Effect of California mission fig consumption on levels of c-reactive protein and antioxidant levels

Rasha Abdrabou

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EFFECT OF CALIFORNIA MISSION FIG CONSUMPTION ON LEVELS OF C-REACTIVE PROTEIN AND ANTIOXIDANT LEVELS

By

Rasha Abdrabou

A Dissertation in Partial Fulfillment of the Requirements for the Degree of Doctor of Public Health in Preventive Care

August, 2009
Each person whose signature appears below certifies that this dissertation, in his/her opinion, is adequate in the scope and quality as a dissertation for the degree of Doctor of Public Health.

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ABSTRACT OF THE DISSERTATION

Effect of California Mission Fig Consumption on Level of C-Reactive Protein and Antioxidant Levels

By

Rasha Abdrabou

Doctor of Public Health Candidate in Preventive Care

Loma Linda University, 2009

Serena Tonstad, MD, MPH, PhD, Chair

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Polyphenols exert anti-inflammatory and antioxidant effects. We investigated the effect of consumption of polyphenol-rich figs on high sensitivity C-reactive protein (CRP) and antioxidant levels in hyperlipidemic subjects. The purpose of this study was to examine the effect of fig consumption on C-reactive protein and antioxidant levels. Analyses were carried out on collected data from September to December of 2008. Using a crossover design, one hundred and two participants were recruited and randomized into an intervention phase (eating figs) and a control phase (not eating figs) for 5 weeks. Blood samples were drawn from each group at the 4th and 5th week of each phase, centrifuged and frozen. Frozen samples were sent to the University of California Davis Clinical Laboratories for CRP analysis. Antioxidant analysis was done on frozen samples in the Nutrition Laboratory at Loma Linda University via a test and retest method for accuracy using the ferric reducing antioxidant power (FRAP) and total antioxidant capacity (ABTS) tests. Data was analyzed using SPSS for paired t-test analysis for CRP,
antioxidant tests and dietary recalls. Mean CRP values did not differ significantly in the intervention phase when compared to control phase (median [interquartile range] 1[0.5, 2.1] mg/L versus 1 [0.6, 2.1]; p=0.31). The FRAP test results did not differ between study phases (0.1± 0.1 mmol versus 0.1± 0.1 mmol; p = 0.96). The ABTS test concentrations likewise did not differ between the two phases (0.2± 0.04 mmol versus 0.2± 0.04 mmol; p= 0.97). An analysis of dietary intake showed participants consumed approximately 187 calories more in the intervention phase (p<0.0001) while consuming 4% less calories from fat (p<0.0001). For men and women with elevated cholesterol levels, fig consumption did not lower CRP or affect antioxidant levels. While overall intake of iron, magnesium, potassium and copper increased significantly, fat and protein intake decreased slightly despite higher caloric intake.
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CHAPTER 1
INTRODUCTION

A. Statement of the Problem

Cardiovascular disease (CVD) has been the leading cause of death in both women and men since the year 1900, with the exception of the 1918 influenza epidemic (American Heart Association [AHA], 2007). Cardiovascular disease is a group of conditions that include heart disease, stroke, high blood pressure, heart failure and several others and in 2004 was identified as the underlying cause of death in 869,724 people (AHA, 2007). When considered separately from other CVD risk factors, such as diabetes and hypertension, coronary artery disease was the leading cause of death, claiming 451,326 lives, while stroke was the third leading cause of death nationally with 150,074 deaths in 2004 (AHA, 2007).

Although the number of deaths to CVD were lower in 2004 than 2003 and have been declining since 1999, overall the numbers are still staggering and the risk factors that cause the disease are on the rise (AHA, 2007). These risk factors, which are mostly lifestyle related and can drastically alter risk if modified, include high blood pressure, high cholesterol, diabetes, smoking, physical inactivity, and obesity. According to the Centers for Disease Control and Prevention (CDC), 37% of adults have at least two of these risk factors (CDC, 2003). Rates of overweight, for example, are on the rise in both adults and children and have been rising for several decades (AHA, 2007). Overweight in adults is defined as a BMI range of 25-29.9 kg/m² while obesity is defined as a BMI value of 30 kg/m² and greater (CDC, 2009). Overweight and obesity in children are
classified according to a growth chart and a percentile range. Children in the 85th percentile and less than the 95th percentile are considered overweight while those in the 95th and higher percentile are considered obese. According to recent statistics, 66% of adults are overweight, while 31.4% of children are obese (AHA, 2007). Among children and adolescents, 12-19 years of age, 17% are overweight, while the percentage of overweight among those between ages 6-11 and 2-5 are 17.5% and 14%, respectively. A staggering 90% of middle aged Americans will develop hypertension in their lifetime, of whom 65% will not be able to keep it within normal ranges. CVD also includes some un-modifiable risk factors, such as age, gender, family health history, and race (AHA, 2007). The damage caused by CVD extends beyond human loss to healthcare cost. Cost for heart disease and stroke is expected to exceed $448.5 billion in 2008, of which $152.1 billion alone is due to loss of productivity (Centers for Disease Control [CDC], 2008). Hypertension treatment spending reached $69.4 billion in 2008, while among Medicare beneficiaries cost of hospitalization reached $29 billion in 2001 (CDC, 2008).

