body. This creates an energy imbalance and excess energy is stored in the body as adipose tissue (NHLBI, n.d.b). Obesity is usually correlated with increased VAT, in which the excess energy is stored as fat around the intra-abdominal organs (Matsouka et al., 2017). The DXA machine was used to assess changes in body composition. The EWO treatment group showed a significant decrease in VAT volume from baseline to final. A study conducted on Japanese adults by Matsouka et al. (2017) found that lactic-fermented egg white consumption after 12 weeks significantly reduced visceral fat area compared to baseline and visceral fat area was significantly lower compared to the control group. They suspect that the ovalbumin and ovotransferrin proteins present in egg whites, which are known to prevent lipid absorption in the small intestine, contributed to reducing visceral fat area.

Endothelial dysfunction is a major predictor of CVD, especially in overweight or obese individuals. Obesity contributes to endothelial dysfunction by promoting an environment in the endothelium that disrupts blood pressure and flow patterns, resulting in an inability to maintain vascular homeostasis (Lobato et al., 2012). This study evaluated endothelial function, represented by an RHI value, as assessed by the EndoPAT. The RHI value for both the MEWB and EWO treatment groups were maintained throughout the course of the study. However, while the RHI value displayed no difference in trend in the MEWB group, the EWO treatment group showed an incline trend. It is possible that the data received from the EndoPAT is unreliable as the accuracy of the EndoPAT was highly dependent on several variables. The RHI value obtained relied on the participant lying completely still, how relaxed the participant was, proper placement of the blood pressure cuff and probe, and the room being silent and completely dark.

sICAM-1 and sVCAM-1 are both cellular adhesion molecules that contribute to CVD by facilitating the interaction between the endothelium and monocytes, a type of white blood cell that can differentiate into foam-ladened macrophages (Mulhem et al., 2021). sP-selectin is another cellular adhesion molecule that is involved in the interaction between platelets or endothelial cells with leukocytes and facilitates leukocyte rolling along activated endothelium (Hope & Meredith, 2003; Mulhem et al., 2021). Increased circulation of CAMs are positively correlated with CVD. A cell study found that white button mushrooms consistently reduced adhesion molecule expression under pro-inflammatory conditions associated with CVD (Martin, 2010). This suggests that dietary mushrooms may be protective against CVD as they are able to inhibit adhesion molecule expression and the binding of the monocytes to the endothelium. In the current study, no statistically significant changes were detected in sICAM-1, sVCAM-1, or sP-selectin values in either treatment group. However, the findings suggest that MEWB product

consumption may help improve levels of sVCAM-1. A clinical review investigating the role of cellular adhesion molecules in atherogenesis found that although circulating levels of cellular adhesion molecules have all been associated with the presence of atherosclerotic disease, the findings from different studies have not been consistent (Hope & Meredith, 2003). Current studies investigating the effects of dietary mushrooms on adhesion molecules and cardiovascular health have utilized mushroom extracts or mushroom powder, which may provide a more concentrated amount of the beneficial properties of the mushroom. The current study used fresh whole mushrooms prepared into a MEWB patty. Perhaps the amount of mushrooms participants consumed daily was too small or the relatively short duration of the study contributed to the lack of significant changes seen in the MEWB treatment group.

TC, HDL cholesterol, LDL cholesterol, and TG are all lipid biomarkers commonly utilized as predictors of CVD. Dyslipidemia, the imbalance of one or more of these lipids, plays a significant role in endothelial dysfunction and the development of CVD. Although no significant effects were observed in levels of lipid biomarkers after the consumption of either the MEWB or EWO products, the data suggests consumption of a MEWB product for 6 weeks had a positive effect on all lipid biomarkers. Levels of TC, LDL cholesterol, and TG in the MEWB treatment group all display a downward trend from baseline to final. As for HDL cholesterol, the EWO treatment group showed a downward trend in HDL cholesterol from baseline to final while the MEWB treatment group showed no change. This suggests that consumption of mushrooms can act against decreasing HDL cholesterol levels in those who consume egg whites. Although the change was not significant, egg white alone caused a slight decrease in HDL cholesterol. On the other hand, adding mushrooms with the egg white may take away the negative effects of

eating egg white by itself. Potentially, egg white could affect lipids negatively and may be attenuated by the addition of mushroom.

The researcher acknowledges that the study had a few limitations that may have impacted the outcomes. The drop-out rate for the study was much higher than anticipated. The study was unexpectedly halted due to the unprecedented COVID-19 pandemic. A majority of participant drop outs were due to COVID-19 restrictions halting the study, thus losing 11 of the 40 participants that initiated the study. This may have contributed to significant changes being more difficult to detect. The effect size was also not achieved, which was a minimum of 24. The high drop-out rate caused the effect size to be smaller, shifted the power analysis, and ultimately impacted the statistical results of our study. This study also depended on the participant compliance with daily consumption of the MEWB and EWO products. A few participants withdrew from the study due to gastrointestinal discomfort and some finding the palatability and/or texture of either product undesirable. Reformulation of the products may be necessary for future studies to make them more desirable for long-term consumption in a clinical trial of longer duration. A few participants also withdrew due to difficulties with blood collection and the amount of blood draws per clinic visit. This study was conducted in tandem with another study detecting the effects of MEWB and EWO products on satiety, which required multiple timed blood draws. In future studies, it may help to disclose the number of blood draws with participants prior to the start of the study. One notable strength of this study is that simple, accessible, and versatile ingredients were used to create the treatment products. Mushrooms and egg whites are commonly found in most grocery stores and can be used in a variety of ways in meal preparation. Another strength is that the control and treatment products were cooked then flash frozen to -80 °C in a food-grade blast chiller to maintain the optimal texture, flavor, and

quality. Lastly, all participants met with a registered dietitian at the baseline visit of each treatment arm. Each participant was able to receive personalized nutrition recommendations during the 20 minute consultation.

