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THE EFFECTS OF THE ACUTE CONSUMPTION OF PEANUTS ON GLYCEMIC CONTROL AND ENDOTHELIAL FUNCTION IN THE POSTPRANDIAL STATE

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ABSTRACT

Cardiovascular disease is the leading cause of death in the United States, killing more than 600,000 people in 2013 alone. Certain risk factors, such as postprandial triglyceride response, glycemic control, and endothelial function, can increase the risk for atherosclerosis and cardiovascular disease. The postprandial state, i.e. the four hours following the consumption of a meal, is a key time when assessing risk factors for this disease because of its association with the onset of atherosclerosis and because many Americans spend most of their waking hours in this state. A high-fat, high-sugar meal can initiate inflammation during the postprandial state due to elevated triglyceride and glucose responses; this inflammation can lead to the narrowing of the arteries and endothelial dysfunction. Peanuts have certain cardioprotective qualities that may aid in the prevention of cardiovascular disease. This study aimed to assess acute triglyceride response, glycemic control, and endothelial health in the postprandial state in a population of 15 healthy, overweight male participants after the consumption of peanuts. Five blood samples (one baseline sample and four samples in the postprandial state) were taken from participants after the consumption of a shake containing peanuts and a control shake containing no peanuts during two separate visits to test glyceride and glucose responses. Endothelial function was assessed through an ultrasound flow-mediated dilation recording. The results showed that peanuts, when included in a high-fat meal, helped to blunt the triglyceride response and preserve endothelial function postprandially in healthy, overweight males.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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Chapter 1

Literature Review

According to the Centers for Disease Control and Prevention, cardiovascular disease (CVD) is the leading cause of death in the United States, accounting for approximately 23.5% of total deaths nationwide (CDC 2014). CVD has not only had a significant impact on the number of deaths in this country but also on the United States economy. The prevalence of heart disease throughout the US has led to spending that accounts for 17% of the total national health expenditures. The United States spends the most amount of money on medical costs compared to every country in the world, and these costs only continue to increase (Heidenreich et al. 2011). Identifying ways to prevent the onset of cardiovascular risk factors and disease may help to decrease the prevalence of deaths caused by CVD and money directed toward CVD-related medical costs. Furthermore, since the diet plays such an important role in the pathogenesis for cardiovascular disease, it is crucial to analyze which specific foods and nutrients may be beneficial to the prevention of disease.

Risk Factors for Cardiovascular Disease

There are certain risk factors for CVD, such as postprandial lipid and glucose levels, endothelial function, and blood pressure, which are affected by the diet. The American diet is typically high in sugar, fat, and calories; meals like this lead to a spike in postprandial glucose and triglycerides levels following consumption of the meal. Because most people spend most of

their waking hours in the postprandial state (the four hours following a meal), this is a crucial physiological state to be studied and observed. Chronically increased blood glucose and triglyceride levels over time may increase the risk for cardiovascular disease. Foods high in fat and sugar also elicit an inflammatory response during the postprandial period following a meal, which may also contribute to endothelial dysfunction and atherosclerosis (Widlansky, Gokse, Keaney, Vita 2003). This is because spikes in postprandial glucose and triglycerides levels cause higher levels of blood coagulation in the blood vessels and an excess of free radicals, which elicits an inflammatory response. A meal loaded with fat (which is comprised of triglycerides, phospholipids, and cholesterol) and carbohydrates (which are comprised of starch, sucrose, lactose and simple sugars such as glucose and fructose) disrupts the redox balance of the cellular reactions, renders the cells more susceptible to oxidation, and increases the likelihood of oxidative damage (Sies, Stahl, Sevanian 2005).

Inflammation occurring multiple times per day (after several high fat, energy-dense meals) over an extended period of time may cause the hardening of the arteries through plaque buildup on the endothelium walls, and this eventually leads to endothelial dysfunction and the progression of atherosclerosis. Evidence of postprandial inflammation occurs through elevated C-reactive protein, cytokines, and endothelin-1, which are all markers of an inflammatory response. This inflammation leads to increased low-density lipoprotein (LDL) oxidation and vasoconstriction, which over time contributes to the hardening of the arteries and constricted blood flow (O'Keefe, Gheewala, O'Keefe 2008).

In a cross-over study done by Jiang et al., it was found that there is an inverse relationship between nut consumption and the inflammatory markers C-reaction protein, interleukin-6, and fibrinogen. This reduction in inflammation is now viewed as one of the

potential mechanisms behind nut consumption and the reduction of risk for cardiovascular disease. When the endothelium of the artery is damaged (through many high-fat meals), inflammatory markers aggregate at the site of injury on the vascular wall and stimulate plaque buildup; this plaque buildup can lead to clotting and potentially a more serious cardiovascular event such as stroke or myocardial infarction due to constricted blood flow. With reduced levels of inflammation, there is less of a risk for endothelial dysfunction. Inflammation is a key factor in every stage of atherosclerosis, from the beginning stages of endothelial injury, to plaque buildup, and finally to plaque ruptures. Preventing endothelial injury and dysfunction may be attained through more frequent nut consumption, and this has the potential to prevent life-threatening cardiovascular events in the future (Jiang et al. 2006).

Postprandial Glucose Response

It is hypothesized that certain dietary modifications can improve postprandial glucose and lipid levels, decrease inflammation, and lower the risk for cardiovascular disease. Following a high-fat and high-sugar meal, blood glucose levels increase; in a healthy adult, homeostatic mechanisms of the body normalize and regulate blood glucose levels in the postprandial state so that there is not excess glucose in the bloodstream (O'Keefe, Gheewala, O'Keefe 2008). Blood glucose levels, however, are poorly regulated in millions of adults in the United States. According to the National Diabetes Statistics Report of 2014, 29 million (or 9.3% of the population) people have diabetes, and 37% of the population has prediabetes, having a high risk for developing type 2 diabetes (CDC 2014). This equates to almost half of all American adults are partially or completely resistant to insulin, meaning that their cells have decreased capacity to

absorb glucose from the bloodstream and into the cells to be used as energy or stored as fat. When the body is resistant to insulin's signals, blood glucose levels remain elevated following a meal. Elevated postprandial glucose levels have been shown to increase the risk for cardiovascular disease (O'Keefe, Gheewala, O'Keefe 2008). One prospective cohort study found that of the 1,199 participants who had acute coronary syndrome, 57% had abnormal glucose metabolism, demonstrating the connection between unregulated blood glucose levels and cardiovascular risk. Many of these individuals had relatively normal fasting blood glucose levels but had elevated blood glucose levels in the postprandial state (Conaway, O'Keefe, Reid, Spertus 2005).

In a meta-analysis of 38 different cohort studies with blood glucose levels measured and cardiovascular disease or mortality as an endpoint, it was found that blood glucose is a significant indicator for cardiovascular disease progression, even in individuals who do not have diabetes. The exact mechanism for this causation is still unclear; however there are several consequences of elevated blood glucose levels that act as plausible explanations for the increased risk for cardiovascular disease. Poor glycemic control, or in other words unregulated blood glucose levels, can lead to the glycosylation of low-density lipoprotein, rendering it more readily susceptible to being oxidized, which can lead to plaque build-up in the arteries and enhance the progression of atherosclerosis. Elevated glucose levels in the bloodstream in the postprandial state following a meal also generate free radicals, which cause oxidative damage; this leads to impaired vasodilation and endothelial dysfunction (Sies, Stahl, Sevanian 2005). Excess glucose can also activate protein kinase C, an enzyme which plays a role in signal transduction cascades, which decreases nitric oxide levels. Decreased nitric oxide levels impair vascular tissue tone and

increase blood coagulation, causing endothelial dysfunction and enhancing the progression of atherosclerosis and risk for cardiovascular disease (Levitan, Song, Ford, Liu 2004).

Postprandial Lipid Response

In addition to poor glycemic control, consistently eating a diet composed of foods high in saturated fat and sugar can lead to dyslipidemia, which is another risk factor for cardiovascular disease. Hyperlipidemia in the postprandial state includes elevated triglyceride levels, chylomicrons, and remnant lipoproteins; these elevated lipid levels can lead to oxidative stress and inflammation. Oxidative stress occurs when there is an increase of reactive oxygen species; the oxidation of low-density lipoproteins in the vascular endothelium can lead to plaque buildup, a fatty acid streak, and the hardening of arteries or a myocardial infarction (O'Keefe, Gheewala, O'Keefe 2008). An increase in triglyceride levels and inflammation following a meal leads to higher systolic blood pressure, endothelial dysfunction, and vasoconstriction in the arteries, increasing the risk for a cardiovascular event. One crossover study done by Jakulj et al. found that, relative to consuming a low-fat meal, even just a single high-fat meal could actually increase systolic and diastolic blood pressure and increase peripheral resistance in healthy participants with a normal blood pressure. Higher levels of saturated fat and cholesterol can lead to a buildup of plaque in the blood vessels, which impairs blood flow due to a blockage of the artery. This blockage of the artery leads to cardiovascular events such as a stroke or myocardial infarction after the plaque in the artery has ruptured and caused completely constricted blood flow to the heart (Jakulj et al. 2007).

In another seminal study testing the cardiovascular effects of a high-fat meal in a healthy population, the results showed that one high-fat meal (50 g fat, 14 g saturated fat) caused endothelial dysfunction in healthy, normocholesterolemic volunteers in the postprandial state following the consumption of the meal. The flow-mediated vasoactivity of the brachial artery was impaired by 50 percent only four hours after consuming the high-fat meal, whereas vasoactivity was not impaired in participants who consumed a meal with equal calories but low in total fat content (0 g fat). This study showed a significant correlation between elevated triglyceride levels, specifically in the postprandial state as opposed to the fasting state, and coronary disease risk through the mechanism of endothelial dysfunction. It is important to note that triglyceride levels following a high-fat meal in the postprandial state significantly impaired endothelial function, whereas fasting triglyceride levels showed no significant change of endothelial function, thereby showing the importance of the postprandial state as opposed to the fasting state when assessing the risk for atherosclerosis (Vogel, Corretti, Plotnick 1997).

Foods high in saturated fats may interrupt the signaling process of nitric oxide synthase and thus disrupt nitric oxide production; this leads to impairment of vascular tone and the ability of the arterial walls to relax and dilate as blood flow increases. Triglyceride-rich lipoproteins such as LDL remaining in the blood for extended periods of time in the postprandial state eventually cause damage to the endothelium. Chronic hyperlipidemia following a meal can lead to endothelial dysfunction and plaque formation; thus the postprandial state and specifically hyperlipidemia in this state are major indicators of atherosclerosis progression (Sies, Stahl, Sevanian 2005).

The Connection between Hyperlipidemia and Hyperglycemia

Furthermore, hyperlipidemia (elevated triglyceride, lipoproteins, and chylomicron levels) can exacerbate the effects of postprandial hyperglycemia as well because free fatty acids in the plasma disrupt the insulin signaling pathway, which leads to insulin resistance (Boden and Laakso 2004). One study involving healthy, nondiabetic women showed that elevated levels of plasma free fatty acids decreased insulin-stimulated glucose uptake by about 40 percent only four hours following the elevation of free fatty acids. A meal high in triglycerides will be metabolized into fatty acids, and an elevation in fatty acids may create some insulin resistance, leading to hyperglycemia in the postprandial state. Furthermore, diacylglycerol (DAF), which is an intermediate in the process of triglyceride metabolism and thus would be available after a high-fat meal, is a known activator of the enzyme protein kinase C. Protein kinase C is a key player in several signaling pathways, and it is involved in the phosphorylation of certain residues of insulin receptors that are essential for the insulin signaling pathway. This phosphorylation of the residues can cause destruction of the receptors needed in insulin signaling and thus can lead to insulin resistance, causing elevated levels of glucose to remain in the blood during the postprandial state. It is evident that there is a connection between hyperlipidemia and hyperglycemia because both free fatty acids in the plasma and phosphorylation steps in triglyceride metabolism exacerbate insulin resistance, which leads to poor glycemic control (Homko, Cheung, Boden 2003).