These risk factors, although diverse, have a common effect on the endothelial function of the heart (Vita, 2005). Endothelial health plays a key role in regulating the development of atherosclerosis, which is the underlying cause of all heart disease (Vita, Keaney, 2002). Atherosclerosis, a chronic inflammatory disease characterized by plaque formation in medium sized arteries, can be present for years before it manifests itself in a variety of CVD forms, such as MI or even sudden cardiac death (Ross, 1999). According to Ross (1999), these cardiac events usually occur when plaque is exposed to flowing blood as a result of a ruptured artery. Even though the exact mechanism of action is not well understood, local inflammation within the plaque is considered an important factor
Plaque formation and eventual development of CVD affect the homeostatic balance of the vascular system by producing factors that act on the vessel wall, as well as the lumen. Other endothelium derived products that affect vascular homeostasis are fibrinolytic factors, substances that influence vascular tone, as well as factors that affect coagulation and proinflammatory factors, to name a few (Widlansky, Gokce, Keaney, Vita, 2003). These observations lead to the conclusion that the state of the endothelium is directly related to vascular health and thus is implicated in the development of CVD. Therefore, maintaining a healthy endothelium will have a great impact not only on heart disease rates, but will indirectly impact healthcare costs.

The environment, genetics, and diet are all factors affecting the extent of the influence CVD risk factors have on endothelial function (Leeson, Hingorani, Mullen, et al., 2002). According to the American Heart Association, CVD risk reduction requires maintaining an ideal body weight, monitoring lipid levels, and maintaining an exercise program for 30-60 minutes on most days of the week. Proper nutrition is also vital in achieving ideal weight, but obesity rates nationwide reflect a grim picture of our ability to do so. Data from the 2005 Behavioral Risk Factor Surveillance System (BRFSS) established that fewer than one in three U.S adults consume fruit two or more times per day and only 27.2% ate vegetables three or more times per day (CDC, 2007). The USDA guidelines for consumption of fruits and vegetables for adults recommend the equivalent of 2 cups of fruit and 2 and a half cups of vegetables per day (USDA, 2005). Despite the well documented benefits of eating fruits and vegetables and the association with decreased risk of chronic disease (USDA, 2005), Americans are still reluctant to increase their intake of these nutrient-dense foods. In 1999, only 17% of 15,000 Americans
surveyed from different stages of life ate the recommended servings (Thompson, et al., 1999).

Although benefits of nutrient-dense fruits and vegetables have been credited largely to their high content of vitamins and beta carotene, recent studies have attributed greater benefits to their phenol antioxidant content, both monophenols and polyphenols (Yusuf, Dagenais, Pogue, Bosch, & Sleight, 2000; Ascherio, et al., 1999). Apples, for example, have high levels of phenols in the form of flavonoids, and according to a long term Finnish study conducted on 10,000 men and women, were linked to a decrease in the risk of lung cancer in that population (Knekt, Jarvinen, Seppanen, & Hellovaara, 1997). Flavonoids and other phenols, such as proanthocyanidins and phenolic acids, have been shown to have anticancer effects (Seeram, 2008), as well as being protective for heart disease (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993) and stroke (Keli, Hertog, Feskens, & Kromhout, 1996). Berries, also rich in polyphenols, also have been shown to have anticancer effects (Seeram, 2008).

The significance of the effect of phenol oxidants on decreasing the risk of several chronic and lifestyle diseases makes them a worthy candidate for further studies. Polyphenols are not only the most abundantly available antioxidants, they also can be ingested in higher amounts (Scalbert, Johnson, & Saltmarsh, 2005). Intake can be as high as 1g/d, an amount 10 times higher than the intake of vitamin C (Scalbert, Johnson, & Saltmarsh, 2005), for example. Flavonoid consumption from tea, apples and onions among 805 elderly men from the Zutphen Elderly study yielded a 68% decrease in cardiovascular risk among those consuming more than 29mg/d (Hertog et al., 1993). Another study assessed the relationship between consumption of tea and coffee and the
incidence of myocardial infarctions among participants of the Boston Area Health Study (Sesso et al., 1999). Among those who drank more than one cup of tea a day, there was a 44% reduction in cardiovascular events (Sesso et al., 1999). Polyphenols also demonstrated benefits among individuals with established cardiovascular disease. The Myocardial Infarction Onset study demonstrated that moderate and heavy tea drinkers reduced cardiovascular risk by 31% and 39% respectively, after adjusting for other risk factors (Mukamal, Maclure, Muller, Sherwood, Mittleman, 2002). The study included 1900 participants who were hospitalized for acute myocardial infarctions (Mukamal, Maclure, Muller, Sherwood, Mittleman, 2002).

B. Purpose of the Study

Since costs associated with cardiovascular disease extends beyond the material loss to the devastating loss in human life, finding a treatment that is cost effective and available to most individuals is most essential. Oxidative stress and inflammation are two processes implicated in the development of CVD. Though not all research has been supportive of their effect or clear regarding the action pathway, enough studies have established a relationship to warrant further investigation into the matter.

Inflammation is the process by which the body signals the presence of injury or infection. Factors such as cigarette smoking, hypertension and obesity all signal a release of chemicals, such as cytokines, and the activation of the inflammatory process that eventually leads to the formation of plaque and blood clots (AHA, 2007). Inflammation then manifests itself through production of markers such as C-reactive protein (CRP; Patrick & Uzick, 2001). Inflammation plays a critical role in the development of atherosclerosis through the accumulation of monocytes and T-cells in the endothelium of
the vessels that eventually proliferate and differentiate, forming lesions and fibrous caps (Van Dyke, & Kornman, 2008).