In summary, this cross-over study investigated the effects of MEWB consumption on improving endothelial and cardiovascular health in owerweight and obese individuals. Although the outcomes of this study did not show significant effects of a MEWB treatment on endothelial and cardiovascular health, the findings still provide worthwhile contributions to this area of research. Findings suggest that egg white consumption had positive effects on endothelial function while consumption of a MEWB had positive effect on endothelial and lipid biomarkers; therefore, improving overall cardiovascular health of overweight and obese individuals. To the researcher's knowledge, this is one of the only clinical studies investigating the effects of whole mushrooms on endothelial function and cardiovascular biomarkers. Despite the numerous *in vivo* and *in vitro* studies, more advanced clinical trials are necessary to confirm the efficacy of polyphenols and dietary fiber in the treatment of atherosclerosis-related vascular diseases. Further research also needs to be done with a larger sample size, longer duration of both treatment periods, and the improved palatability of the treatment product to see if significant changes in endothelial and cardiovascular health can be achieved with a MEWB product.

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Table 1

Participant Screening and Drop Out Rate

Participants Screened	Qualified and Initiated Study	Completed Both Treatment Arms	Participant Drop Out due to COVID-19
104	40	15	11[a]

Note. [a] Participants lost due to COVID-19 restrictions: 3 were in washout, 2 were in Arm 2, 6 were in Arm 1

Table 2

Drop Out Rate of Each Treatment Group

Treatment Group	Qualified and Initiated Treatment	Completed Treatment	Participant Drop Out	Drop Out Rate
EWO	38	18	20	52%[a]
MEWB	33	19	14	42%[b]

Note. [a] of the 20 drop out, 9 were due to COVID-19 restrictions

[b] of the 14 drop out, 8 were due to COVID-19 restrictions

Table 3

Demographics

Females	Males	Age Range	Average Age
32	8	19.5-41.1	28.68

Table 4

Effect of EWO and MEWB Products on Body Composition

	EWO		MEWB	
Body Composition	Baseline	Final	Baseline	Final
BMI (kg/m^2)	30.8±0.8	30.3±1.0	31.5±0.7	31.9±0.8
VAT Mass (lbs)	2.3±0.3	1.9±0.4	2.1±0.3	2.2±0.4
VAT Volume (in^3)	68.6±9.6*	55.1±10.5**	62.2±9.7	67.7±12.4
Android Fat %	46.8±1.5	44.4±1.8	45.9±1.6	47.0±1.6***
Gynoid Fat %	43.8±1.2	43.2±1.4	44.3±1.1	44.0±1.3
A/G Fat Ratio	1.07±0.03*	1.04±0.04	1.04±0.03	1.08±0.04***
Total Body Fat %	42.5±1.0	41.2±1.2	42.6±1.0	42.8±1.1
Fat Free Mass (lbs)	108.1±3.5	106.8±4.2	106.2±4.3	108.4±4.3

Note. Mean ± SEM. $n = 18$ for EWO group. $n = 19$ for MEWB group.

* significance as compared to baseline in MEWB group ($p \leq 0.05$)