Characteristics of the Peanut

The peanut, while technically in the legume family, is the most consumed nut in the United States; in fact, peanuts are consumed more than every other nut combined (Alper and Mattes 2003). While peanuts provide a substantial amount of plant-based protein and fiber, they are also an excellent source of fat, containing 47-50% fat; it is their fatty acid composition that gives them cardioprotective attributes. Since peanuts are often chosen as a snack in the United States, they may be a beneficial dietary strategy to target in an effort to improve cardiovascular health throughout the nation. Peanuts are a rich source of monounsaturated fats (approximately 55% of total fat content), specifically oleic acid, and polyunsaturated fatty acid (approximately 25% of total fat content), specifically linoleic acid (Ozcan and Seven 2003). While saturated fats (which are low in peanuts) are known to raise serum cholesterol levels, the fatty acids most abundant in peanuts (mono- and polyunsaturated fatty acids) are known to lower serum cholesterol levels and improve cardiovascular health. Monounsaturated fats also increase HDL levels (the good cholesterol), and although saturated fats also increase HDL levels, they also raise LDL levels (the bad cholesterol) (Ros and Mataix 2006). When saturated fats are replaced with unsaturated fats, such as when peanuts or other nuts are consumed in place of other foods high in saturated fats, serum triglyceride levels also decrease. Furthermore, while saturated fats tend to increase insulin resistance, increasing the risk for hyperglycemia and the development of type 2 diabetes, monounsaturated and polyunsaturated fats can improve insulin resistance (Ros and Mataix 2006).

Besides their fatty acid composition, peanuts are also a good source of micronutrients that aid in cardiovascular health. Peanuts are a rich source of magnesium, which is being consumed of less and less in today's society because magnesium is frequently lost through the processing

of foods. The national average of magnesium consumption does not meet the designated RDA (recommended dietary allowance), so consuming peanuts, a rich-source of magnesium, would help to improve magnesium deficiency. Low levels of serum magnesium lead to reduced activity levels of lipoprotein lipase and lecithincholesterol acyltransferase (LCAT). Lipoprotein lipase breaks down triglycerides into free fatty acids and glycerol which the body can use for energy or store for later usage; with decreased lipoprotein lipase activity, serum triglyceride levels are elevated. LCAT is an enzyme which esterifies cholesterol into a form that is more efficiently transported to the liver by lipoproteins to be expelled from the body or used in other body tissues; decreased LCAT activity leads to elevated levels of free cholesterol in the blood or deposited in tissues, manifesting into other clinical problems. Therefore, magnesium plays a role in lowering blood cholesterol and triglyceride levels, and thus aids in improving cardiovascular health (Alper and Mattes 2003).

Peanuts also are an excellent source of folate, and folate reduces the level of serum homocysteine. This is beneficial because homocysteine increases vascular smooth muscular cell proliferation, which can increase plaque buildup on the endothelial walls of the artery and increase the chance for thrombosis (Chiang J et al. 2011). When endothelial cells are damaged, which is often the cause of the start of plaque buildup, homocysteine also impairs repair of the endothelial cells. By peanuts providing folate (and actually four times the amount of folate as any other nut), the consumption of peanuts can inhibit the harmful effects of homocysteine and reduce the risk of cardiovascular disease (Alper and Mattes 2003).

Peanuts and Risk for CVD

In a prospective Nurses' Health Study, frequent peanut consumption (consuming a one ounce serving of nuts at least five times per week) was associated with a reduced risk for fatal coronary heart disease and non-fatal myocardial infarction events in women. When compared to women who rarely consumed nuts, there was an approximate 35% reduction in risk for coronary heart disease in women who frequently consumed nuts. The endothelium-preserving and lipid-lowering properties of nuts have been shown to lower the risk of coronary heart disease as well as fatal cardiovascular events (Hu et al. 1998).

Peanuts, when incorporated into the diet, have been shown to decrease susceptibility for LDL oxidation when compared to the American diet. Polyphenolic compounds in peanuts have antioxidant properties, further enhancing the cardioprotective effects of peanuts (Kris-Etherton, Hu, Ros, Sabate 2008). It is important to note, however, that most nuts (including peanuts) contain most of their antioxidants in the skin of the nut; thus, removal of the skin removes about 50% of the antioxidants of peanuts and therefore eliminates an important cardioprotective effect of the nut (Blomhoff, Carlsen, Andersen, Jacobs 2006). Reactive oxygen species have the potential to non-enzymatically oxidize certain cellular components, such as cell membranes, proteins, and lipoproteins, which can affect their structure and function and eventually lead to disease pathogenesis. It is the role of the antioxidant to inhibit these reactive oxygen species and prevent oxidative damage. Fortunately, through certain dietary modifications, it is possible to increase antioxidant levels in the body to scavenge or neutralize free radicals, which may decrease the risk for many diseases caused by or enhanced by oxidative damage (Blomhoff, Carlsen, Andersen, Jacobs 2006).

Endothelial Dysfunction

Arterial endothelial dysfunction is often detected through both a decrease in the availability of nitric oxide, which is a vasodilator that is derived from the amino acid L-arginine and improves blood flow, and the increase of inflammatory cytokines and cellular adhesion molecules. These are signs that the endothelium of the artery has been damaged, the structure has been altered, and function is impaired; the loss of endothelial function leads to the hardening of the arteries and eventually atherosclerosis. Certain cardiovascular risk factors or the progression of vascular disease can lead to the decrease of nitric oxide production and availability, which will inhibit vasodilation and disrupt normal blood flow, as well as increase smooth muscle cell proliferation and blood cell adhesion. Monocytes will attach to the endothelium wall at the site of endothelial cell injury through adhesion molecules, and then smooth muscle cells, oxidized LDL, and macrophages will form foam cells, which creates plaque and a fatty streak along the walls of the artery (Kris-Etherton, Hu, Ros, Sabate 2008).

Vascular endothelium plays a large role in the progression of atherosclerosis and heart disease; impaired endothelial function, and thus impaired flow-mediated dilation, is actually now considered a risk factor for cardiovascular disease (Adams et al. 1997). The vascular endothelium is a dynamic tissue that functions to regulate vascular tone by producing vasodilators and vasoconstrictors, control blood fluidity and coagulation, and produce molecules that regulate inflammatory responses (Brown and Hu 2001). Furthermore, certain foods have certain effects on vascular reactivity, or affect the ability of the vascular endothelium to properly react to the shear stress caused by blood flow (Kris-Etherton, Hu, Ros, Sabate 2008). Because the vascular endothelium plays such a significant role in the progression of cardiovascular disease, it is important to understand how certain foods will affect its structure and function.

The Arterial Endothelium and Inflammation

The endothelium tissue is activated in response to inflammatory events such as LDL activation, oxidation by free radicals, and cytokine stimulation. The duration and intensity of the inflammatory state is typically well-regulated in normal conditions. However, ongoing, chronic inflammation initiates disease pathogenesis and impairs the homeostatic functions of endothelial tissue. This activated state of the endothelium during inflammation contributes to the development of the fatty streak that can develop in the endothelium and result in atherosclerosis. During inflammation, monocytes (a type of white blood cell) will circulate near the site of injury and attach to adhesion molecules, where they will then become macrophages. The macrophages will then scavenge oxidized LDL, creating foam cells, which will then develop into a fatty streak. Fatty streak development will eventually develop into atherosclerosis and increase the risk for cardiovascular events such as stroke or myocardial infarction (Brown and Hu 2001).

L-Arginine and Nitric Oxide

Vascular tone is established through the proper balance between vasoconstriction, the narrowing of the arteries through contraction of the muscular walls of the arteries, and vasodilation, the widening of the arteries through relaxation of the muscular walls. Nitric oxide, which is derived from L-arginine, is one of the main factors responsible for vasodilation, and it is released in response to shear stress to relax the muscular tissues of the vessels to ensure normal blood flow. It also aids in the inhibition of monocyte attachment to adhesion, the reduction of platelet aggregation, and the inhibition of smooth muscle cell proliferation, thus playing a vital role in normal endothelium function (Palmer RMJ, Ashton DS, Moncada S 1988).

L-arginine is an amino acid which acts as a substrate for nitric oxide synthase, which is an essential step for the production of nitric oxide. Impaired artery dilation and increased monocyte adhesion to the walls of the arterial endothelium both contribute to endothelial dysfunction and are both affected by levels of L-arginine in the blood (Palmer RMJ, Ashton DS, Moncada S 1988). One study showed that oral L-arginine therapy given to young men with coronary artery disease improved nitric-oxide dependent endothelial function and also reduced the adhesion of monocytes to the endothelial cells, thus improving vascular function and decreasing risk for further atherogenic problems. In patients with atherosclerosis, there is a decrease in nitric oxide production, thus inhibiting vasodilation and normal blood flow; L-arginine may be used as therapy to improve this problem (Adams et al. 1997).

Resveratrol

Resveratrol is a type of natural polyphenol that is found in red wine, grape skins, and peanuts; more attention has been drawn to this dietary component because of its potential cardioprotective benefits (Bradamante, Barenghi, Villa 2004). It acts as an antioxidant in that it scavenges reactive oxygen species, and it aids in prevention of LDL oxidation. Normally, oxidized LDL contributes to the formation of foam cells, which create the fatty streak that leads to endothelial dysfunction and atherosclerosis; consumption of resveratrol may be one method to slow or prevent this process. Vascular smooth muscular cells can inappropriately proliferate and also contribute to plaque formation; resveratrol, however, is antiproliferative in nature and can inhibit the proliferation of smooth cells and thus reduce the risk of thrombosis.

Resveratrol has other cardioprotective effects such as an ability to reduce platelet aggregation, which can reduce blood clotting in the arteries and reduce the risk for thrombosis (Conaway, O'Keefe, Reid, Spertus 2005). It also reduces the expression of the tissue factor gene, which expresses tissue factor. Tissue factor is a glycoprotein which is a signal for a blood coagulation cascade that can contribute to decreased blood flow. Normally, in healthy arteries, tissue factor is not present; however, in arteries with plaque buildup (during the progression of cardiovascular disease), there is a significant amount of tissue factor. Tissue factor is specifically located within plaques in macrophages, smooth muscle cells, and oxidized lipids, all of which form the atherosclerotic plaque. An increased level of tissue factor within plaque buildup is thought to increase the risk of thrombosis (Pendurthi, Williams, Rao 1999).

One study demonstrated that resveratrol significantly suppressed the induction of tissue factor gene expression in endothelial cells and monocytes. This was done through resveratrol-mediated inhibition of transcription of the tissue factor gene, thus reducing the amount of tissue factor that is expressed and inhibiting tissue factor activity. This would reduce smooth muscle cell proliferation, platelet aggregation, plaque buildup, and the risk for thrombosis. Therefore it is evident that resveratrol acts through multiple mechanisms to lower the risk for cardiovascular disease or a potentially fatal cardiovascular event (Pendurthi, Williams, Rao 1999).

Flow-Mediated Dilation

Structural change of the artery leading to atherosclerosis is one of the key predictors of cardiovascular disease. This structural change involves the hardening and narrowing of the artery, which leads to endothelial dysfunction and compromised vasodilation and blood flow. If

vasodilation is compromised and the artery vessel becomes narrowed, thrombosis may occur, which is when a blood clot causes the obstruction of a blood vessel and potentially a myocardial infarction. Fortunately, there is a way to measure arterial diameter and thus endothelial function, which can aid in the detection of atherosclerosis at an early stage. The arterial diameter should change in response to an increase in shear stress, which in the case of arteries is blood flow that causes frictional force as it passes by the endothelium of the blood vessel. As blood flows through the artery, the artery should expand; this demonstrates endothelium-dependent dilation (Silver and Vita 2006). Measuring flow-mediated dilation (FMD) is a non-invasive ultrasound procedure which measures the diameter of the artery before and after an increase in shear stress and therefore can provide insight on endothelial dysfunction and the possible progression of atherosclerosis. FMD typically measures changes in the brachial, radial, or femoral vessels because these are conduit arteries which will stretch in response to blood flow. If structural changes in the artery are detected, or if the artery does not dilate in response to increased shear stress, atherosclerosis can be detected at an earlier stage (Raitakari and Celermajer 2000).

Nitric oxide is released by vascular endothelial cells, which causes the vascular tissue to relax, the artery to dilate, and blood flow to improve. This relaxation of vascular tissue aids in the prevention of blood clotting and adhesion molecules from attaching to the wall of the artery and forming a thrombus that could obstruct the flow of blood (Palmer, Ashton, Moncada 1988). The amino acid L-arginine is a precursor necessary for the synthesis of nitric oxide, and L-arginine is present in peanuts. Nitric oxide is highly responsive to flow-induced shear stress; when the walls of the artery narrow, causing blood flow to increase in velocity, nitric oxide is released from endothelial cells as a mechanism to relax the muscular tissue and induce vasodilation (Böger, Bode-Böger, Frölich 1996). In a study done by Bode-Böger where ten

healthy males were intravenously infused with L-arginine, the mean arterial blood pressure was significantly reduced and platelet aggregation was reduced by about 33% when compared with the subjects receiving a placebo treatment. This is because the increased supplementation of L-arginine increased production of nitric oxide, which then relaxed vascular tone, decreased blood pressure, and inhibited platelets from aggregating along the arterial walls, thus preventing plaque buildup (Bode-Böger et al. 1997).