Patients with immune-inflammatory diseases have been shown to have a higher risk of mortality and morbidity due to cardiovascular disease (Wilson, 2008). This higher risk was not found to be associated with a higher rate of traditional risk factors such as hypertension and hyperlipidemia, but to nontraditional ones such as levels of inflammatory markers (Wilson, 2008). Evidence in the current literature support the use of CRP as a tool in the assessment of CVD (Patrick & Uzick, 2001) as a part of a group of markers that researchers are shifting towards assessing to improve global cardiovascular risk prediction (Torres & Ridker, 2003). CRP has been shown to predict future risk of cardiovascular disease at a wide range of values of low-density lipoprotein cholesterol, Framingham Risk Score and metabolic syndrome and even among healthy women and men, CRP enabled the stratification of individuals into low, moderate and high risk of CVD (Torres, Ridker, 2003).

A study conducted in Denmark among individuals without major CVD at baseline demonstrated that CRP is significantly related to the risk of developing CVD later in life (Jeppesen, et al. 2008). Based on a study conducted in China, Li and Fang (2004) concluded that CRP can no longer be thought of as playing a passive role in CVD prediction, but that it is a direct cause of CVD and that treatments reducing CRP should be used for both primary and secondary prevention of the disease. This conclusion was due to evidence presented through data showing that monocytes increased production of interleukin-6 (IL-6) in response to CRP, which was inhibited by the ingestion of a statin drug.
In another study conducted on 17,802 men and women with normal blood lipid levels and elevated CRP, ingestion of rosvastatin reduced CRP levels and in turn significantly decreased the occurrence of cardiovascular events (Ridker, 2008). From the evidence provided by the literature, it is clear that CRP is a strong predictor of heart disease among healthy individuals and that it should serve as a global assessment tool to prevent future incidence of CVD. Statins were not the only interventions researched by the scientific community for their effect on CRP levels. Studies examining the effect of dark chocolate rich in flavnols, a subclass of polyphenols, versus white chocolate without flavnols on blood pressure and insulin sensitivity found an improvement in both measurements in healthy individuals (Grassi, et al., 2005). Polyphenol-rich wine has long been thought of as heart protective. According to recent studies, polyphenols in wine stimulate eNOS production, a heart-protective enzyme (Wallerath, 2005). In a literature review of epidemiologic studies on the health benefits of polyphenols and their effect on cardiovascular function, researchers concluded that though there were inconsistencies in the human data reported, the studies still showed a reduction in inflammatory markers when foods rich in polyphenols were ingested (Vermuri, Kelley, & Erikson, 2008). Cherries, for example, when ingested by healthy participants for 28 days yielded a 25% decrease in CRP levels (Kelley, Rasooly, Jacob, & Kader, 2006).

In a study conducted in Scranton, Pennsylvania, researchers sought to determine the amount and quality of phenol antioxidants in dried fruit compared with fresh fruit (Vinson, Zubik, Bose, Samman, & Proch, 2005). As a dried, concentrated form of the fruit, researchers sought to investigate how the quality, as well as the quantity, of phenol antioxidants would differ between the dried and fresh apricots, cranberries, dates, figs,
raisins and prunes (Vinson et al., 2005). The analysis of the data showed that dates had the highest concentration of polyphenols, while figs and dried plums had the best nutrient score among the dried fruit, as well as a higher quality of antioxidants (Vinson et al., 2005).

Among the dried fruit tested, figs were shown to enrich lipoprotein plasma and protect against oxidation for up to 4 hours after consumption (Vinson, et al., 2005). Figs were also shown to overcome inflammation caused by consuming a carbonated beverage, and when ingested, figs are in vivo antioxidants (Vinson, et al., 2005). Thus figs, with their higher quality phenols, are the most suitable fruit to use for further study of the effect of phenol oxidants on inflammatory processes in the human body.

C. Research Questions

- What is the effect of fig consumption on CRP levels in the body?
- What is the effect of fig consumption on antioxidant levels in the body?

D. Theoretical Justification

Polyphenols exert both an anti-inflammatory and an anti-oxidant effect on the body that ultimately affect risk of cardiovascular disease. One mechanism of action for the antioxidant effect of polyphenols is thought to be through a decrease in oxidized LDL levels. The anti-inflammatory effect of polyphenols has not been studied extensively and therefore an established mechanism of action is not available for use. The hypothesized connection is based on available experimental data only. Figs are a rich source of polyphenols and are easily available to the public. If figs indeed affect levels of inflammation as well as oxidative stress in the body then they would have important treatment as well as prevention implications.
The method employed to assess the effect of polyphenol-rich figs on oxidative stress and inflammatory processes in the body was a randomized crossover design. The randomization eliminates any calendar time effect as well as confounding between the groups. The crossover design allows for repeated testing on the individual in both conditions (eating and not eating figs) with each participant acting as their own control, thus requiring a smaller sample size to achieve an adequate level of precision. Multiple measurements on the same individual produces results that may vary but the variation found within an individual’s measurements is less than that found between individuals in a group. The minimized variability allows for a more precise comparison of differences.

Antioxidant levels will be assessed using two methods to ensure accuracy and consistency of results in the Nutrition Laboratory at Loma Linda University. The ferric reducing antioxidant power (FRAP) test is highly reliable, fairly inexpensive and easy to conduct (Benzie & Strain, 1999). The total antioxidant (ABTS) test is also fairly inexpensive, fast and highly reliable (Re et al., 1999). Reagents involved in both tests are easy to prepare. C-reactive protein was tested in laboratories at the University of California at Davis.