** significance as compared to baseline within group ($p \leq 0.05$)

Table 5

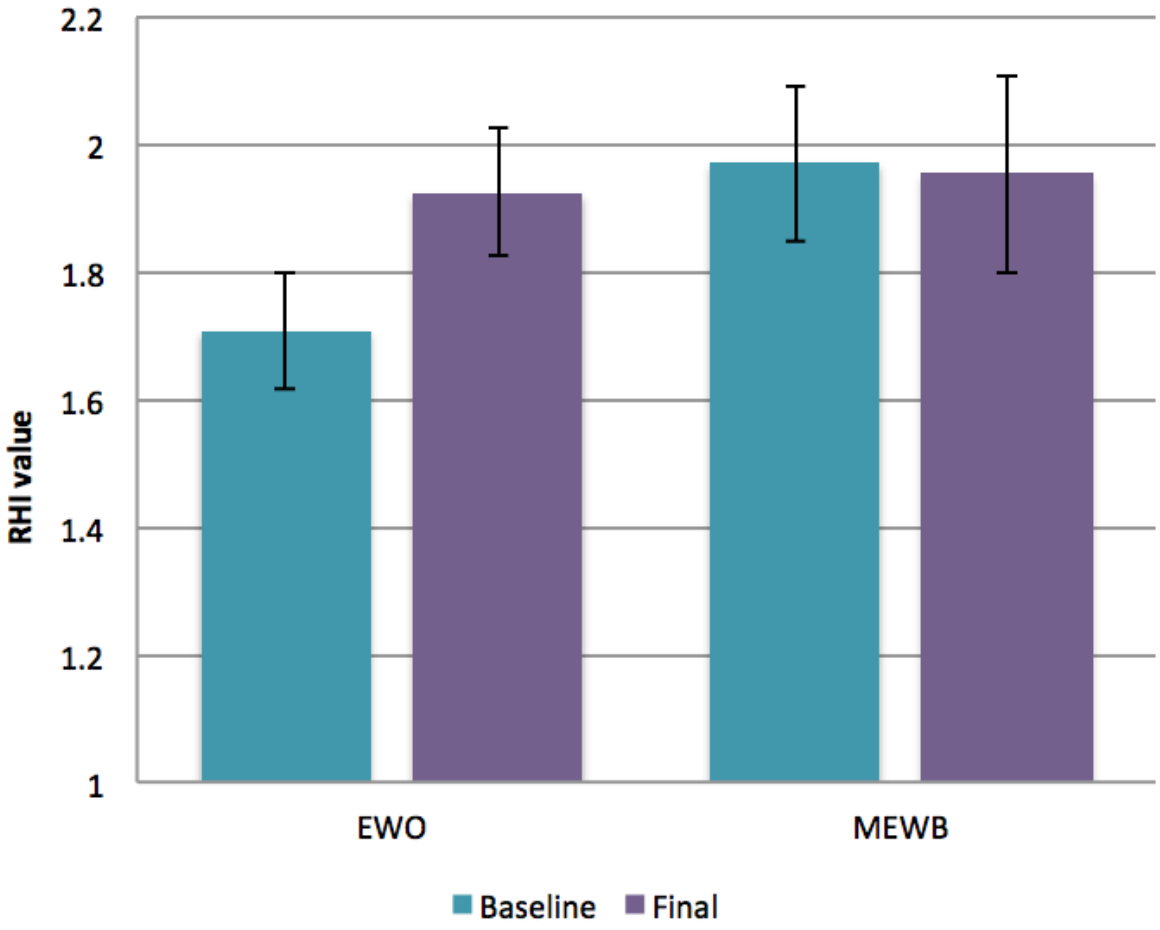
Effect of EWO and MEWB Products on CAMs

	EWO		MEWB	
Analyte	Baseline	Final	Baseline	Final
sICAM (ng/mL)	1.25±0.18	1.07±0.08	1.25±0.08	1.35±0.18
sVCAM (ng/mL)	7.21±0.42	7.30±0.42	7.30±0.36	7.22±0.45
P-selectin (ng/mL)	1.73±0.12	1.52±0.19	1.75±0.18	2.21±0.50

Note. Mean ± SEM. $p \leq 0.05$. $n = 18$ for EWO group. $n = 19$ for MEWB group.

Figure 1

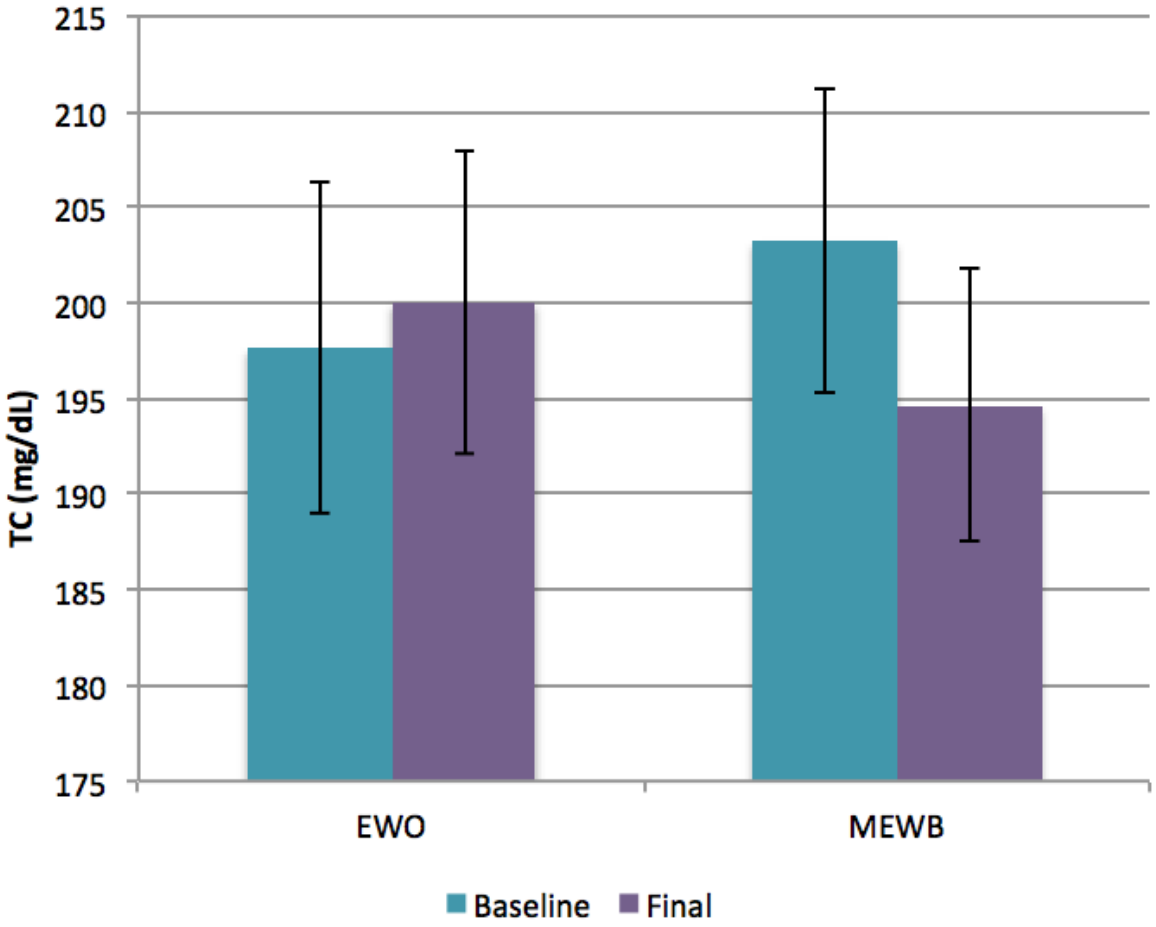
Effect Of EWO and MEWB Products on RHI Value



Note. Mean ± SEM. $p \leq 0.05$. $n = 18$ for EWO group. $n = 19$ for MEWB group.