Chapter 2

Introduction

In 2013 alone, over 600,000 people in the United States died from cardiovascular disease. Millions of people are at risk for the disease because of the Western diet (CDC 2014). Over one-third of the adults in this nation are obese, and this is caused by the excessive amounts of energy, which contribute to a high intake of saturated fat and sugar, consumed by Americans daily. Not only does this type of diet lead to weight gain and obesity, which increases the risk for cardiovascular disease, but it also elevates serum triglyceride and cholesterol levels. Furthermore, it can lead to unregulated blood glucose levels, insulin resistance, and eventually type 2 diabetes (O'Keefe, Gheewala, O'Keefe 2008).

It is important to look at the postprandial state when analyzing the risk factors for atherosclerosis, the narrowing and hardening of the arteries, which increases the risk for a thrombosis and a stroke or myocardial infarction. The postprandial period, i.e. four hours following consumption of a meal, is when inflammation occurs, causing endothelial injury (Widlansky, Gokse, Keaney, Vita 2003). High-fat meals increase the prevalence of reactive oxidative species, which can cause oxidative damage of the endothelium. Injury to the endothelium leads to an influx of inflammatory molecules which will bind to adhesion molecules, increase platelet aggregation, and form a foam cell composed of oxidized LDL, macrophages, and smooth muscle cells. This foam cell will lead to the development of arterial plaque along the walls of the endothelium, which may rupture, constricting blood flow and

increasing the risk for a potentially fatal cardiovascular event (Kris-Etherton, Hu, Ros, Sabate 2008).

Flow-mediated dilation occurs when the arterial walls relax, causing a widening of the artery vessels in response to the shear stress of blood flow. This homeostatic function is maintained in normal conditions, but will eventually be impaired after chronic consumption of high-fat meals. Chronic consumption of foods high in saturated fat will lead to endothelial dysfunction and disrupted flow-mediated dilation. This is a prominent indicator of the progression of atherosclerosis, which is why flow-mediated dilation has received a lot of attention in recent decades (Raitakari and Celermajer 2000).

One method of the possible prevention of cardiovascular disease is through replacing saturated fat in the diet with monounsaturated and polyunsaturated fats. This can be done through frequent consumption of nuts instead of foods high in saturated fats, since most nuts are high in mono- and polyunsaturated fatty acids. Previous research has highlighted the cardiovascular benefits of frequent nut consumption such as lower levels of inflammation and oxidative damage, reduced serum triglyceride and cholesterol levels, and increased nitric oxide production (Ros and Mataix 2006).

Furthermore, regarding nut consumption, the peanut is of particular interest because it is the most consumed nut in the United States and has several cardioprotective attributes (Alper and Mattes 2003). While it is high in fat, the peanut's fatty profile, composed of mainly monounsaturated fats and some polyunsaturated fats, reduces the risk of cardiovascular disease when consumed frequently. The peanut is also particularly interesting because it contains resveratrol, a natural polyphenol which acts as an antioxidant to scavenge free radicals and prevent LDL oxidation, as well as reduce platelet aggregation and smooth muscle cell

proliferation (Bradamante, Barenghi, Villa 2004). Furthermore the peanut contains the amino acid L-arginine, which acts as a precursor in the synthesis of nitric oxide, a vasodilator that improves vascular tone and blood flow. Peanuts also contain magnesium, which lowers serum cholesterol and triglyceride levels, and folate, which reduces smooth muscle cell production by inhibiting the actions of homocysteine (Alper and Mattes 2003).

While previous research has been done on the cardiovascular benefits of peanuts, there is no research based on our knowledge that has demonstrated the effects of the acute consumption of peanuts on the lipid profile, glycemic control, and vascular function in the postprandial state. Since the postprandial state has such a significant impact on the progression of atherosclerosis, and since most Americans spend most of their waking hours in the postprandial state, this is a crucial area of research that needs to be further explored. This study aims to examine the postprandial state of 15 healthy, overweight male participants following consumption of a high-fat meal containing peanuts. The lipid profile, glucose levels, and vascular function tested through flow-mediated dilation will be the main endpoints of study. Our hypothesis is that the acute consumption of peanuts will show cardiovascular benefits by decreasing serum lipid levels, improving glycemic control, and improving vascular function in the postprandial state.

My role in this study included participant recruitment, scheduling and managing screening visits, making the test and control meals, managing testing visits, collecting data, and analyzing the data.

Chapter 3

Study Design and Methods

Participant Eligibility

Participants were recruited through flyers hung throughout Pennsylvania State University's campus as well as local stores and restaurants throughout State College, Pennsylvania. An email describing the study aims and general eligibility requirement was also sent out to previous participants in studies within the Department of Nutritional Sciences, as well as other research teams at Penn State. Lastly, an ad was placed in Penn State's student newspaper, *The Daily Collegian*, describing the study and participant eligibility.

Potential participants for the study were interviewed over the phone at first to undergo the initial screening for general eligibility requirement. If they met the initial requirements, they were scheduled for a second screening visit, where blood pressure, height, and weight were recorded, and blood was drawn to be submitted to the Quest Lab for analysis. If all general health requirements, blood pressure, BMI, and lab values met the study's eligibility requirements, the participant was then scheduled for testing visits.

For a participant to be eligible for this study, the participant must have been a male between the ages of 20-50 with a BMI between 28 and 39 kg/m^2. The participants must not have been taking any cholesterol-, blood pressure-, or glucose-lowering medication, and could not be a current smoker. Participants must all have been generally healthy; participants with heart disease, liver disease, gastrointestinal disease, kidney disease, peripheral vascular disease, stroke,

or high blood pressure were disqualified. Participants allergic to peanuts or dairy products or intolerant to high-fat meals were disqualified; participants taking steroid, over-the-counter cholesterol-lowering substances (such as fish oil), or anti-inflammatory or immunosuppressant supplements were also disqualified.

The participants must have had a blood pressure lower than 140/90 mm/Hg; otherwise they were considered hypertensive and were disqualified from the study. The participant must have had a fasting triglyceride level below 350 mg/dL and an LDL level lower than 160 mg/dL; otherwise they were considered hyperlipidemic and thus disqualified from the study (Kris-Etherton et al. 2011).

All participants completed a medical history form and were given the informed consent. Once the participant fully understood the purpose of the study, study design, and requirements as a participant, informed consent forms were signed by and obtained from the participants. A total of 15 eligible participants qualified for the study and successfully completed it. Each participant was awarded $125 for completion of the study.

Study Design and Procedure

The study design was a crossover study consisting of two single testing visits per participant, each lasting about 5-6 hours, with each testing visit separated by a minimum of one week. Before each testing visit, the participants were asked to avoid foods high in antioxidants 48 hours prior to the visit and were also asked to record their food intake during those 48 hours to ensure that they were not consuming foods high in antioxidants that could affect the results of the study. Participants were also required to not consume any alcohol in those 48 hours leading

up to the testing visits, and were asked not to take any vitamins, supplements, or medications (unless it was a prescription medication that was already approved by the study investigators). For the 24 hours leading up to each testing visit, the participants were asked not to engage in any vigorous physical activity that would affect their heart rate or blood pressure. The participants were asked to fast (no food or drink except water) for the 12 hours leading up to the test visit. All participants complied with these requirements.

The testing visits were conducted on the Pennsylvania State University's campus in the Clinical Research Center. Upon arrival, the blood pressure of the participant was recorded, and a topical skin anesthetic was applied to prevent any discomfort when the catheter was later inserted into the arm. The participant then began the flow-mediated dilation procedure, which lasted about 30 minutes. In the case of this clinical study, flow-mediated dilation was employed as the primary method to test endothelial function.

Since peanuts contain resveratrol, which stimulates the production of nitric oxide, it is hypothesized that the acute consumption of peanuts will increase production of nitric oxide, thus dilating the arteries, improving blood flow, and lowering the risk for atherosclerosis. Flow-mediated dilation was measured using an ultrasound machine, and the test was administered by a trained professional. The diameter of the brachial artery of the upper right arm was measured before and after the inflation of a blood pressure cuff on the forearm; this procedure typically dilates the artery. Since the participants consumed the control meal (with no peanuts) during one visit and the peanut meal (with peanuts) during the other visit, while following the same FMD procedure for both visits, it can be determined whether or not the acute consumption of peanuts improved endothelial function. The FMD test was performed at baseline prior to consuming the meal and four hours after consuming the meal.

Following the baseline FMD recording, a catheter was inserted in the participant's arm or hand, and a baseline blood sample was drawn. The participant was then asked to consume either the test shake (containing peanuts) or the control shake (containing no peanuts) within a fifteen minute time period. Each participant consumed the control shake during one visit and the test shake during the other, and the two different shakes were randomly assigned to each testing visit using a computer randomization generator.

After the participant finished the shake, a timer was started, and blood samples were drawn by a nurse 30 minutes, 1 hour, 2 hours, and 4 hours after the shake was finished; thus a total of 5 blood samples (including the baseline sample) were collected during each testing visit. Participants were asked to either sit or lay down during the testing visit, while refraining from any physical activity. They were free to use their laptops, read, or watch television throughout the course of the visit. Following the 4-hour mark, the catheter was removed, the participant's blood pressure was measured and recorded again, and the participant was prepared for the second FMD recording. This FMD recording followed the same protocol as the recording at the baseline testing visit. After the FMD recording was completed, the participant was given a free lunch and was free to leave. All results from the visit were recorded, and all blood samples were centrifuged and pipetted into small valves. The blood samples were frozen and then sent to the Quest Lab for analysis after all participants' samples had been collected.

Blood samples were tested for triglycerides, lipoproteins (LDL-C and HDL-C), glucose, insulin, markers of oxidative stress (e.g. plasma 8-iso-prostaglandin F2 α), inflammatory markers (IL-6), and the following endothelial function adhesion molecules: soluble forms of intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1), and E-selectin as surrogate markers of endothelial activation (Kris-Etherton et al. 2011).

The same procedure was repeated again for each participant during his second testing visit; the only change was that he consumed the other type of shake during the second visit (test shake or control shake). This study was approved by the Institutional Review Board (IRB).

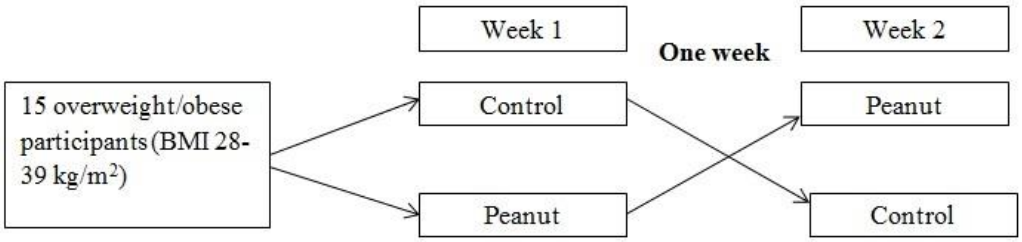

Figure 1: Study Design Model

Shake Composition

The participants each consumed a control shake during one test visit and the peanut shake during the other test visit (randomly assigned by a computer generator). The control shake contained no peanut products while the peanut shake contained raw peanuts; however, each shake was matched for macronutrient, fatty acid, protein, fiber, and energy content. The matched macronutrient, fatty acid, protein, fiber, and energy profiles of the two shakes allowed the investigators of the study to isolate and test the postprandial effects of peanuts on cardiovascular risk factors such as blood lipids and blood glucose as well as flow-mediated dilation of the artery.

The control shake contained the following ingredients: 34.8 grams (g) glucose, 150 g heavy whipping cream, 39 g chocolate syrup, 15 g sunflower oil, 22 g safflower oil, 27 g powdered egg whites, 9.6 g of a fiber supplement, water and crushed ice.

The peanut shake contained the following ingredients: 3.0 ounces ground peanuts (including skin), 34.8 g glucose, 137 g heavy whipping cream, 39 g chocolate syrup, water and crushed ice (Kris-Etherton et al. 2011).