E. Significance to Preventive Care

Initially, inflammation is a transitory condition, but often it becomes chronic leaving the body vulnerable to a host of diseases and conditions, such as diabetes, heart disease and much more. Measuring inflammation in the body will help identify those who are at risk for developing disease, which will serve as an integral part of prevention. Identifying natural foods that can affect inflammation levels in the body is vital for a preventive care practitioner in that it will be an effective and simple tool to ward off
disease. Polyphenols also have an antioxidant effect in which they have shown to reduce oxidative stress in the body and provide vascular protection (Stoclet, et al., 2004). Figs are inexpensive to grow and are available worldwide. If figs indeed decrease inflammation and oxidative stress in the body, they will provide a simple and more cost effective treatment for many diseases at the same time. Rather than addressing conditions such as diabetes, heart disease, and Alzheimer's individually, figs can be a way to treat several at the same time. In the field of prevention, figs reducing inflammatory marker levels in the body, as well as the damages caused by oxidative stress could be a valuable assessor for lifestyle diseases that would ultimately result in reducing health care costs and rates of cardiovascular disease. This has international implications as well, since figs can be grown in a variety of climates, making them accessible to many developing, as well as developed, countries.
CHAPTER 2
LITERATURE REVIEW

A. Overview

1. The Epidemic of Cardiovascular Disease (CVD)

Cardiovascular disease, which includes coronary heart disease (CHD), stroke, and peripheral artery disease, claims the lives of approximately 700,000 Americans every year, therefore accounting for almost 29% of all deaths (CDC, 2008). Coronary heart disease is the most prevalent cardiovascular disease, followed by stroke (CDC, 2008). The American Heart Association’s goal of reducing death rates from these two leading cardiovascular diseases by 25% by the year 2010 is proving to be attainable. Although much of the progress made can be attributed to advances made in medicinal therapies (Capewell, Beaglehole, Seddon, & McMurray, 2000), the progress achieved in decreasing the rates of disease is diminished by the fact that the underlying risk factors for CVD are still on the rise (AHA, 2007).

2. Establishing the Risk Factors for CVD

Three major observational studies conducted over a prolonged period of time sought to determine the risk factors that lead to CVD. These studies include the Framingham Study, the Multiple Risk Factor Intervention Trial (MRFIT), and the Chicago Heart Association Detection Project in Industry. The Framingham Study assessed the lifetime risk of developing CVD in a sample of approximately 7,733 participants that were initially free from disease and ranged in age from 40-94 years (Lloyd-Jones, Larson, Beiser, & Levy, 1999). The Chicago Heart Association Detection
Project in Industry followed a sample of 38,642 participants, all of whom were working adults ages 18 to 59, and the Multiple Risk Factor Intervention Trial (MRFIT) consisted of 347,978 men ages 35 to 57 years and a population-based sample of 3,295 men and women ages 34 to 59 years (Greenland, et al., 2003). Collectively these three longitudinal studies included 380,000 participants, of whom 21,000 participants died as a result of CVD (Greenland, et al., 2003). Follow-up lasted 21 to 30 years across the studies. Risk factors for CVD were determined to be total cholesterol (≥240 mg/dL; ≥6.22 mmol/L), systolic blood pressure (≥140 mmHg), diastolic blood pressure (≥90 mmHg), smoking, and diabetes. Excluding smoking, all other risk factors identified can be directly impacted by nutrition.

The worldwide INTERHEART study, which included participants from 52 countries, identified nine modifiable risk factors that accounted for 90% of all MI events. These nine risk factors included smoking, dyslipidemia, hypertension, diabetes, abdominal obesity, psychosocial factors, daily consumption of fruits and vegetables, regular alcohol consumption, and regular physical activity (Yusuf, et al., 2004). In another report released by the American Heart Association, age, gender, family history, obesity and physical inactivity were all determined by other studies also to be risk factors for CVD. Although most of the risk factors that cause CVD can be modified through proper dieting and exercise alone, a few, such as family history, age and gender, require the addition of medicinal therapies to effect change.

Cardiovascular risk factors affect both genders, but at different rates and vary by age. For example, incidence of heart disease is 50% higher in diabetic women than in diabetic men (Huxley, Barzi, & Woodward, 2006). More women than men who have
CVD have a family history of the disease (Sullivan, et al., 1994). Incidence of heart disease was also shown to be greater among the offspring of the Framingham Study participants when at least one of the parents had a history of heart disease (Lloyd-Jones, et al., 2004). Smoking also poses a greater risk for women than men, having been linked to approximately half of all coronary events occurring in this group (Rich-Edwards, Manson, Hennekens, & Buring, 1995). Hypertension is a major risk factor for developing heart disease, and according to the Chicago Heart Association Detection Project in Industry study, young adult males’ elevated blood pressure readings were significantly related to death from a coronary incident (Miura, et al., 2001).

According to the National Health and Nutrition Examination Survey, (NHANES) obesity rates have increased 15-30% between the years 1960 and 2000 (Gregg, et al., 2005). Weight plays a significant role in controlling blood pressure and lipid levels. Excess weight has been shown to promote atherogenic risk factors such as hyperlipidemia, and physical inactivity worsens the outcome while speeding the disease process (Hung, Whitford, Parsons, & Hillman, 1990). Men who began a vigorous or moderate exercise program resulted in decreased rates of mortality due to heart disease (Paffenbarger, et al., 1993).