Figure 2

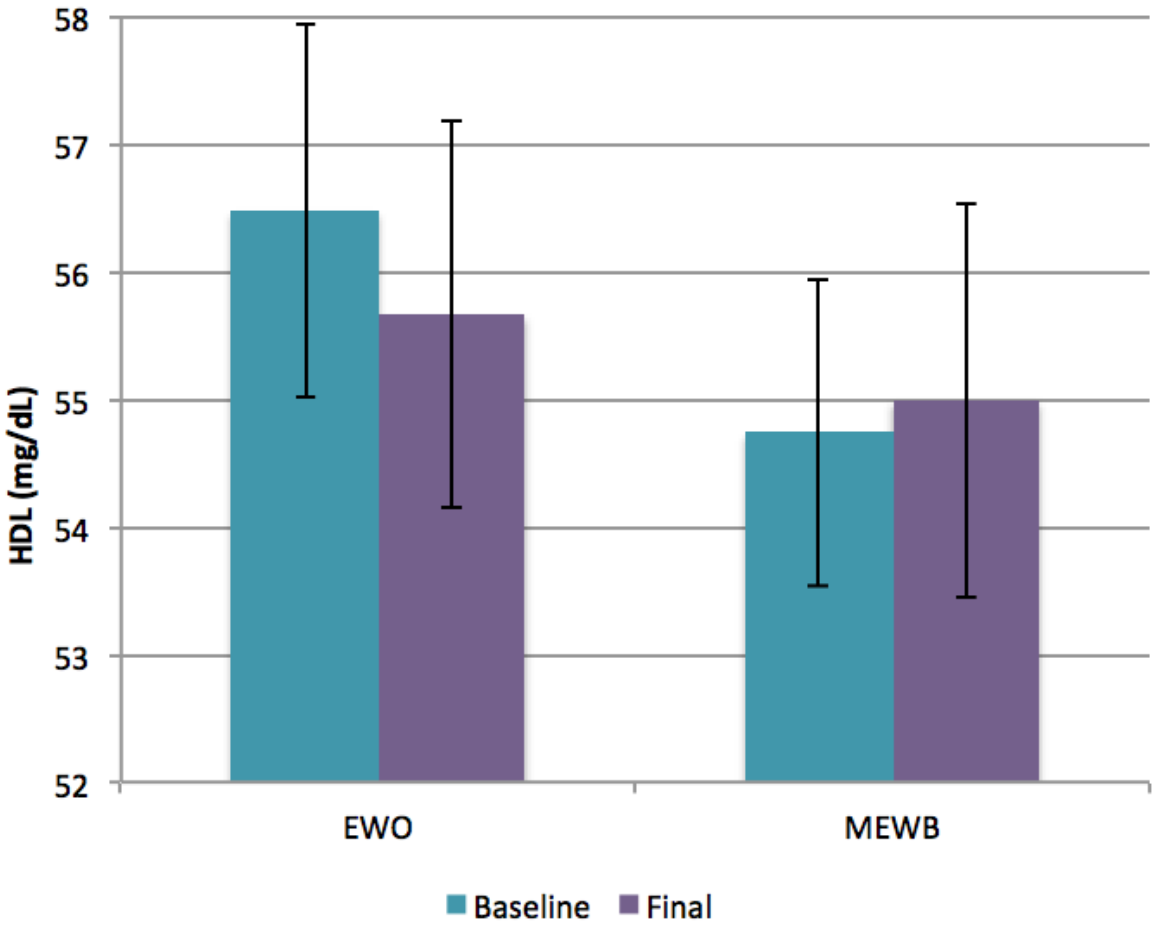
Effect Of EWO and MEWB Products on Plasma TC Concentration



Note. Mean ± SEM. $p \leq 0.05$. $n = 18$ for EWO group. $n = 19$ for MEWB group.

Figure 3

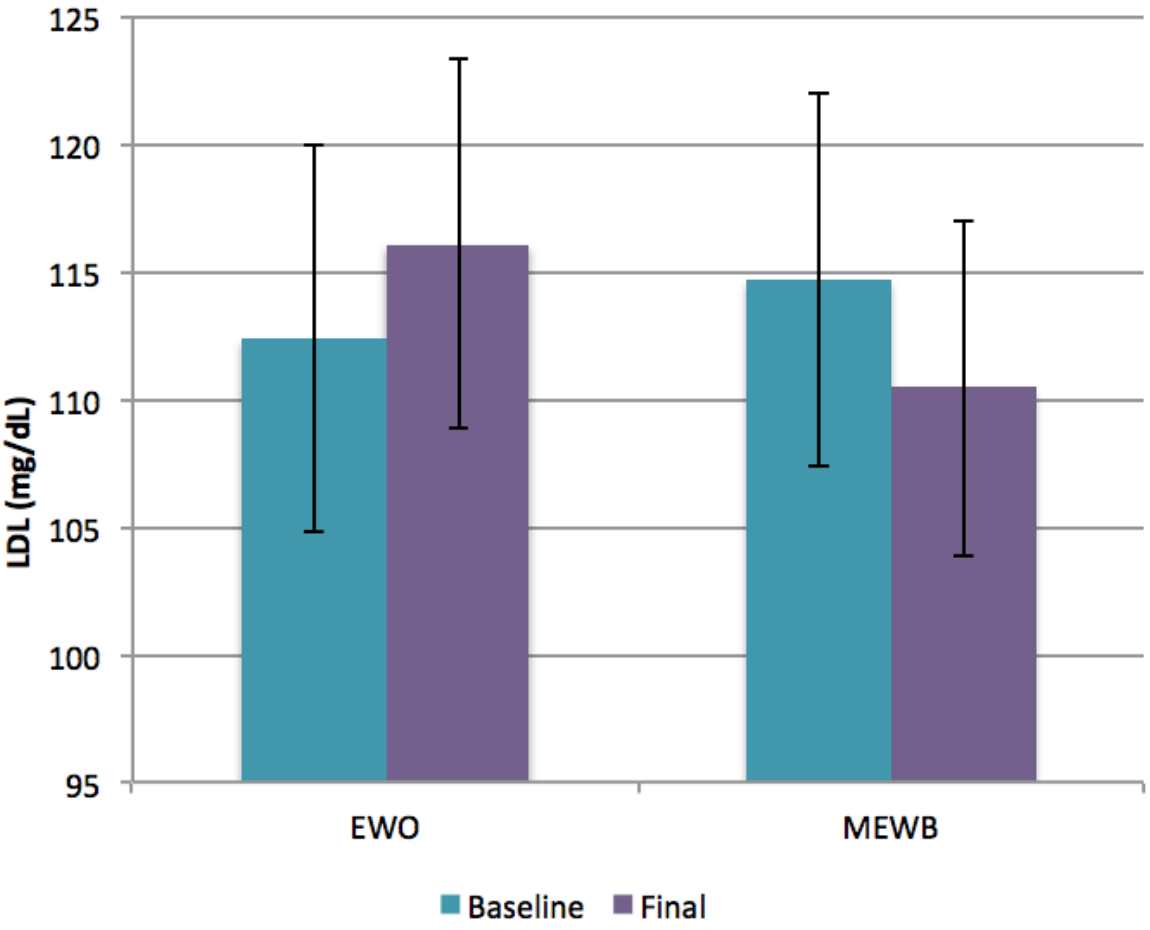
Effect Of EWO and MEWB Products on Plasma HDL Cholesterol Concentration