Table 1: Shake Composition by Percentage

TABLE 1: SHAKE COMPOSITION (%)				
	Percentage by Weight		Percentage by Energy (kcal)	
Macronutrient	Control	Peanut	Control	Peanut
Carb	**24.8**	**25.9**	**24.5**	**24.9**
Fat	**68**	**69**	**67.3**	**67**
-SFA*	27.6	28.2	-	-
-MUFA*	26	26.7	-	-
-PUFA*	11.1	11.3	-	-
Protein	**8.4**	**8.5**	**8.3**	**8.2**
Fiber	-	-	-	-
Energy (kcal)	1229	1198	1229	1198

*SFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

Table 2: Shake Composition by Weight and Energy

TABLE 2: SHAKE COMPOSITION				
	Grams		Energy (kcal)	
Macronutrient	Control	Peanut	Control	Peanut
Carb	**76.1**	**77.1**	**304.4**	**310.8**
Fat	**92.9**	**93**	**836.1**	**837**
-SFA*	37.7	37.6	339.3	338.4
-MUFA*	35.5	35.5	319.5	319.5
-PUFA*	15.1	15.1	135.9	135.9
Protein	**25.8**	**25.6**	**103.2**	**102.4**
Fiber	8.2	8.2	-	-
Energy (kcal)	1229	1198	1229	1198

*SFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

Flow-Mediated Dilation Ultrasound Analysis

To analyze and measure the ultrasound recordings of flow-mediated dilation in the 15 participants, a software program called Brachial Analyzer and Imager was used. Through this, the images could be paused and analyzed at every frame during the cuff inflation period and the cuff deflation period. The diameter of the brachial artery was measured from the M-line (the center black line) at the top wall of the artery down to the M-line of the bottom wall of the artery. This diameter, in theory, gets larger after the cuff is deflated, the artery walls relax, and vasodilation occurs. The M-line distance, as well as peak diameter and average diameter, was recorded during the inflation and deflation periods for both the baseline and 4-hour time point recordings. This same procedure was done for both of the participant's testing visits (the control visit and the peanut visit) and done in the same way for each of the 15 participants. The

recordings were measured by two different people at two different times to ensure accuracy of the results.

In the baseline inflation period, multiple frames were inspected to find a clear frame to analyze. Once a frame was chosen, then the clearest part of the artery was chosen. This exact same part of the artery (determined by coordinates) was analyzed for both the inflation and deflation period to maintain consistency. The largest, or peak, diameter was recorded, as well as the average diameter. The percent change in the M-line, representing the change in arterial diameter, from the inflation to deflation was recorded for both time point 0 and time point 4 hours for each participant and for each testing visit. This helps to measure whether or not flow-mediated dilation is affected by the consumption of peanuts.

Figure 7 shows an example of the brachial artery ultrasound image of one of the participants. In this image, the green box denotes the clearest region of the artery that was chosen to be analyzed for both the inflation and deflation states. The pink lines on the top wall and bottom wall of the artery denotes the M-lines, which help to measure the arterial diameter before and after the shear stress of blood flow.

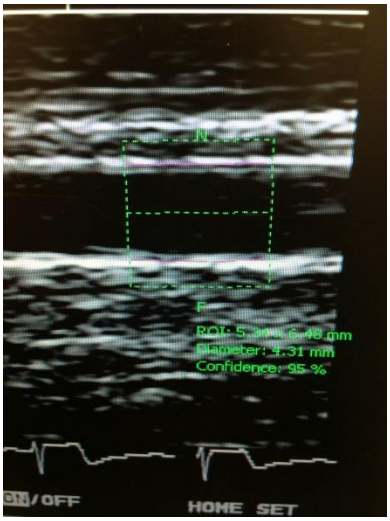

Figure 2: Example of Brachial Artery Ultrasound Image

Chapter 4

Statistical Analysis

Statistical analyses were performed by using SAS (version 9.2; SAS Institute, Cary, NC). The mixed models procedure (PROC MIXED) in SAS was used to test the effects of treatment, period, and treatment by period interactions on each variable. Subject was treated as a random effect, and all other factors were fixed effects. When period effects were significant they were included in the model. When treatment by period interaction was not significant they were excluded from the model. Tukey-Kramer was used for post hoc comparison. Values are expressed as means±SEM (standard error mean).

To test significance of glucose, a t-test analysis was performed between the change of glucose concentrations at each time point, comparing the two treatment meals (peanut and control). The p-value was found to be greater than 0.05 ($p=0.15$). This demonstrates that no significance was found in average change in glucose concentrations between the peanut meal and the control meal during the postprandial state.

Chapter 5

Results

Participant Baseline Characteristics

Table 3 reports the baseline characteristics for the 15 male participants who successfully completed the study. The average age of the participants was 26.7 years, with an average BMI of 31.4 kg/m^2. This means the participants on average were in class I of obesity. Blood pressure levels, both systolic and diastolic, were on average normotensive in all participants. Furthermore, the average triglyceride level (90.6 mg/dL) fell into the normal triglyceride range, which is less than 150 mg/dL. Participants were also normocholesterolemic, with an average cholesterol level of 159.7 mg/dL, with a desirable cholesterol level being below 200 mg/dL. Optimal LDL cholesterol level is below 100 mg/dL, and the average LDL level of the participants was 99.1 mg/dL. The participants also had a desirable average HDL cholesterol level of 42.4 mg/dL, where anything above 40 mg/dL for men is considered normal. Average fasting glucose concentration for the 15 participants was 92.5 mg/dL, which falls into the normal range of 70-99 mg/dL. Overall, the average baseline characteristics of the 15 participants of the study demonstrated that all participants were all generally healthy (no diseases, normotensive, normocholesterolemic, and normal glycemic control), aside from being overweight or obese.

Table 3: Participant Baseline Characteristics

TABLE 3: BASELINE CHARACTERISTICS	
Average Baseline Characteristics	
Age (years)	26.7±1.6
Weight (kg)	100.0±3.7
BMI (kg/m^2)	31.4±0.8
Systolic blood pressure (mmHg)	125±2
Diastolic blood pressure (mmHg)	83±1
Fasting glucose (mg/dL)	92.5±0.7
Cholesterol (mg/dL)	159.7±7.1
HDL (mg/dL)	42.4±1.9
LDL (mg/dL)	99.1±6.4
Triglyceride (mg/dL)	90.6±8.9

Effect of Peanuts on Postprandial Triglyceride Concentrations

Table 4 lists the average postprandial triglyceride concentrations (mg/dL) of the 15 participants over the 4 hours following the consumption of the test meal. Time point 0 denotes the baseline concentration (the fasting concentration before the meal was consumed), time point 0.5 denotes a half-hour after meal consumption, time point 1 is one hour after meal consumption, time point 2 is two hours after meal consumption, and time point 4 is four hours after meal consumption. These time points all reflect the postprandial state following a meal.

The average postprandial triglyceride concentrations (mg/dL) throughout the postprandial state show a substantial increase in concentration with both the peanut meal and control meal from the baseline concentration until time point 2. From time point 2 to time point 4, there is a slight decrease in concentration with the control meal and a slight increase in concentration with the peanut meal.

Table 4: Average Postprandial Triglyceride Concentrations (mg/dL) over 4 hours

TABLE 4: AVERAGE POSTPRANDIAL TRIGLYCERIDE CONCENTRATIONS OVER 4 HOURS (mg/dL)				
Time Point	Control	SEM	Peanut	SEM
T0	82.2	7.4	93	11.4
T0.5	100.5	9.5	106.3	10.5
T1	124.1	12.1	132.1	10.8
T2	197.5	20.6	188.9	19.4
T4	197.3	18.4	189.9	24.3

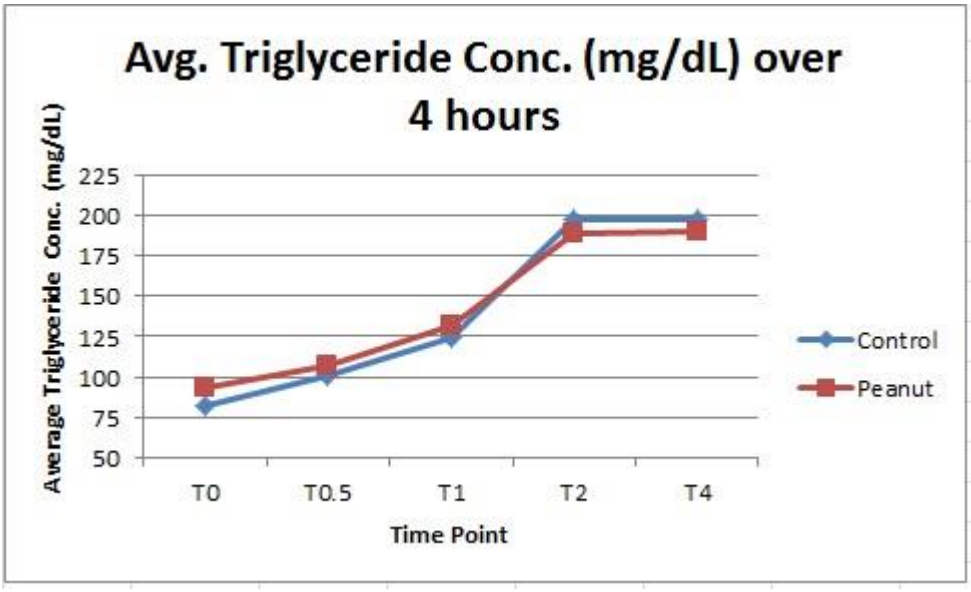

Figure 3: Average Postprandial Triglyceride Concentrations (mg/dL) over 4 hours

Table 5 displays the average change in postprandial triglyceride concentrations (mg/dL) during the four hour postprandial state following meal consumption. The triglyceride response continued to increase almost until the end of the postprandial state. While there was no statistical significance from time point 0 and 4 when comparing control versus peanut meals, there was a statistical difference in the change in triglyceride response at time point 2 (2 hours post-meal consumption) when comparing the control versus the peanut data (p=0.034). Since

two hours after meal consumption is typically known to be the peak of triglyceride response following a high-fat meal, this is a crucial time point to analyze (Cohen, Noakes, Benade 1988). These results show that the addition of peanuts to the high-fat meal aided in blunting the triglyceride response in the postprandial state at the 2-hour time point during the postprandial state.

Table 5: Average Change in Postprandial Triglyceride Concentration (mg/dL) over 4 hours

TABLE 5: AVERAGE CHANGE IN POSTPRANDIAL TRIGLYCERIDE CONCENTRATION OVER 4 HOURS (Δ mg/dL)				
Time Point	Control	SEM	Peanut	SEM
T0	0	0	0	0
T0.5	18.3	5.3	13.3	2.7
T1	41.9	8.1	39.1	6.3
T2	117.7	13	94.1	13
T4	117.3	13	95.1	13

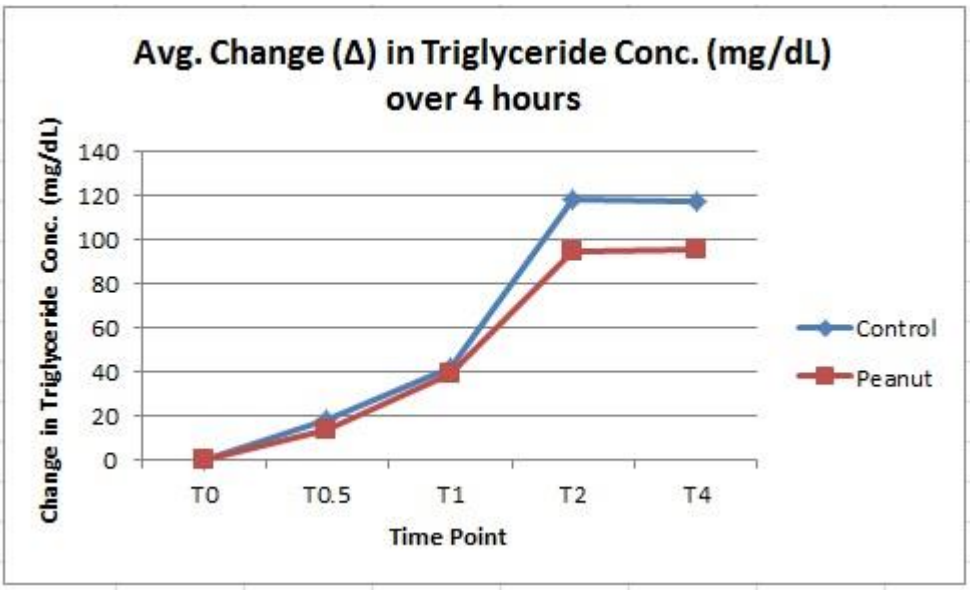

Figure 4: Average Change in Triglyceride Concentration (mg/dL) over 4 hours

Table 6 shows the average postprandial glucose concentrations (mg/dL) over the four hours following the meal consumption. For both the peanut and control meal, the glucose concentration peaks around time 0.5 hours and then decreases from that point forward. The glucose concentration at time point 4 hours for both the peanut and control meals was similar to the baseline glucose concentration at time point 0, showing a return to normal levels.