3. **C-reactive Protein and CVD**

C-reactive protein (CRP), an inflammatory marker, has been implicated in the development of heart disease and has been used as a tool to predict the occurrence of a first myocardial infarction (MI), ischemic attack or peripheral arterial disease in healthy men and women (Ridker, Glynn, & Hennekens, 1998). Levels of CRP are affected by many factors including disease, exercise, and weight (Libby, Bonow, Mann, Zipes
Individuals with CRP levels less than or equal to 1.0mg/L are considered low risk for developing CVD, while those with levels between 2.0mg/L and 3.0mg/L are at moderate risk and those with levels greater than 3.0mg/L are considered high risk (Libby, Bonow, Mann, Zipes, 2007). According to Ridker et al., (1998), results from the Physician’s Health Study demonstrated the importance of C-reactive protein levels in predicting incidence of heart attacks. In the 9 year follow-up study, baseline levels of CRP were measured for a sample of 245 participants who developed a first MI and 372 participants who never developed the disease. Those who had elevated levels of CRP at baseline had a higher risk of developing an MI. In another study conducted in Augsburg, Germany, Koenig and colleagues (1999) examined the association between CRP levels and the incidence of a first major CVD event in 936 men ages 45 to 64 who were randomly selected from the general population. Data for the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) survey was collected from 1984 to 1985 and the participants followed for 8 years. The investigators concluded that there was a statistically significant positive correlation between CRP values and CVD incidence in the population.

The Framingham Score provides a way to assess cardiovascular disease risk together with borderline values of blood pressure, pre-diabetes, and lipid levels (Vasan, et al., 2005). CRP alone or in combination with any risk factors was found to be a better predictor of cardiovascular events (Kervinen et al., 2001). In the Helsinki Heart Study conducted in Finland, Kervinen et al. (2001) measured baseline and pre-cardiac event CRP levels in men with elevated lipid levels who eventually developed heart disease.
during the 8.5 year follow-up. Results showed higher CRP levels in those who developed the disease versus those in the control group.

4. Polyphenols

Despite making strides in understanding risk factors for CVD, practical change has been slow to occur, which can be attributed mainly to the fact that modification of most of the risk factors requires a major commitment to behavior modification. Nutrition plays an important role in the etiology of CVD since it is implicated in most of the risk factors that cause a myriad of cardiac diseases. The scientific community has made many advances in understanding the importance of nutrition not only in the treatment, but the prevention of CVD (Kris-Etherton & Keen, 2002).

Moving from examining macronutrients and whole foods, scientists are now making advances in examining the effects of the different bioactive factors in foods and how they affect health (Kris-Etherton et al., 2002). Polyphenols, which are natural compounds abundant in plant foods, have received a lot of attention of late. According to a report published by Ray Sahelian, MD (2008) on the health benefits of polyphenols, they are large molecules that are widely distributed in food. Polyphenols are ingested in greater amounts than other substances with similar effects, with consumption being usually around 1g per day. Vitamin C and E’s intake, for example, is often at about 100mg per day.

Rich sources of polyphenols in the American diet include onions, cocoa, tea, apples, soy and red wine. Polyphenols are classified into two main groups: phenolic acids and flavonoids. Phenolic acids account for about a third of all ingested
polyphenols, while flavonoids account for the remaining two thirds. Phenolic acids are widely distributed in plants as simple molecules, such as caffeic acid, which is available abundantly in coffee. Other simple molecules within the phenolic acid group include vanillin and coumaric acid, as well as widely distributed groups such as hydroxybenzoic acid, which occurs mainly as glucosides and hydroxycinnamic acids that most often occur as simple esters.

Flavanoids, which are the most widely distributed form of polyphenols available in nature, consist of several subgroups. Anthocyanins, a subgroup of flavonoids, are a group of large molecules that give different fruits and vegetables a variety of red to purple pigmentations. Grapes and especially berries have a high content of anthocyanins, while proanthocyanidins are found in grapes, red wine, and pine bark. Another subgroup of flavonoids are flavonols or catechins, which are abundantly found in tea, as well as grape seeds and cocoa. Broccoli has the highest concentration of flavonols, but they are also available in onions, apples, red wine and tea in high concentrations. Isoflavones are available in soy and have been shown to mimic the hormone estrogen on post menopausal women. Each of the above mentioned subgroups has many other phytochemicals within them, which is a testament to the wide distribution of each of the polyphenols in plant foods.

B. Antioxidant Effect of Polyphenols

1. Role of Oxidative Stress in Cardiovascular Disease

Several studies have examined the effect of polyphenols on the incidence of cardiovascular disease through an antioxidant pathway. Oxidative stress has been shown to play a critical role in causing endothelial dysfunction, which leads to
atherosclerosis (Davignon & Ganz, 2004). According to Steinberg, Parthasarathy, Carew, Khoo and Witztum (1989), high plasma levels of low density lipoproteins (LDL) accumulate into the arterial intima which are then oxidized, forming fatty streaks that result in endothelial injury. The injury to the endothelium causes adherence of blood platelets and the eventual release of platelet-derived growth factor, which together with other growth factors causes cell proliferation and the formation of lesions.

2. Antioxidant Effect of Vitamin C

Antioxidants protect against the oxidization of LDL in the vascular cells (Steinberg et al., 1989). A few substances have been identified as antioxidants, and previous studies have linked an increase in antioxidant levels to a decrease in the risk of developing CVD, but the mechanism of action is not yet understood. Vitamin C, for example, has been shown to reverse oxidative stress induced by smoking (Harats, et al., 1990). In a study conducted by Weber, Erl, Weber, and Weber (1996), plasma levels of vitamin C were decreased among smokers while such a difference did not exist with retinol, vitamin E or beta carotene levels. Supplementation with vitamin C restored normal plasma concentrations and led to a decrease in monocyte adhesion with the endothelium. The NHANES study found a 34 percent decreased mortality rate among 11,500 American adults consuming 50mg or more of vitamin C a day than those consuming less than that amount (Enstrom, Kanim, & Klein, 1992). Unfortunately, not all studies concluded positively on the impact of vitamin C on CVD risk reduction, and some studies did not find any significant benefit of the vitamin on the disease. Rimm et al. (1993) studied 39,910 male health professionals who ranged in age from 40-75 years and who were free of diagnosed heart disease, diabetes and hyperlipidemia to assess the
impact of vitamin C intake on the development of heart disease. After four years of follow-up, it was concluded that a high intake of vitamin C was not associated with a lower risk of CVD. Similarly, a 20 year study by Gale et al., found that though vitamin C levels were associated with risk of death from stroke, it was not associated with a risk of mortality from heart disease.