Note. Mean ± SEM. $p \leq 0.05$. $n = 18$ for EWO group. $n = 19$ for MEWB group.

Figure 4

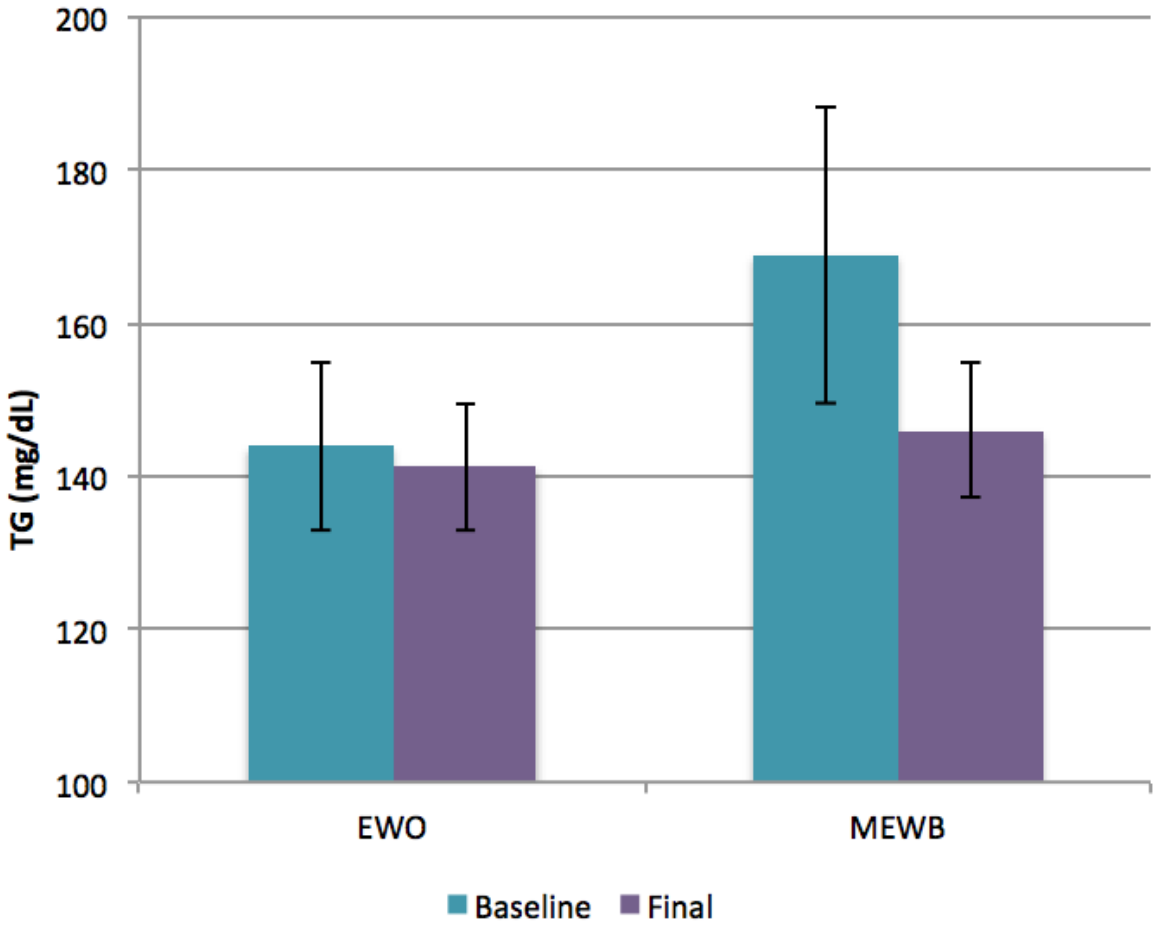
Effect Of EWO and MEWB Products on Plasma LDL Cholesterol Concentration



Note. Mean ± SEM. $p \leq 0.05$. $n = 18$ for EWO group. $n = 19$ for MEWB group.

Figure 5

Effect Of EWO and MEWB Products on Plasma TG Concentration



Note. Mean ± SEM. $p \leq 0.05$. $n = 18$ for EWO group. $n = 19$ for MEWB group.

APPENDIX A

PARTICIPANT RECRUITMENT FLIER

Need Research Volunteers

Are you struggling with weight management?