Table 6: Average Postprandial Glucose Concentration (mg/dL) over 4 hours

TABLE 6: Average Postprandial Glucose Concentrations (mg/dL) over 4 Hours After Meal Consumption				
	Control	SE	Peanut	SE
Time 0	89	1.24	86	1.74
Time 0.5 hours	103	2.34	106	2.76
Time 1 hour	100	4.07	97	3.65
Time 2 hours	88	4.00	100	3.86
Time 4 hours	93	3.15	89	1.91

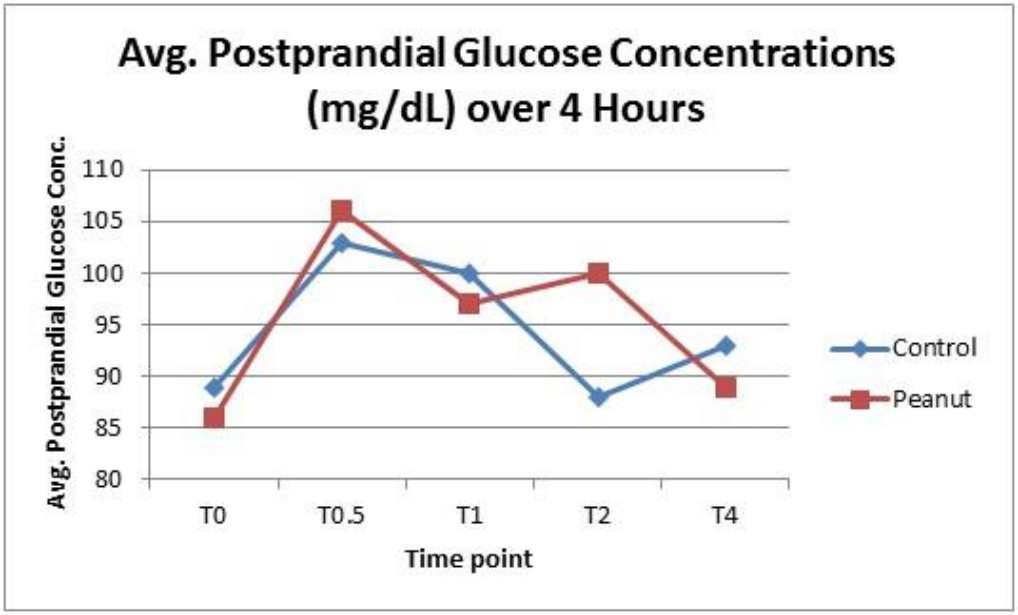

Figure 5: Average Postprandial Glucose Concentration (mg/dL) over 4 hours

Table 7 displays the average change in postprandial glucose concentrations (mg/dL) over the four hours following the meal consumption. No statistical significance was found between the control and peanut meal regarding glycemic control for each time point ($p > 0.05$). This shows that in this particular study, peanuts did not have a significant effect on glycemic control in overweight and obese men. Further research should be done to confirm these results.

Table 7: Average Change in Postprandial Glucose Concentration (mg/dL) over 4 hours

TABLE 7: AVERAGE CHANGE IN POSTPRANDIAL GLUCOSE CONCENTRATION OVER 4 HOURS (Δ mg/dL)					
Time Point	Control	SEM	Peanut	SEM	P-value
T0	0	0	0	0	-
T0.5	14.5	2.5	20.1	2.6	0.15
T1	14.6	2.9	15.3	3.8	0.88
T2	11	4.0	15.1	2.2	0.33
T4	6.9	1.6	6.8	3.2	0.99

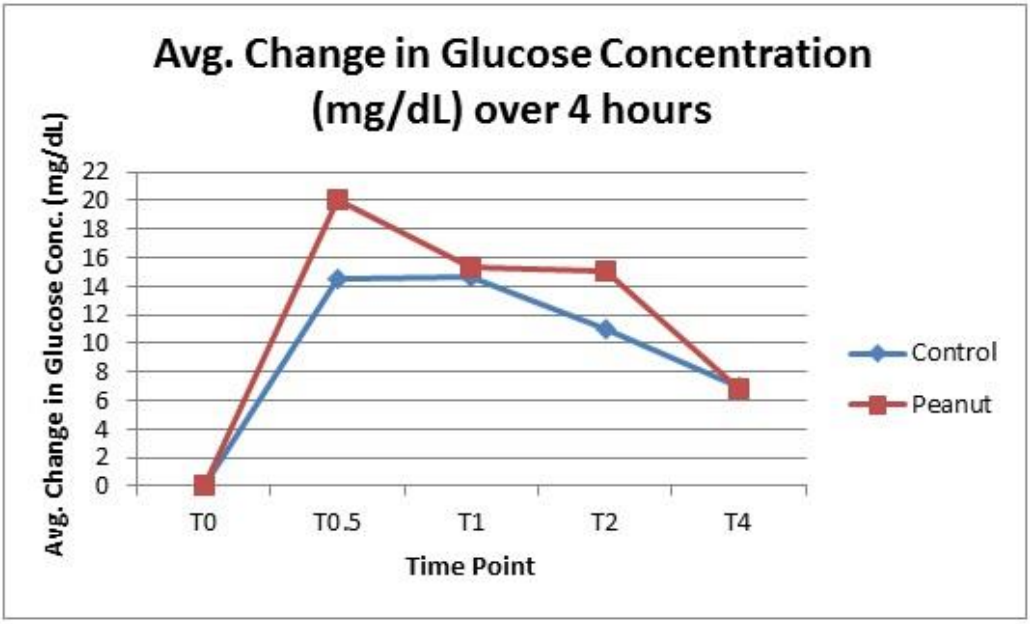

Figure 6: Average Change in Postprandial Glucose Concentration (mg/dL) over 4 hours

Effect of Peanuts on Flow-Mediated Dilation

Table 8 displays the average percent change in arterial diameter of the brachial artery in the 15 participants, representing the average change in flow-mediated dilation after consumption of the test meal. To measure this change in flow-mediated dilation, the ultrasound procedure was performed twice: once during baseline (before meal consumption) and once at time point 4 (four hours after meal consumption). This made it possible to measure change in dilation before a high-fat meal without peanuts, and after a high-fat meal without peanuts (control), as well as before a high-fat meal *with* peanuts, and after a high-fat meal with peanuts. Thus, the effects of peanuts on postprandial flow-mediated dilation could be assessed. During this procedure, a blood pressure cuff was placed on the upper arm of the participant. The cuff was inflated for five minutes to increase blood pressure of the brachial artery in the upper arm. Then, the cuff was deflated for two and a half minutes. An ultrasound image of the artery was recorded throughout the entire procedure. This recorded the dilation that occurred after the shear stress of blood flow in the artery. After all of the images of all participants had been recorded, the ultrasound images were analyzed and measured using a software system (procedure of analysis is described above) to calculate the percent change in dilation before the meal was consumed (baseline) and after the meal was consumed (postprandial).

The measured results (Table 8) show a statistically significant ($p=0.03$) decrease in flow-mediated dilation when comparing the baseline flow-mediated dilation recording to the recording made four hours after consumption of the control meal. This demonstrates that a high-fat meal reduces dilation, thus narrows the artery diameter, and disrupts optimal blood flow. The peanut meal did show a decrease in flow-mediated dilation from the baseline recording to the recording made four hours after the peanut meal was consumed, however the difference was not

statistically significant (p=0.3). This finding shows that the control meal statistically decreased flow-mediated dilation, while the peanut meal was able to preserve endothelial function, and thus flow-mediated dilation and blood flow.

Table 8: Average Percent Change in Flow-Mediated Dilation over 4 hours

TABLE 8: AVERAGE % CHANGE IN ARTERIAL DIAMETER OVER 4 HOURS				
	Control	SE	Peanut	SE
0 hours post meal	5.84	0.56	5.42	0.64
4 hours post meal	4.44	0.54	5.02	0.56

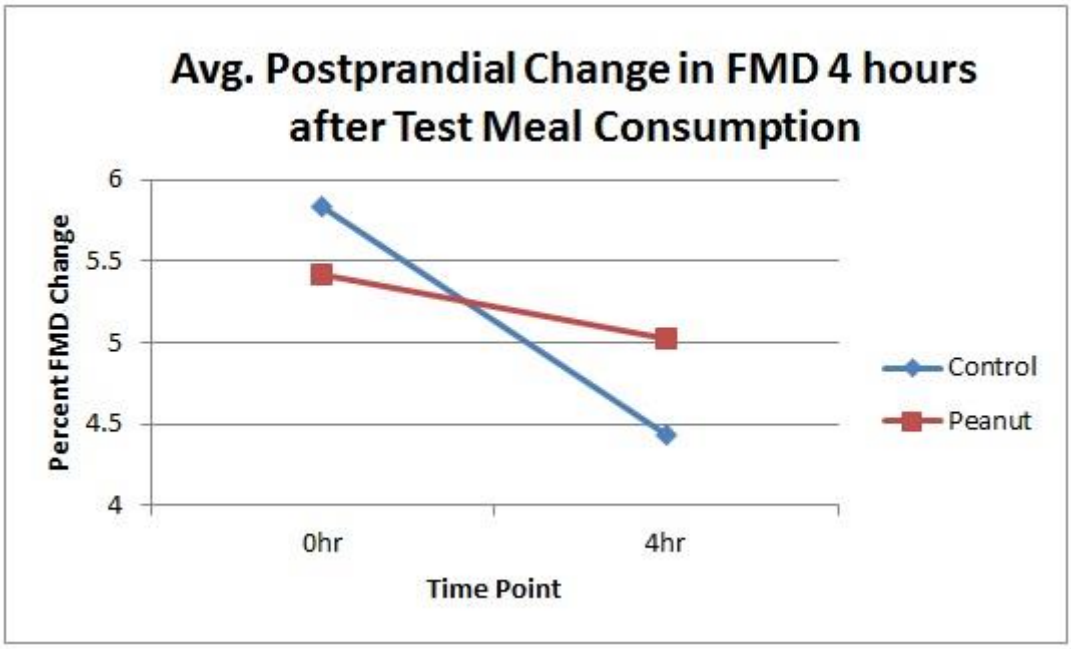

Figure 7: Average Percent Change in Flow-Mediated Dilation over 4 hours after Meal Consumption

Chapter 6

Discussion

The postprandial state is an extremely important time to analyze because most Americans spend a large portion of their day (when not asleep) in the postprandial state. Furthermore, previous research has shown the connection between the postprandial state and risk for cardiovascular disease. This is because the inflammatory response caused by consuming a high-fat meal increases the triglyceride and glucose responses that occur in the postprandial state, as well as increases damage caused to the endothelium of the arteries (Sies, Stahl, Sevanian 2005). This study provided further insight on the effects of the acute consumption of peanuts on these risk factors of cardiovascular disease that occur in the postprandial state.

When consumed with a high-fat meal, peanuts may be a good strategy for preventing impaired flow-mediated dilation and may aid in blunting the triglyceride spike in the postprandial state. Spiked triglyceride levels following a high-fat meal cause injury to the artery endothelium and thus an inflammatory response. This inflammatory response includes smooth muscle cells, oxidized LDL, and macrophages, which can form a foam cell and eventually a fatty streak lining the artery endothelium. This narrowing and hardening of the artery causes a decrease of blood flow that increases the risk for a cardiovascular event such as a stroke or myocardial infarction (Jiang et al. 2006). This study showed that a high-fat meal containing peanuts, when compared to the control meal without peanuts, significantly blunted the triglyceride response in the 2-4 hour range following consumption of the meal. This decrease in triglyceride response would then decrease the inflammatory response occurring in the artery and

reduce risk for endothelial injury. With less endothelial injury, there is a decreased risk for plaque buildup lining the walls of the arteries.

While peanuts did not improve flow-mediated dilation, the incorporation of peanuts into a high-fat meal helped to preserve endothelial function and maintain normal blood flow. A high-fat meal with no peanuts (the control meal) proved to actually significantly impair flow-mediated dilation and blood flow. Impaired flow-mediated dilation is now considered to be a major risk factor for cardiovascular disease. The incorporation of peanuts into the high-fat meal has been shown to increase nitric oxide production, which stabilizes vascular tone, promotes endothelial dilation upon shear stress, and maintains normal blood flow. This shows that it is possible that if peanuts are incorporated into a high-fat meal, endothelial function can be preserved rather than impaired, and the risk for cardiovascular disease will decrease (Adams et al. 1997).