3. **Antioxidant Effect of Vitamin E**

Studies with vitamin E supplementation yielded similar conflicting results as with vitamin C. Oxidation of LDL varies according to the density of the species and subspecies involved. Small dense LDL species are more susceptible to becoming oxidized than large subspecies and thus are more deeply implicated in the atherosclerotic process (Tribble, Holl, Wood, & Krauss, 1992). This difference was attributed to the lower antioxidant content of the smaller more dense subspecies of LDL, specifically of alpha–tocopherol levels (Tribble, Thiel, van den Berg, & Krauss, 1995). Tribble et al. found that in the presence of vitamin C, alpha-tocopherol depletion slowed down from small LDL and became more rapid in large LDL species. In a study of 22 healthy male smokers by Neunteufl et al. (1992), supplementation with vitamin E decreased the extent of transient impairment of endothelial function in heavy smokers by improving oxidative status. The Nurses’ Health Study found that supplementation with vitamin E daily was associated with a reduced risk of heart disease among middle-aged women (Stampfer, et al., 1993). However, the Finnish Alpha Tocopherol Beta Carotene Cancer Prevention Study concluded that daily supplementation with vitamin E did not have a significant effect on decreasing the incidence of death due to MI or cardiac arrest in 27,271 Finnish male smokers (Virtamo, Rapola, & Ripatti, 1998).
4. Conclusions from Available Data on Vitamin C and E

Despite demonstrating promising results in observational studies for the primary prevention of CVD, randomized trials with vitamin E and vitamin C have yielded disappointing results. These conclusions are consistent with the recommendations of the third U. S. Preventive Services Task Force (USPSTF) not to use supplements of vitamin A, C, E, beta carotene or antioxidant combinations for the prevention of cardiovascular disease due to insufficient evidence of their benefit. Though supplementation studies have yielded conflicting results regarding the role antioxidants play in preventing heart disease, dietary intake studies have reached different conclusions. In a prospective study to assess the relationship between plasma ascorbic acid levels and all-cause mortality, Khaw and colleagues (2001) recruited 19,496 men and women ranging in age from 45-79 years and followed them for 4 years. Risk of all-cause mortality was found to be lowest among individuals with the highest levels of plasma ascorbic acid. The researchers concluded that increasing the intake of fresh fruits and vegetables by one serving may have an impact on disease prevention. A similar study was conducted in which 34,486 postmenopausal women free of cardiovascular disease were followed for 7 years to assess the impact of dietary intake of vitamin E on the risk of mortality due to heart disease Kushi, et al. (1996) concluded that there is an inverse relationship between eating foods high in vitamin E and risk of death from heart disease. The antioxidant effect of vitamins E and C as well as beta carotene exerted on the body is limited by the amount that can be ingested daily. The recommended daily allowance for vitamin C is set at 60-95 mg per day, and the allowance for vitamin E is set at 10mg per day (DeBruyne, Pinna, & Whitney, 2008).
5. *Antioxidant Role of Polyphenols*

Polyphenols, which are the most abundant form of antioxidants in food, have received a significant amount of attention from researchers since 1995 (Scalbert, et al., 2005). The delay of interest in the effects of these compounds can be attributed to their complexity and variability (Stoclet et al., 2004). Abundance in food was not the only aspect of polyphenols that attracted the scientific community however, but also the quantity that can be safely ingested warranted further investigation for possible disease prevention. Polyphenol intake can easily reach 1 g daily, which is roughly 10 times the amount recommended for vitamin C and 100 times greater than the amount recommended for vitamin E (Scalbert, et al., 2005).

Recent epidemiologic studies supported the role of polyphenols as antioxidants through the ingestion of different foods such as fruits, vegetables, cocoa and olive oil and soybeans or beverages such as wine and tea (Stoclet, et al., 2004). Though cocoa and its many products have received considerable attention in the scientific community recently for their potential antioxidant effects, there are only a few studies to date that have been done on humans that examined the antioxidant effect of this substance on oxidative stress.

6. *Antioxidant Effect of Cocoa*

A randomized crossover study by Wan and colleagues (2001) was conducted over a 2 year period to evaluate the effect of cocoa powder and dark chocolate intake on LDL oxidative susceptibility and total serum antioxidant capacity as well as urinary prostaglandin concentrations. The study consisted of 23 subjects who were given two different diets to follow. The first diet was an average American diet that was
controlled for fiber, caffeine, and theobromine. The second diet was the same as the first, with the addition of 22g of cocoa powder and 16g of dark chocolate. The results of the study indicated a slight reduction in LDL oxidation susceptibility when participants ingested cocoa powder and dark chocolate. Another randomized study examining the effect of dark chocolate on vascular and platelet function in heart transplant patients found that eating 40g of dark chocolate not only improved coronary vascular and platelet function, but also caused a reduction of serum oxidative stress (Flammer et al., 2007).