- **Are you a healthy adult?**
- **Are you in between 20 – 45 years old?**
- **Are you not physically active (exercises > twice per week)?**
- **Are you currently struggling with weight loss or maintaining a healthy weight?**
- **Would you be willing to participate in a study where you may be asked to consume an egg white product and an egg white mushroom blended product for 6 weeks for each product?**

If you have answered **YES** to all of the above, then you may be eligible to participant in a 4-month research study to look at the beneficial effect of mushroom in assisting weight management and improving satiety (feeling full).

Criteria include meeting the requirements listed above and willing to consume both egg white/ mushroom blend product and egg white product for 6 weeks for each product (total of 12 weeks with 4 weeks wash-out period, during which no product is consumed). There will be blood draws at the beginning and the end of each treatment period (0-week, 6-week, 10-week, 16-week time point). Satiety will be assessed using an appetite questionnaire prior and post feeding the study products at the beginning and the end of each treatment period. Weight, body composition, and heart health will also be assessed at the beginning and the end of each treatment period. The total time you need to spend for the study is approximately 12 hours over 4 months involving 4 study visits.

Benefits include: Upon completion, you will receive a compensation of $250 for your time in partial payment of $100 at completion of the first treatment period (6-week time point) and $150 at completion of the second treatment period (16-week time point). Other benefits include promotion of substituting mushrooms for animal proteins in diet, promotion of health benefits from naturally achieved weight management by satiety effects of foods. Blood markers of satiety and metabolism will be evaluated.

If interested, please email or call for more information:

Shanil Juma, PhD Department of Nutrition and Food Sciences

sjuma@twu.edu ; 940-898-2704

There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

APPENDIX B

SCREENING QUESTIONNAIRE

Appendix B **Screening Questionnaire**

ID:		
Sex:	**Age:**	
Weight:	**Height:**	**Calculated BMI:**
Do you smoke:	Cigarettes per day:	
Do you drink:	Alcohol per day:	
Any medical conditions? Heart Diseases_____ Hypertension_____ Hyperlipidemia _____ (including high cholesterol) Diabetes _____ Kidney Diseases _____ Lung Diseases _____ Liver Diseases _____		
Any medications, drugs, prescription drugs, over the counter drugs that you are taking? List the amount (mg) and times taken (daily, weekly etc.)		
Any vitamins or dietary/food supplements you are taking? List the amount (mg) and times taken (daily, weekly etc.)		
Dietary information: Are you on a special diet? Yes or No If yes, what type of special diet? Vegetarian_____ Low fat diet _____ Low carb diet _____ Weight loss diet _____ Low salt diet _____ Others (specify) _____		
Do you have any food allergies? Yes or No If yes, specify foods that you are allergic to_____		
Are you allergic to either egg white or mushroom? Yes or No		
Physical Activity: What is your occupation? _____ How much physical activity is involved in your daily work? _____ Outside of your job, how much exercises do you do weekly? Times per week _____ Duration each time _____ Type of activity_____ Additional notes about physical activities _____		
Here is the list of items (drugs/foods) you, as the participant, will be exposed to during the study: egg white, mushroom		
(Number 1) Are you OK with consuming an egg white and mushroom blend product or an egg white product for breakfast and dinner over a 6-week period? Yes or NO		

APPENDIX C

IRB APPROVAL EXTENSION LETTER

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378
email: IRB@twu.edu
https://www.twu.edu/institutional-review-board-irb/

DATE: January 24, 2020

TO: Dr. Shanil Juma
Nutrition and Food Sciences

FROM: Institutional Review Board (IRB) - Denton

Re: Extension for Effect of Mushroom and Mushroom-Egg Blend on Satiety, Satiety Hormones, and Weight Management (Protocol #: 20364)

The request for an extension of the IRB approval for the above referenced study has been reviewed by the TWU IRB (operating under FWA00000178). This study was originally approved on January 28, 2019 and has been renewed. Approval for this study expires on January 27, 2021.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. If subject recruitment is on-going, a copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any unanticipated incidents. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Dr. Shane Broughton, Nutrition and Food Sciences

APPENDIX D

CONSENT TO PARTICIPATE IN RESEARCH FORM

Texas Woman's University

Appendix C **Consent to Participate in Research**

Title: Effect of Mushroom and Mushroom-Egg Blend on Satiety, Sateity Hormones, and Weight Management

Investigators:	Shanil Juma, PhD.....	940-898-2704	sjuma@twu.edu
	Xiaofen Du, PhD.....	940-898-2667	xdu@twu.edu
Collaborator:	Parakat Vijayagopal, PhD.....	940-898-2009	pvijayagopal@twu.edu

Summary and Key Information about the Study

- You are being asked to participate in a research study conducted by Shanil Juma, PhD at Texas Woman's University.
- The purpose of the study is to find out if eating a mushroom and egg white blend product for 6 weeks will cause weight loss, decrease body fat, and increase satiety, therefore helping with weight management.
- You have been invited to the study because you meet the inclusion criteria of being overweight or obese, and otherwise healthy.
- As a participant, you will be asked to consume 100g total of mushroom and egg white blend product or 100g total of egg white product daily for 6 weeks. Then, you will be asked to not consume any study products for 4 weeks. After that, you will be asked to consume 100g total of mushroom and egg white blend product or 100g total of egg white product daily for another 6 weeks, during which period, the product you consumed will be different from what you consumed during the first 6 weeks. If you received a mushroom and egg white blend during the first 6 weeks, you will receive an egg white product during the second 6 weeks of study product consumption period.
- During this study, you will be asked to come to the TWU Human Research Laboratory for 4 visits, at baseline and at the end of each treatment course (baseline, 6 week, 10 week, 16 week time point). During each study visit, we will measure your weight, height, waist and hip measurements, body fat and muscle percentage, heart health as measured by EndoPAT, and afterward draw blood. You will also be asked to consume 100 g of the study product prior to blood draw, and be asked to fill out satiety questionnaires.
- A code name will be assigned to you at the beginning of the study. Your personal information and blood specimen will be identified via your code name to protect your confidentiality.
- The total time commitment for this study is approximately 35 hours.
- Following the completion of the study, you will receive $250 in cash, within which $100 will be paid at the completion of the first 6 weeks, and another $150 will be paid at the completion of the whole study.