Even though in this particular study, there was no statistically significant improvement of glycemic control when comparing the peanut meal with the control meal, previous research has shown a link between triglyceride response and glycemic response. Further research may be needed to see if the acute consumption of peanuts has any significant effect on glucose concentration in the postprandial state.

One possible limitation of the study was the smaller sample size (n = 15). With a larger sample size, a greater or lesser effect may have been observed after the consumption of the peanut meal. Another limitation of the study was the acute consumption of peanuts. Each participant was only consuming one meal that contained peanuts over the course of the study. Had the participants been consistently consuming peanuts over a longer period of time, this may have also influenced the results. This study's participant sample included overweight and obese, but otherwise healthy, men. It may be interesting to test the effects of the acute peanut

consumption on glycemic control and endothelial function in a population with a greater risk for cardiovascular disease. For instance, the results seen in the study may have been more pronounced if the sample population had multiple risk factors for cardiovascular disease, such as smoking, hypertension, or hypercholesterolemia. It would also be interesting to test these endpoints with either healthy women or overweight women as well, since this particular study only focused on males. Since this study's sample population tested a younger population (mean age of 26.7 years), it would be enlightening to test the effects of the acute consumption of peanuts on an older population as well since the risk for cardiovascular disease increases with age. Future research with this type of population may provide more insight on the possible benefits of including peanuts in the diet and decreasing the risk for cardiovascular disease.

Chapter 7

Conclusion

With cardiovascular disease being the leading cause of death in the United States, it is vital to explore possible methods of prevention of the disease. Previous research has demonstrated how significant of a role the diet plays in disease pathogenesis. The American or Western diet is one high in saturated fat, sugar, and energy. There are certain dietary modifications that can be made, however, to improve cardiovascular disease and decrease risk for the disease. Peanuts are a food source of mono- and polyunsaturated fats, as well as folate, magnesium, and L-arginine, which all have shown cardioprotective benefits.

This study showed that peanuts, when incorporated into a high-fat meal, can limit endothelial injury and preserve flow-mediated dilation in the postprandial state in healthy, overweight men. Peanuts also aided in blunting the triglyceride response two to four hours after consuming a meal with peanuts compared to consuming a meal without peanuts. This improvement in triglyceride response may decrease endothelial injury and inflammation, which lowers the risk for plaque buildup that could lead to atherosclerosis.

Therefore, including peanuts into the diet could be one possible dietary intervention to lower the risk for atherosclerosis and cardiovascular events such as stroke or myocardial infarction. The fatty acid composition and micronutrient profile of peanuts have been shown to provide cardioprotective benefits that decrease inflammation and increase flow-mediated dilation in the postprandial state. Consuming peanuts with a high-fat meal can decrease the triglyceride response in the postprandial state, which is hypothesized to limit inflammation and decrease risk

for cardiovascular disease. Eating peanuts may also limit endothelial dysfunction, through the production of nitric oxide to increase vasodilation and through a decreased risk of endothelial injury, which would prevent plaque formation and the progression of atherosclerosis.

Appendix A

Peanut Study Informed Consent

INFORMED CONSENT FOR CLINICAL RESEARCH STUDY

The Pennsylvania State University

Title of Project: The effect of peanuts and peanut products on glucose control and vascular function

Principal Investigator: Penny Kris-Etherton, PhD RD
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Jennifer Fleming, MS, RD, Clinical Coordinator
Email: jas58@psu.edu
Phone: 863-8056

(Please print your name) _____ so that the person in charge of the research, Dr. Penny Kris-Etherton, would know that you have had a chance to read the information below. This form may contain words you do not understand. Please ask the study personnel to explain any words or information you do not clearly understand.

PLEASE READ EVERY PAGE CAREFULLY AND INITIAL THE BOTTOM OF EACH PAGE WHEN YOU HAVE HAD ALL OF YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION.

Purpose of the Study

You have been invited to participate in a clinical research study to test the effects of peanut consumption on the health of your blood vessels and other important cardiovascular disease (CVD) risk factors. To be enrolled in this study, your screening results indicated you were healthy with a body mass index (BMI) between 28-39 kg/m^2. Frequent nut consumption is associated with a

reduced risk for type II diabetes and cardiovascular disease (CVD). This reduction in risk may be due to improvements in artery health, blood lipids (fat), and glucose.

The overall aim of this study is to determine the effects of acute peanut consumption artery health, glucose, and lipids. These effects will be evaluated by measuring fasting and post meal responses. Post meal responses are important as most individuals spend the majority of the day in this state. Furthermore, having abnormally high blood glucose and fats following a meal greatly increases risk for CVD, possibly due to impaired artery function. Peanut consumption may therefore be an important strategy for lessening the rise in blood glucose, fats and impairments in artery function that may occur after eating. During the study, your blood will be tested for the levels of lipids, glucose, inflammation, oxidative stress (tissue damage caused by free radicals) and markers of vascular health.

Procedures to be Followed

Screening Tests

If you decide to participate in the study and are considered suitable after the telephone screening, you will be further screened to determine your eligibility during a visit to the Clinical Research Center (CRC) at Penn State. The visit will consist of filling out forms (informed consent, medical history, personal information); measuring height and weight so your BMI can be calculated; and measuring blood pressure (BP). If after these measurements it is determined you are still eligible, a blood sample will be taken from your arm, or hand, and a complete blood count, including liver and kidney function and a blood fat panel will be performed (approximately 15 mls of blood or ~1 tablespoon will be taken). If the initial blood draw is unsuccessful, it may need to be repeated, with your permission**.** You will be contacted in about a week with the results of the screening blood sample. A clinician at the CRC will review all of the screening data and if you are still eligible for the study, you will be contacted to set up your testing schedule. If you agree to participate in this study, you will agree to check with the study staff before participating in any other research studies. Also, you will agree to refrain from donating blood or plasma during the entire study.

Study Scheme

You will be required to perform 2 single day visits of 5-6 hours (separated by a minimum one week break). During the two days prior to each test visit, you will be asked to record your food intake to ensure no high antioxidant foods are consumed. You will be provided with a list of foods to avoid during this time. In addition you must not consume any alcohol during these 48 hours and any medications (including non-prescription) that you may have taken should be reported to the study coordinator. During the 24 hours prior to your test visit you will not be allowed to engage in any vigorous exercise (e.g. activity that greatly increases heart rate and breathing, such as running, cycling, and team sports).

On each day of testing, following an overnight fast (12 hours with no food or drink except water), you will come to the CRC on the Pennsylvania State University Campus. On the morning you arrive for the test, if desired, a topical skin anesthetic will be applied to the arm or hand, to numb the area in preparation for catheter insertion. You will then undergo a non-invasive procedure known as Flow Mediated Dilation (FMD), which is used to determine the health of your arteries. This procedure is described in detail in the section *Vascular Ultrasound Test by Flow-mediated dilation (FMD)*. Once this test is finished, and after 30 min, a catheter will be inserted into your arm, or hand

(the needle is removed and the catheter, a flexible plastic tube, will remain in your arm, or hand) by trained CRC staff. If the initial insertion attempt is unsuccessful, it may need to be repeated, with your permission. A blood sample will be drawn, and you will then be asked to consume a test meal in the form of a shake (see below), which must be consumed within 15 minutes

You will not be allowed to consume any other food or drinks (other than water, caffeine-free, diet soda, and diet jello) for the remainder of the testing period (~5.5 hrs). You also must refrain from any physical activity (you must remain seated or lying down) during the testing period. Sedentary activities such as reading, computer work, watching television or talking on the phone will be allowed. Following the shake consumption blood samples will be taken at 30 minutes, 1, 2 and 4 h (see Table 1 below). If the catheter fails during the experiment, it will be removed. We will ask you if you wish to continue with the experiment. If you agree to do so, you will be given the option of having another catheter inserted or of having a needle stick for the remaining blood samples.

At each visit your weight and blood pressure will also be recorded. At the end of the 4 hour post-meal period you will have the catheter removed and the 2nd FMD test will be performed. You will be briefly evaluated for safety before leaving the study site. You will be offered a small meal before leaving. Approximately 150 ml (about 10 tablespoons) of blood will be collected during each of the postprandial tests (total of 300 ml for the 2 postprandial tests). This is less than a typical Red Cross blood donation, which is 1 pint (500 ml).

Test Meal Details – Each Shake delivers ~1200 kcals

1. Control (C): Ingredients for this shake will be 34.8 g glucose, 150 g heavy whipping cream, 39 g chocolate syrup, 15 g sunflower oil, 22 g safflower oil, 27 g powdered egg whites, 9.6 g of a fiber supplement, water and crushed ice.
2. Peanut (P): Ingredients for this shake will be 3.0 oz ground peanuts (including skin), 34.8 g glucose, 137 g heavy whipping cream, 39 g chocolate syrup, water and crushed ice.

Table 1: Estimated blood collection protocol relative to meal consumption

TIME	***8:00am***	***8:30am***	***8:45am-9:00am***	***9:30am***	***10:00am***	***11:00am***	***1:00pm***
ACTION	• You arrive at CRC after overnight fast. • FMD testing	• An IV catheter is inserted. • Blood draw (~30 mls)	• You consume one of the shakes	• Blood draw (~30 mls)	• Blood draw (~30 mls)	• Blood draw (~30 mls)	• Blood draw (~30 mls) • FMD
BLOOD DRAW		1 (Baseline)		2 (30 min post shake)	3 (1 hr post shake)	4 (2 hr post shake)	5 (4 hr post shake)

Assessments

Fasting Blood Sampling Reminder:

You cannot consume any food or drinks except water for 12 hours, and cannot drink alcohol during the 48 hours prior to having your blood taken. You also cannot engage in vigorous physical activity 24 hours prior to having your blood taken.

A fasting blood sample will be taken from you at screening. Approximately 15 ml (~1 tablespoon) of blood will be collected at this visit. During each postprandial visit: you will have a blood sample taken from your arm, or hand, at baseline (time 0) as well as at 30 minutes, 1, 2 and 4 hours after consumption of the test meal. About 30 minutes before the catheter is inserted, you may choose to have a small amount of topical skin anesthetic cream put on the site to reduce any feeling of pain from the insertion of the needle and catheter. If the catheter fails during the experiment, it will be removed. We will ask you if you wish to continue with the experiment. If you agree to do so, you will be given the option of having another catheter inserted or of having a needle stick for the remaining blood samples. At the end of the 5.5 hour testing period you will have the catheter removed and you will be briefly evaluated for safety before leaving the study site. Approximately 150 mLs (30 mLs or 2 tbsp at each time point) of blood will be collected during each of the post meal tests for a total of 300 mLs for the 2 tests. Blood samples will be frozen, and analyzed at the end of the study (when all subjects have completed the study). The results of the study will only be available at the end of the entire study (which may take up to a year). Your blood will be tested for the following: blood fats (triglycerides), lipoproteins (LDL-C, HDL-C), glucose, insulin, markers of oxidative stress (e.g. plasma 8-iso-prostaglandin F2 α) inflammatory markers (IL-6), and endothelial function adhesion molecules: soluble forms of intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1) and E-selectin as surrogate markers of endothelial activation; nitrosoglutathione

Vascular Ultrasound Test by Flow-mediated dilation (FMD):

In addition to collecting blood samples, you will undergo a test that determines the health of your arteries and their reactivity to mild stressors, which are described below. This test will be performed using an ultrasound machine. Ultrasound is often used to see images of babies in the womb. We will use ultrasound to measure the diameter of an artery in your upper arm, before and after the inflation of a blood pressure cuff on the forearm. In most people, this procedure produces dilation (opening up) of the artery. The purpose of this test is to test whether addition of peanuts into the diet will improve blood vessel function. The test will be performed at baseline (prior to consuming the test meal) and 4 hours following the test meal. The FMD test is described below.

Ultrasound will be used to collect images of the brachial artery in your right arm. This procedure is known as Flow Mediated Dilation (FMD). The FMD test takes about 30 minutes and is performed as follows:

1. In a private room, you may be asked to remove your shirt and put on a hospital gown. (You will not be asked to remove any clothing below the waist). You will lie quietly on a bed in a quiet, darkened room.
2. Your right arm will be extended at a 45-degree angle from your shoulder and rest on some foam cushion supports. A blood pressure cuff will be placed on your forearm.
3. A research assistant will place 3 EKG electrodes (stickers) on your upper chest and stomach.
4. You will be asked to rest for 10 minutes.
5. A technician, trained in medical ultrasound, will sit at the head of the bed. The technician will apply ultrasound gel on your arm and will place an ultrasound probe (which looks like a microphone) on that arm.
6. An image of the blood vessels in your arm will be viewed on the ultrasound equipment next to the bed. The technician may need to move the probe over a small area of your upper arm to obtain the clearest image. The image of the artery will be videotaped for 5 minutes.