7. Antioxidant Effect of Grapes and Red Wine

The healthful effects of the Mediterranean diet on the risk of cardiovascular disease have been attributed to the abundance of olive oil and red wine in the diet (Zern & Fernandez, 2005). Red wine intake also was said to explain the “French Paradox” in which the French show low heart disease rates despite a diet high in saturated fat (Zern & Fernandez, 2005). This effect was found to be mainly due to the high flavonoid and polyphenol content of grapes (O’Bryne, Devaraj, Grundy, & Jialal, 2002). Concord grape juice and red wine were shown to have similar polyphenol content and thus they both produced similar effects in decreasing oxidative stress, which in turn decreased the risk of developing CVD (Durak, et al., 1999). Grape juice would be the more appealing beverage to promote for its antioxidant effect since it does not have the addiction factor associated with alcohol. A study by O’Bryne et al., (2002) compared the effect of supplementation with 400 IU alpha-tocopherol with ingestion of Concord grape juice and found both substances increased serum antioxidant capacity and protected against LDL oxidation. Concord grape juice was found also to decrease plasma protein oxidation, which led the investigators to conclude that it is a more potent antioxidant than
alpha-tocopherol. Polyphenols from red wine have also been shown to increase antioxidant capacity through rapid absorption (Garcia-Alonso, et al., 2008). In another study by Chou, et al., (2001), purple grape juice was given to participants alone or in combination with vitamin E to assess endothelial function as well as antioxidant effects. The results showed an improvement in endothelial health, as well as an increase in antioxidant levels without affecting lipid or glucose metabolism.

8. **Antioxidant Effect of Olive Oil**

Olive oil is another substance that is high in polyphenols and has been shown to affect oxidative stress levels. In a randomized trial to assess olive oil’s role as an antioxidant, researchers randomly assigned participants to three sequences of daily intake of the oil 3 times a day. The sequences referred to the phenolic content of the oil and were as follows: sequence 1: high medium and low polyphenol olive oil; sequence 2: medium, low and high polyphenol olive oil; and sequence 3: low, high and medium polyphenol olive oil. Oxidative stress, measured as oxidized LDL, was found to have an inverse linear relationship with polyphenolic content (Covas et al., 2006). Another study to determine the effects polyphenol-rich olive oil has on oxidative stress yielded similar results as the earlier study, with an additional increase in high density lipoprotein (HDL) levels than other types of olive oil (Marrugat et al. 2004).

9. **Antioxidant Effect of Tea and Soy**

Tea is the most consumed beverage in the world and consists of more than 400 bioactive chemicals (Yung, et al., 2008). Recently, tea had been linked to CVD protection due to its high antioxidant content (Yung et al., 2008). Japanese green tea, for example, was shown to decrease plasma oxidized LDL levels in 40 healthy adult
volunteers, ultimately decreasing the risk associated with heart disease (Inami et al., 2007). Ingestion of green tea by 12 healthy male volunteers for 4 weeks caused a significant decrease in oxidized LDL levels, suggesting a potential benefit of influencing the development and progression of atherosclerosis (Sung, et al., 2005). Black tea has also been linked to coronary artery disease as demonstrated by a metanalysis of available data between 1990 and 2004 that found that consumption of three or more cups of black tea per day decreased CVD risk through tea’s polyphenol action (Gardner, Ruxton, & Leeds, 2007). Soy has also been shown to reduce oxidative stress, as well as the risk of cardiovascular disease, though it has not been studied as extensively as other polyphenol-rich products (Mota et al., 2007). Several studies have concluded that more studies on the antioxidant effect of soy are needed to make more concrete recommendations (Clair & Anthony, 2005).

C. Anti-inflammatory Effect of Polyphenols

1. Role of Inflammation in Cardiovascular Disease

The antioxidant effect of polyphenols is not the only factor that affects the development of atherosclerosis. Inflammation is also a major part of the atherosclerotic process and has been shown to lead to more complications such as MI and stroke (Paoletti, Gotto, & Hajjar, 2004). Oxidized LDL elicits an inflammatory response that leads to the formation of fatty streaks on the arterial wall (Berliner et al., 1995). The inflammatory response involves the release of a group of molecules, referred to as cytokines, which in turn affect the levels of different biological markers in the body, such as C-reactive protein (CRP; Berliner, et al., 1995). Of all the inflammatory biomarkers, CRP is the most studied and has been linked to cardiovascular disease in numerous
studies, even though the exact mechanism of action was not clear (Ridker & Blake, 2003; Ridker, 2007).

2. CRP’s Role in Cardiovascular Disease

Recent studies have demonstrated the importance of the inflammatory process in predicting cardiovascular events and how CRP can be used as a tool to assess the extent of this inflammation, which would improve outcome and prevent disease. A study by Ridker, Cushman, stampfer, Tracy and Hennekens (1997) sought to measure inflammation and its effect on the occurrence of a cardiac event. The study consisted of 543 otherwise healthy men from the Physician’s Health Study, who eventually developed heart disease, and compared them to an equal number of participants who had never developed the disease. The participants were randomly assigned to either an aspirin regiment or a placebo one at the beginning of the trial and were followed for 8 years. Assessment of inflammation was done by measuring plasma CRP levels. Among the participants who eventually developed heart disease, CRP levels were elevated at baseline with those in the highest quartile having a threefold increase in risk. Aspirin reduced the risk of an MI in those with the highest levels of CRP, which the researchers concluded could have clinical implications in preventing heart disease. In another study conducted on a different participant sample of almost 15,000 men from the Physician’s Health Study found that CRP measurements, along with measurements of total cholesterol and HDL, were also significant in predicting a cardiovascular event (Ridker, Glynn, & Hennekens, 1998).