Participant Initials

- The greatest risks of this study include loss of confidentiality, exposure to low-level radiation associated with body composition assessment, allergic reactions to study products, discomfort from blood drawings, and coercion.

Explanation and Purpose of Research

We are asking you to participate in a research study at Texas Woman's University. The purpose of the study is to find out if eating a mushroom and egg white blend product for 6 weeks will cause weight loss, decrease body fat, increase lean body mass, and increase satiety, impact satiety hormones, and therefore help with weight management and improve health. Mushrooms are low in energy, fat, sodium, and cholesterol, but high in protein, carbohydrate, dietary fiber, and contain bioactive compounds. These properties, along with their "meaty" flavor, serve as a great option for substituting animal protein. In the research, we will ask the following questions:

a) Will eating a mushroom and egg white blend product daily for 6 weeks, as compared to eating an egg white product alone, improve satiety and impact satiety hormones?
b) Will eating a mushroom and egg white blend product daily for 6 weeks, as compared to eating an egg white product alone, produce weight loss and body fat loss?

Inclusion and Exclusion Criteria

Inclusion criteria: We are recruiting adult men and women, regardless of ethnicity, between age 20-45 years with a BMI between 25 to 35 kg/m2, and who are otherwise healthy. You will need to have a history of physical activity < twice/week, < 20 minutes/session, and have a sedentary occupation for at least one year prior to enrollment in this study.

Exclusion criteria: Any form of pre-existing disease, e.g. cancer, heart disease, diabetes, liver, or renal disorders, anemia, pregnancy and lactation, taking mega doses of antioxidants/fish oil supplements, abnormal Hb, WBC, or platelets, hyperlipidemia, hypo/hyperthyroidism, abnormal liver enzymes, abnormal kidney function, smoking, and heavy alcohol drinking (> 2 drinks per day for men; > 1 drink per day for women) will be excluded from the study due to their potential influence on weight management and satiety hormones. If you refrain from eating egg or egg-based products, have egg allergies, and/or cannot tolerate mushrooms, you will also be excluded from the study.

Research Procedures

For this study, we will ask you to consume either a mushroom and egg white blend product or an egg white blend product daily for 6 weeks. Then, you will stop consuming either product for 4 weeks (wash-out period).

After the 4 weeks, we will ask you to consume a different product as compared to what you consumed in the 1st 6 weeks, either a mushroom and egg white blend product or an egg white blend product daily for another 6 weeks.

Each product is packaged individual in a 50 g portion. A 50 g portion of the product will appear to be approximately half the size of an English muffin. You will be asked to consume the product twice daily, one portion at the first meal of the day and the other portion at the last meal of the day. You will receive a two week supply of product at the baseline visit. We will ask you to come to TWU campus to pick up supplies every two weeks.

During the study, you will be asked to come to TWU human research laboratory for 4 visits, at baseline and at the end of each treatment course (baseline, 6 week, 10 week, 16 week time point). Prior to each study visit, we will ask you not to eat any food after 6 PM and to appear the next day at a certain place in the university.

On the day of a study visit (total of 4 study visits, which are at baseline and final point for each treatment course), a female researcher (for female participants) or a male researcher (for male participants) will take your weight, height, waist and hip measurements, and body fat and muscle percentage measurements. Also, your heart health will be examined using EndoPAT, a non-invasive way to measure heart health. A researcher will put a plastic tube on your index finger. Within the tube, there is a sensor that will detect the blood vessel pressure, which reflects your heart health. This measurement procedure will take 15 minutes to complete. Then, a phlebotomist (person taking the blood) will place a temporary catheter in the vein in one arm, and draw 3 tablespoons of your blood, at 0 minutes (pre-meal). We will then provide you breakfast (either egg white product or mushroom egg white blend product in the quantity of 100g) and at 15, 30, 60, 90, and 120 minutes after the breakfast the phlebotomist will again draw 3 table spoons of your blood. We will take a total of 18 tablespoons of blood from you. Filtered water and a light snack will be available for you at the study site. At the same time, you will be asked to fill out an appetite questionnaire presented on an iPad starting at 0 minute (pre-meal), you will fill the questionnaire every 15 minutes, over a period of 60 minutes, then every 30 minutes, up to 150 minutes.

At your first laboratory visit, you will meet with a registered dietitian for a nutrition consultation session. You will remain at the university for about 3.5 hours for each laboratory visit and during that time you cannot leave. We will ask you to fill out a 3-day dietary record (2 weekdays and 1 weekend day), prior to your first laboratory visit, during each treatment course (6-week periods), and during the wash-out period (4-week period). You will need to bring your 3-day dietary record to the following laboratory visit.

Study Schedule Table

Time Points	Baseline of treatment course 1	6 weeks	Final of treatment course 1	4 week washout period	Baseline of treatment course 2	6 weeks	Final of treatment course 2
Research Activities	Lab Visit* • Anthropometric measurements • Heart health measurements • Product feeding (100 g; 2 of the 50g products) • Blood draw • Satiety questionnaires	Consume assigned feeding products on your own twice daily (50g/each) with first and last meals of the day	Lab Visit*	No products need to be consumed	Lab Visit*	Consume assigned feeding products on your own twice daily (50g/each) with first and last meals of the day	Lab Visit*
Other research activities	Dietitian consultation	3-day dietary records		3-day dietary records		3-day dietary records	
Note: *All lab visits contacts the same research activities							

Time Commitment

The study will include two treatment courses, each treatment course is 6 weeks. In between the two treatment courses, there is a 4-week wash-out period (no study products need to be consumed during this time). Total of 16 weeks (4 months) will be involved in this study. Your time commitment includes initial phone screening (~ 15 minutes), consent form (~ 20 minutes), prior to the baseline visit, you will be asked to fill out 3-day dietary record (~ 20 minutes) and bring it to the baseline visit.