7. Next, the blood pressure cuff on your lower arm will be tightly inflated (to a pressure of 250 mmHg) and it will remain inflated for 5 minutes.
8. While the cuff remains inflated, the technician will continue to record the image of the artery in your upper arm. At the end of 5 minutes, the cuff will be deflated and images will be captured and recorded for an additional 2.5 minutes. It is very important throughout the recording that you rest quietly and keep your arm as still as possible.

Compliance with Study Protocol

*****Please note: Successful completion of this study depends on the total cooperation of the participants. If during the study, you cannot comply with study procedures (such as attending clinic visits, medication requirements), you will be asked to leave the study. Every effort will be made to give you a chance to comply with the study requirements, but if you do not follow the above study protocol you may not continue in the study.**

In addition, please advise us of any medical events (such as illness, injury, surgery etc) that arise during the course of the study. Depending on the event, we may require you to obtain a medical clearance before continuing with the study. Some medications may also interfere with our study outcomes so please inform us of any medication changes.

Time Commitment for the Study

Your total involvement in the study will last for approximately 2 weeks. The following is an estimate of the amount of time you will spend in study activities:

- Screening: Forms, BP, weight, blood draw – 45-60 min
- Postprandial tests x 2 5.5 hrs each visit (11 hrs total)

Total time for study is approximately 720 minutes or about 12 hours

Discomforts and Risks

Post Meal Study

The meals provided in this study will be prepared according to accepted standards of sanitation and provisions are made to ensure the safety of foods provided for off-site consumption. However, it is possible that incorrect food handling during shipping, storage or preparation, if not detected, could result in food-borne illness. Every effort will be made to safeguard against this possibility. You may experience gastrointestinal (e.g. stomach) upset from the foods provided in the meal challenge, possibly due to the high fat content. This will likely subside within a few hours of eating the meal challenge.

Peanut Allergies

You will be asked to report a peanut allergy during the telephone screen, however it is possible that an unknown peanut allergy may manifest during the study. You will be provided with a list of possible symptoms associated with peanut allergies, and instructions to seek medical advice should any of these symptoms occur after you leave the CRC.

Blood Sampling

The risks involved with taking blood from you include some local pain and bruising where the blood is taken. Well-trained and experienced nurses will be performing your blood draws. Blood sampling can also cause light-headedness and dizziness. If this occurs, the symptoms will be alleviated by having you lie flat with your feet raised. As with any procedure involving taking blood, infection is possible (less than 1 in 10,000). All precautions will be taken to avoid infection. There is a slight risk of developing a blood clot at the blood draw site.

Topical Anesthetic Cream

Numbing cream will not be used in those individuals who have increased sensitivity to lidocaine. Eye contact should be avoided. When used, all sensations within the treated area are blocked. For this reason, unintentional trauma to the area, such as scratching, rubbing or exposure to hot or cold temperatures should be avoided until complete sensation has returned. During or immediately after application, mild swelling, skin redness or abnormal sensation may develop at the site of treatment. In clinical studies, no serious reactions resulted from the use of the cream. Allergic reactions can occur and will be managed. Whole body adverse reactions following appropriate use are unlikely due to the small dose absorbed. If effects do occur, they are similar in nature to those seen with other local anesthetic agents and may include lightheadedness, nervousness, apprehension, dizziness, drowsiness, twitching and vomiting. Reactions may be brief or not at all.

Vascular Ultrasound Test (FMD)

There are no known risks associated with ultrasound. However, because the blood pressure cuff on your right forearm is inflated tightly, it is likely that your hand and arm below the blood pressure cuff will experience "pins and needles" (tingling and pricking sensations) while the cuff is inflated and for a few minutes after it is released. This feeling is similar to what you feel when your hand or foot "fall asleep." During the 5 minutes that the blood pressure cuff is inflated on your forearm, your arm could become numb and we will ask you not to move it. This might be moderately painful. However, any discomfort or numbness should go away within minutes of cuff deflation and there are no known long-term risks associated with this test. There is a possibility for red blotching or mild bruising (petechiae) appearing on the skin above and below the location of the blood pressure cuff. Studies indicate that petechiae are rare (occurring in less than ½ of 1% of patients) and it is typically not uncomfortable and it does not require treatment. There are no risks associated with measurement of blood pressure, heart rate, or EKG as long as the participant is not allergic to adhesive tape. Temporary redness at the site of the electrode placement is possible.

All video tapes from the ultrasound will have no personal identifying information associated with them and will be stored in a locked closet indefinitely since there is no indication on the tape of who the subject is.

Benefits to You

You will receive screening laboratory analysis, including a blood count, interpretation of liver and kidney function, and blood fat values, at no cost to you. The final results of the study will not be available until all of the analyses are completed which may take up to a year. No benefit from participation in this study is guaranteed.

Potential Benefits to Society

It is hoped that the information gained from this study will increase our understanding of the effects of peanuts on triglycerides and heart disease risk factors.

Study Funding Source Information

This funding for this study is provided by the Peanut Institute. However, the funding source will not be involved in data analysis. They will have the right to review all publications before submission. There are no contractual agreements that allow them to restrict the publication of results.

Statement of Confidentiality

Your participation in this research is confidential. All records are coded with a unique ID number and no names are used. Records containing names or other identifying information are kept under lock at the PI's research office. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records. In the event of publication of this research, no personal identifying information will be disclosed. Your blood specimens will be coded with your unique ID number and will be maintained until three years after the date from when the study is published, and then destroyed unless (see end of document) you give permission for use to keep your blood samples for future use. At the end of the study (after all subjects have completed the study), you will be given your laboratory results without cost, and informed of the study results, and advised of the implications for your future care.

The following administrations may review and copy records related to this research: The Office of Human Research Protections in the U.S. Dept. of Health and Human Services; The Penn State University Institutional Review Board; The Penn State University Office for Research Protections.

Right to Ask Questions

Please contact Dr. Skulas-Ray or Dr. Kris-Etherton at (863-8622 or 863-2923) with questions, complaints or concerns about this research. You can also call this number if you feel this study has harmed you. If you have any questions, concerns, problems about your rights as a research participant or would like to offer input, please contact Penn State University's Office for Research Protections (ORP) at (814) 865-1775. The ORP cannot answer questions about research procedures. Questions about research procedures can be answered by the research team.

If the Primary Investigator or study staff becomes aware of new information or research findings that might impact your willingness to participate in this study, you will be given that information. You will be given the opportunity to ask any questions you might have and to decide if you want to continue to participate in the study. You will be informed of any new information that may affect your willingness to participate.

Compensation

For your time and participation in the study you will receive monetary compensation of $125, pro-rated as follows. All compensation will be given to you at the completion of your participation in the study. If you drop out of the study for whatever reason before its completion your compensation will be the following:

Completion of postprandial meal challenge1=$50

Completion of postprandial meal challenge 2=$75 ($125 total)

If your total payments within one calendar year exceed $600, this will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

Injury Statement

Medical care is available in the event of injury resulting from research but neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or the investigators.

Voluntary Participation

Your participation in this study is voluntary; you may decline to answer any questions during the screening process or during the study. Please be aware that refusing to answer a question may keep you from being able to participate in the study. You may withdraw from this study at any time by notifying the investigators or other study personnel. Refusal to take part in or withdrawing from this study will involve no penalty or loss of benefits you would receive otherwise. You may be asked to leave the study at any time if you do not comply with the study protocol.

In the event that abnormal lab test results are obtained during initial screening or subsequently throughout this study, you will be informed as quickly as possible of these results and instructed to contact your private physician for further assessment. The lab test results will be made available to your private physician at your request.

This is to certify that you consent to and give your permission for your participation as a volunteer in the study entitled **"The effect of peanuts and peanut products on glucose control and vascular function".** You certify that you are 18 years of age or older. You will receive a signed copy of this consent form.

_____ _____

Signature of Volunteer Date

_____

Printed Name of Volunteer

I, the undersigned, have defined and explained the study involved to the above volunteer

_____ _____

Signature of Investigator Date

Do we have permission to keep your personal information and contact you about your interest in participating in future studies for Drs. Skulas-Ray and Kris-Etherton?

_____ Yes _____ No _____ Initials

In addition to the main part of the research study, there is an optional part of the research. You can participate in the main part of the research without agreeing to take part in this optional part.

Storage of Leftover Blood Samples for Future Research Studies

As part of this study, we are obtaining blood from you. If you agree, the research team would like to store leftover samples of your blood that is collected so that your blood can be studied in the future after this study is over. These future studies may provide additional information that will be helpful in understanding cardiovascular disease, but it is unlikely that these studies will have a direct benefit to you. Neither your doctor nor you will receive results of these future research tests, nor will the results be put in your health record. If you have any questions, you should contact Drs. Skulas-Ray or Kris-Etherton at 814-863-8622 or 814-863-2923.

Your leftover samples will be labeled with a code number and stored in Drs Skulas-Ray and Kris-Etherton's locked laboratory. If you consent to the collection of samples of your blood for future research, the period for the use of the samples is unknown. If you agree to allow your blood to be kept for future research, you will be free to change your mind at any time. You should contact Drs. Skulas-Ray or Kris-Etherton at 814-863-8622 or 814-863-2923 and let them know you wish to withdraw your permission for your blood to be used for future research. If you do this, any unused blood will be destroyed and not used for future research studies.

You should initial below to indicate your preferences regarding the optional storage of your leftover blood for future research studies.

a. Your samples may be stored and used for future research studies to learn about, prevent, treat or cure cardiovascular disease and obesity and other health problems.

_____ Yes _____ No

b. Your samples may be shared with other investigator/groups without any identifying information.
_____ Yes _____ No

Participant: By signing below, you indicate that you are voluntarily choosing to take part in this optional part of the research.

_____	_____	_____	_____
Signature of Participant	Date	Time	Printed Name

Person Explaining the Research: Your signature below means that you have explained the optional part of the research to the participant/participant representative and have answered any questions he/she has about the research.

_____	_____	_____
Signature of person who explained this optional research	Date	Time

Appendix B

Telephone Screening Form

Telephone Interview Form
PPNUT Study

Date _____
Interviewer_____
Screen ID: ScPPNUT __ __ __
Screening Appt. _____ _____ _____

Before asking any questions, please read the following paragraph to obtain verbal consent to conduct the telephone interview:

"We received your message that you are interested in participating in the 'Peanut Study". I have a brief paragraph that I would like to read to you about the study. After that I can answer any questions you may have about the study. If you are still interested in participating, I will then ask you some questions about your current lifestyle and past medical history.

This study consists of two meal tests designed to evaluate the effects of peanuts on cardiovascular risk factors. For these tests, you will come to the Clinical Research Center (CRC) on the Penn State Campus after an overnight fast. We will measure your blood pressure and perform a test of your artery function called Flow Mediated Dilation (FMD) test. This test is similar to having your blood pressure taken but the cuff stays inflated for a longer time and a sonogram records blood vessel flow. You will then have an IV catheter inserted into your arm. The catheter is a flexible plastic tube through which blood samples will be taken. You will then consume either a control or peanut test shake, comprised mainly of sugar, cream and chocolate syrup, with and without peanuts. Over the next 4 hours 4 blood samples will be collected. Another FMD test will be performed at the 4 hour mark. During this 4 hour period, you will be asked to remain in the CRC. You will not be allowed to consume any food or drinks other than water or caffeine-free, diet soda and diet jell-o during this time. At the end of the testing period, you will be offered a small meal before leaving the clinic.

You will be asked to return to the CRC following a one week washout period to repeat the testing procedure with the alternative test meal.

You should be aware that the compensation for this study ($125) is considered income. Do you have any questions?

"I will now ask you a series of questions about your past medical history and your current lifestyle. If you agree to answer these questions, and it is determined that you meet the criteria for this study, we will schedule you for a screening visit. Are you willing to answer these questions which will take about 15 minutes?"

YES _____ (continue with interview)

NO _____ (thank them for their time and interest)

Upon completion of study the lower portion of this page will be destroyed

1. Please provide:

   Name_____ Date of Birth: _____

   Address _____

   Email_____

   Daytime Phone_____ Evening Phone _____

2. Height (ft and in):_____
   Weight (lbs):_____
   BMI:_____

Age 20 - 50 y	☐Yes	☐ **No**
BMI 28 – 39 kg/m^2	☐Yes	☐ **No**

3. Do you have any food allergies (specifically peanuts, dairy)? ☐ Yes ☐ No

   If yes, please specify foods: _____

4. Have you consumed peanuts and/or peanut products before? ☐ Yes ☐ **No**

   If no, subject is ineligible

5. Do you regularly consume peanuts and/or peanut products? ☐ Yes ☐ No

   If no, why not?

_____

*Evaluate answer for allergy to and/or dislike of peanuts, and exclude if necessary.

6. Do you currently smoke? ☐ **Yes** ☐ No

   If yes, subject is ineligible

   If no, have you been a smoker in the past? ☐ Yes ☐ No

   Explain: _____

7. Do you take any cholesterol, blood pressure or glucose-lowering medication (e.g. Captopril, Lipitor, Zocor, Ezetimibe, Questran, Colestid, Orlistat, insulin, glucophage, benicar)?

If yes, what? _____**If yes, subject is ineligible** ☐ **Yes** ☐ No

8. Do you take any medication prescribed by a doctor? (This includes medications for any diseases, any type of pain medicine, and any drugs for treatment of depression or other mental health problems.)

If yes, please specify the type of medication used, duration of use and reason: ☐ Yes ☐ No

_____

9. Are you taking any OTC cholesterol-lowering substances (e.g.: psyllium, fish oil, soy lecithin, phytoestrogen)?

If yes, what? _____ ☐ Yes ☐ No

If yes, are you willing to discontinue use during the study? ☐ Yes ☐ **No**

10. Do you have a known intolerance (e.g. GI distress) for high fat meals?

If yes, please explain: ☐ **Yes** ☐ No

_____

11. Do you take any medication, nutritional supplement, herb or vitamin **not** prescribed by a doctor?

If yes, please specify the type of medication used, duration of use and reason:

☐ Yes ☐ No

_____

If yes, are you willing to discontinue use during the study? ☐ Yes ☐ **No**

12. Do you have any of the following medical conditions:

a. heart disease ☐ **Yes** ☐ No

b. stroke ☐ **Yes** ☐ No

c. TIA (mini stroke) ☐ **Yes** ☐ No

d. diabetes ☐ **Yes** ☐ No

e. high blood pressure ☐ **Yes** ☐ No

f. renal or kidney disease ☐ **Yes** ☐ No

g. rheumatoid arthritis ☐ **Yes** ☐ No

h. gastrointestinal disease (e.g. Crohn's disease, irritable bowel syndrome, ulcer or history of bowel surgery, lactose intolerance) ☐ **Yes** ☐ No

i. blood clotting disorder ☐ **Yes** ☐ No

j. liver disease or cirrhosis ☐ **Yes** ☐ No

k. any condition that requires the use of steroids ☐ **Yes** ☐ No

l. gout (requiring treatment) ☐ Yes ☐ No

m. anemia (or sickle cell anemia) ☐ Yes ☐ No

n. lung disease (such as bronchitis, emphysema, asthma) ☐ Yes ☐ No

o. cancer within the last 10 years ☐ Yes ☐ No

p. thyroid disease (okay if controlled with meds) ☐ Yes ☐ No

q. Problems with immune system (hepatitis, AIDS) ☐ **Yes** ☐ No

r. Peripheral vascular disease or circulation problems such as Reynaud's ☐ **Yes** ☐ No

s. any other medical condition not specified in this list ☐ Yes ☐ No
Please specify _____

Explain any "yes" answers:

_____

13. Do you donate blood? ☐ Yes ☐ No

If yes, date of last donation? _____

Are you okay with not donating for the course of the study? ☐ Yes ☐ **No**

14. Are you currently trying to lose weight or training for any type of athletic competition? ☐ Yes

☐ No

If yes, please specify: _____

15. Are you allergic to latex? ☐ Yes ☐ No

16. Do you have any food restrictions related to religious practices? Or are there any foods you refuse to eat?

If yes, please specify: ☐ Yes ☐ No

_____

17. Are you willing to avoid alcohol consumption for the 48 hrs prior to each test visit? ☐ Yes

☐ **No**

SUBJECT MAY BE CONSIDERED ELIGIBLE FOR THE STUDY IF NO BOLDED RESPONSES ARE CHECKED

☐ Yes, subject eligible; **schedule visit to GCRC** Date of screening: _____

☐ No, subject is not eligible – Reason: _____

If not eligible, would subject like to be contacted regarding future research studies?

☐ Yes ☐ No

Give the subject directions (including parking instructions) to the Clinical Research Center in Noll Lab (also known as the Elmore Research Wing) on the PSU campus. Subjects should enter the CRC through the door that faces Atherton Street, and continue to the 2nd floor nurse's station where they will meet the research staff. If parking in CRC clinic space, instruct subject to put on their flashers and collect a parking permit from the 2nd floor nurses' station. They should place the permit on their mirror and turn off their flashers. The first visit will take approximately 1 hour to complete all of the paperwork and testing. Remind subjects to drink plenty of water two days prior to their visit as this will facilitate blood sampling.

BIBLIOGRAPHY

Adams MR, et al. Oral L-arginine improves endothelium-dependent dilation and reduces monocyte adhesion to endothelial cells in young men with coronary artery disease. *Atherosclerosis*. 1997; 129(2): 261-269.

Alper CM and Mattes RD. Peanut consumption improves indices of cardiovascular disease risk in healthy adults. *Journal of the American College of Nutrition.* 2003; 22(2): 133-141.

Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR. Health benefits of nuts: potential role of antioxidants. *British Journal of Nutrition.* 2006; 92: S52-S60.

Bode-Böger SM, et al. L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. *Clinical Science (London).* 1997; 87(3): 303-310.

Boden G and Laakso M. Lipids and glucose in type 2 diabetes: what is the cause and effect? *Diabetes Care*. 2004; 27(9): 2253-2259.

Böger RH, Bode-Böger SM, Frölich JC. The L-arginine-nitric oxide pathway: role in atherosclerosis and therapeutic implications. *Atherosclerosis.* 1996; 127: 1-11.

Bradamante S, Barenghi L, Villa A. Cardiovascular protective effects of resveratrol. *Cardiovascular Drug Reviews.* 2004; 22(3): 169-188.

Brown AA and Hu FB. Dietary modulation of endothelial function: implications for cardiovascular disease. *The American Journal of Clinical Nutrition.* 2001; 73(4): 673-686.

Centers for Disease Control and Prevention. Mortality 2013. National Vital Statistics System. 12/31/2014. http://www.cdc.gov/nchs/data/dvs/LCWK9_2013.pdf

Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2014. http://www.cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web.pdf

Chiang J et al. Homocysteine induces smooth muscle cell proliferation through differential regulation of cyclins A and D1 expression. *Journal of Cellular Physiology.* 2011; 226: 1017-1026.

Cohen JC, Noakes TD, Benade AJ. Serum triglyceride responses to fatty meals: effects of meal fat content. *The American Journal of Clinical Nutrition*. 1988; 47: 825-827.

Conaway DG, O'Keefe JH, Reid KJ, Spertus J. Frequency of undiagnosed diabetes mellitus in patients with acute coronary syndrome. *The American Journal of Cardiology.* 2005; 96(3): 363-365.

Heidenreich, PA et al. Forecasting the future of cardiovascular disease in the United States. *Circulation.* 2011; 123: 933-944.

Homko CJ, Cheung P, Boden G. Effects of free fatty acids on glucose uptake and utilization in healthy women. *Diabetes.* 2003; 52(2): 487-491.

Hu FB, et al. Frequent nut consumption and risk of coronary heart disease in women: prospective cohort study. *The BMJ.* 1998; 317: 1341.

Jakulj F, et al. A high-fat meal increases cardiovascular activity to psychological stress in healthy young adults. *The Journal of Nutrition*. 2007; 137(4): 935-939.

Jiang R, et al. Nut and seed consumption and inflammatory markers in the multi-ethnic study of atherosclerosis. *American Journal of Epidemiology.* 2006; 163(3): 222-231.

Kris-Etherton PM, et al. Peanuts and peanut products: effects of glycemic control and vascular health. PSU Collaborate Research Initiative with the Peanut Institute. 2011.

Kris-Etherton PM, Hu FB, Ros E, Sabate J. The role of tree nuts and peanuts in the prevention of coronary heart disease: multiple potential mechanisms. *The Journal of Nutrition.* 2008; 138: 1746S-1751S.

Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. *Archives of Internal Medicine.* 2004; 164(19): 2147-2155.

O'Keefe JH, Gheewala NM, O'Keefe JO. Dietary strategies for improving post-prandial glucose, lipids, inflammation, and cardiovascular health. *Journal of the American College of Cardiology.* 2008; 51(3): 249-255.

Ozcan M and Seven S. Physical and chemical analysis and fatty acid composition of peanut, and peanut butter from COM and NC-7 cultivars. *Fats and Oils*. 2003; 54(1): 12-18.

Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*. 1988; 333: 664-666.

Pendurthi UR, Williams JT, Rao VM. Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 1999; 19: 419-426.

Raitakari OT and Celermajer DS. Flow-mediated dilation. *British Journal of Clinical Pharmacology.* 2000; 50(5): 397-404.

Ros E and Mataix J. Fatty acid composition of nuts – implications for cardiovascular health. *British Journal of Nutrition.* 2006; 96: S29-S35.

Sies H, Stahl W, Sevanian A. Nutritional, dietary, and postprandial oxidative stress. *The Journal of Nutrition.* 2005; 135(5): 969-972.

Silver AE and Vita JA. Shear stress – mediated arterial remodeling in atherosclerosis. *Circulation*. 2006; 113: 2787-2789.

Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. *The American Journal of Cardiology.* 1997; 79(3): 350-354.

Widlansky ME, Gokse N, Keaney JF, Vita JA. The clinical implications of endothelial dysfunction. *Journal of the American College of Cardiology*. 2003; 42(7): 1149-1160.

ACADEMIC VITA

Rachel M. Gabauer

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Education

The Pennsylvania State University - Schreyer Honors College
Bachelors of Science in Biology and Nutritional Sciences — August 2011-May 2015

Montour High School — McKees Rocks, PA
• Salutatorian; Class Rank: 2/245 — 2007-2011

Relevant Experience

The Pennsylvania State University, Department of Nutritional Sciences — University Park, PA
Research Lab Assistant — Spring 2013-Present

• Helped run a clinical study testing the effects of peanuts on risk factors for cardiovascular disease
• Recruited and scheduled participants; managed postprandial test visits; collected and centrifuged blood
• Analyzed lab results and flow-mediated dilation video results

The University of Konstanz, Psychology Department — Konstanz, Germany
Research Intern — May 2014-August 2014

• Assisted with study testing reactance theory involving both restrictive and suggestive health-related messages
• Recruited participants; set up and completed testing visits with participants in German
• Performed literature reviews on relevant research and presented findings at weekly lab meetings

Ohio Valley General Hospital — McKees Rocks, PA
Intern with Inpatient Registered Dietitian — Summer 2012

• Assisted Sara Parr, MS, RD, LDN with gathering information on patients who needed nutritional support
• Visited patient rooms and took notes on their testimonies; completed Nutritional Assessment forms and PES Statements
• Surveyed patients on food and staff management to complete monthly “meal rounds”

Ohio Valley General Hospital McKees Rocks, PA
Nutrition Department Volunteer Summer 2010

• Shadowed Rachel Kingsley RD, LDN, Certified Diabetes Educator and Program Coordinator of "Living Well with Diabetes"
• Observed outpatient nutritional consultations and cardiac rehabilitation; prepared for and observed diabetes education classes.
• Assisted with clerical work and filing

Cafe Laura University Park, PA
Food Service Management Course August-December 2014

• Acted as employee and assisted with cooking, serving the food, and cleaning the kitchen and facility
• Acted as manager and supervised staff, created menus and recipes, and calculated food cost and production estimates

Honors and Awards

• Schreyer Scholar Award
• American Heart Association Grant: Summer Translation Cardiovascular Science Institute (STCSI) Research Program - 2013
• Lola G. Duff and William H. Duff, II Scholarship Award
• Dean's List every semester
• ServSafe Certification in Food Safety – 2013

Extracurricular Activities

• Global Medical Brigades: Medical Service Trip to Panama in Spring 2014
• Penn State IFC/Panhellenic Dance Marathon Pediatric Cancer: Morale Committee, Atlas Merchandise Captain – 2011-2014
• SHOtime (Schreyer Honors College) Freshmen Orientation Mentor – 2012
• Penn State Indoor Tennis Club – 2011, 2012