At baseline visit, dietitian consultation will be provided (~ 20 minutes). Anthropometric measurements will be obtained (~ 5 minutes) (at baseline and at the end of each arm, total of 4 lab visits). Heart health will be examined (~ 15 minutes). Feeding products will be asked to be consumed within ~ 15 minutes. Fasting venous blood will be drawn at 0 (pre-meal) and at 15, 30, 60, 90, and 120 minutes, simultaneously, you will be asked to fill out appetite questionnaires every 15-30 minutes over a period of 2.5 hours (at baseline and at the end of each arm).

Participants will be asked to fill out a 3-day food record during each treatment arm and during the wash-out period (60 minutes total). Products will be asked to be warmed, cooled and consumed daily, which involving ~ 15 minutes per day. Total time commitment for each participant is approximately 35 hours.

Participant Initials

Potential Risks

A potential risk to you as a participant in this study is release of confidential information. Confidentiality will be protected to the extent that is allowed by law. To protect confidentiality, you will be given a code number which will be used in all records. A master list containing participation name and match code name will be used and will be stored away from other data. Only Shanil Juma, PhD and the study coordinator Chen Du will know your identity and have access to the master list. All records will be stored in a locked filing cabinet in Shanil Juma, PhD's office. Blood samples will be stored in a locked -80 ^{0}C freezer in OMB room 305. Paper data (except the copies of the consent form which will be returned to the IRB office) will be shredded within 5 years of completion of the study. And, the original consent forms will be stored as required by IRB for at least 3 years. The blood samples will be sterilized in biological safety bags and disposed in February, 2024. Your name or any other identifying information will not be included in any publication that may result from the study. A DEXA-trained person will know not to enter participants' names into the DEXA in order to protect confidentiality. A code name/number will be used instead. There is a potential risk of loss of confidentiality in email, downloading, and internet transactions.

Identifiers will be removed from the identifiable private information or identifiable biospecimens and that, after such removal, the information or biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from the subject or the legally authorized representative. If you allow your de-identified data to be used in future studies, please initial here_____.

Or, if you would like to participate in the current study but not allow your de-identified data to be used for future research, please initial here _____.

A second risk is that you will be exposed to low-level radiation associated with body composition assessment using the DEXA. Use of DEXA for body composition involves x-ray and exposure to low level radiation. All of us are exposed to unavoidable radiation from the environment. A single DEXA scan will expose the body to approximately what would be 4 days of natural radiation. Potential participants should not be pregnant, lactating, nor at risk of becoming pregnant at the time of the study. The potential subject may consider not participating in the study due to the DEXA scan and withdraw at any time. Trained personnel who have the necessary credentials will be running the DEXA machine.

A third possible risk is that you may not like the food (intervention products) or are allergic to mushroom or egg. If you do not like the food, there is no penalty for not eating it. You are free to quit the study at any time. Before committing to the study, you will be informed that the study products contain mushroom and egg. You will also be informed of common

symptoms of allergic reactions. If you reveal you are allergic to mushrooms or eggs, you will not be considered for participation. If you become allergic to the study products used in the study, you will be asked to stop the study. You will also be advised to consult a physician if you experience any allergic reactions.

Another possible risk to you as a participant in this study includes the discomfort of blood drawings. Blood draws may cause minor pain, bruising, discomfort, swelling, anxiety, infection or fainting. We will use a certified expert for blood draws. This will minimize the possibility of pain, bruising, discomfort, and anxiety. The phlebotomist will clean your arm with alcohol before taking blood and she will use a new needle. This will minimize the possibility of infection.

You will receive time to relax before and after blood draw. A light snack and water will be available to you. This will reduce the possibility of your fainting. If you faint during the blood draw, we will lay you down and make you comfortable. We will carefully watch you until you regain consciousness and will not make another attempt to draw your blood again that day.

You may be allergic to the latex gloves the phlebotomist wears for blood draw. In that case, the phlebotomist will use a different type of gloves.

In addition to the risks above, you may experience anxiety or embarrassment related to weight, height, waist and hip circumference, and body composition measurements. In order to minimize this risk, you will be assured of complete confidentiality before taking these measurements. All measurements will be taken only by an experienced/trained male or female investigator in a private room.

Other possible risks to you are loss of time, fatigue, and allergic reaction. You can watch videos or relax you are waiting. This will help you to overcome boredom and fatigue. If you develop allergy to the food we use in the study, you will not be able to continue in the study.

Coercion may be another potential risk. Before committing to the study, you will be informed that participating of the study is completely voluntary, and you can withdraw from the study at any time. The decision of whether to participate will have no impact on the availability of care during the study.

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Participation Benefits

Your participation in this research study is completely voluntary, and you may discontinue your participation in the study at any time without penalty. As a participant in the study, you will receive the study food for total of 12 weeks. You will also receive a cash incentive of $250 in total by completing the study. A partial payment of $100 will be provided upon completion of one treatment course, and upon completion of the second treatment course, you will receive another $150 in cash. In addition, at completion of the study a summary of results, as well as the results of your blood analysis, will be mailed to you upon request.*

Questions Regarding the Study

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you may ask the researchers; their phone numbers are at the top of this form. If you have questions about your rights as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at 940-898-3378 or via e-mail at IRB@twu.edu.

_____ _____

Signature of Participant Date

This page will be detached and filled separately.

* If you would like to receive a summary of the results of this study, please provide an address to which this summary should be sent:

_____

_____

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_____

Participant Initials