The ability of C-reactive protein to predict the risk of a cardiovascular event goes beyond circulating levels in the blood. Examination of 17 patients who died of an MI
event found CRP deposits in the infarcted tissue along with fragments from the pathway of complement (Lagrand et al., 1997). This conclusion was supported by another study in which CRP and complement were found to be strongly associated with post infarct death and were identified as targets of heart disease therapy (Griselli et al., 1999). Furthermore, C-reactive protein was also found to be elevated in patients with unstable angina when compared to those with stable angina (Berk, Weintraub, & Alexander, 1990). The researchers in the study concluded that high levels of CRP in unstable angina may be linked to future cardiac events (Berk, et al., 1990). Levels of CRP in unstable angina were not found to increase by transient ischemic events (Berk, et al., 1990).

Scientific research was also able to demonstrate the usefulness of CRP levels in not only predicting future cardiac events, but also distinguishing between patients with unstable angina and those with long standing angina who have never been stable. Blood levels of several substances, namely lipoprotein(a), homocysteine, tissue plasminogen activator, plasminogen activator inhibitor-1, CRP, fibrinogen, and von Willebrand factor were compared to determine their ability to distinguish between unstable angina (multiple acute ischemic events) and long standing stable angina (Bogaty et al., 2001). Fifty participants were grouped into one of three condition groups: previous repeated coronary events, stable angina with no coronary events, or control (without any evidence of heart disease; Bogaty, et al., 2001). Only CRP was found to distinguish between those who have unstable angina and those with stable angina. Another study examined the efficacy of two acute phase reactants, CRP and serum amyloid A protein (SAA) in predicting coronary events in patients with stable and unstable angina (Haverkate, Thompson, Pyke, Gallimore, & Pepys, 1997). The results demonstrated that elevated CRP levels were
better predictors of coronary events in stable as well as unstable angina, while SAA was not (Haverkate et al., 1997).

3. Anti-inflammatory Effect of Tea

Although sparse, evidence that polyphenols affect inflammation levels in the body does exist. Tea, a rich source of pranthocyanidin polyphenols, was studied to determine its effect on inflammation. On a molecular level, tea was found to decrease inflammation by inhibiting the release of cyclooxygenase-2 (COX-2) and nitric oxide (NO), which are both important biological mediators implicated in the inflammatory pathway (Hou, et al., 2007). Tea was also found to exhibit anti-inflammatory effects by decreasing the production of IL-6 cytokines that signal the release of inflammatory markers, such as CRP (Tipoe, Leung, Hung, & Fung, 2007).

4. Anti-inflammatory Effect of Olive Oil

Olive oil also was found to have anti-inflammatory effects in cardiovascular disease, which was attributed to its high polyphenol content (Patrick & Uzick, 2001). A study that compared 50ml of two kinds of olive oil (refined and virgin) on inflammatory markers in patients with stable heart disease found that consumption of virgin olive oil decreased CRP, as well as its precursor IL-6, in patients with stable coronary heart disease (Fito et al., 2008).

5. Anti-inflammatory Effect of Cherries

Additionally, dietary intake of cherries has been shown to affect levels of inflammatory markers in the body. Eighteen healthy men and women added Bing cherries to their diets for 28 days. Blood samples were taken at baseline, after 14 days and again at 28 days. Results showed a decrease in both CRP and NO levels 28 days post
study (Kelley, Rasooly, Jacob, Kader, & Mackey, 2006). The common factor between tea, olive oil and cherries is the presence of polyphenol compounds. Although the mechanism of action of the anti-inflammatory effect in this substance is not well understood, the potential benefit warrants further investigation.

**D. Figs and Polyphenols**

Figs are one of the first fruits to be cultivated around the world. In addition to their sweet taste and high fiber content, figs have a very high level of polyphenols compared with other fruits and vegetables. According to the California Fig Advisory Board, a study conducted by the University of Scranton, Pennsylvania, found that the polyphenol content of figs was more than quadruple the amount found in other fruits (California Fig Advisory Board, 2008). Comparison of the polyphenolic content of six different varieties of figs showed darker varieties to have higher antioxidant levels than their lighter counterparts (Solomon et al., 2006). Most of the polyphenols in the fruit were found to be concentrated in its skin, which attributed to its higher antioxidant levels. The Mission fig variety was found to have the highest polyphenolic levels of flavonoids as well as exhibiting the highest antioxidant capacity (Solomon, et al., 2006). Due to their polyphenolic density and superior quality, figs are qualified candidates for further study.

There are two proposed effects that polyphenols can potentially exert on the body to lower the risk of CVD. One is through an anti-inflammatory pathway and the other is through an antioxidant effect. The antioxidant effect of polyphenols has been well explored and documented, while the anti-inflammatory pathway has been less investigated. California Mission figs are rich in polyphenols, but the extent of an anti-
oxidant or anti-inflammatory effect has not been studied. The purpose of this study is to
determine the existence and extent of such an effect.

E. Conclusions

Polyphenols have been shown to affect cardiovascular disease through an
antioxidant and anti-inflammatory pathway. These two pathways are not completely
independent of each other since they involve many of the same foods. Studies involving
the antioxidant pathway demonstrated a polyphenolic effect on oxidative stress through a
decrease in levels of oxidized LDL, which in turn decreased the formation of fatty streaks
that lead to atherosclerosis. The anti-inflammatory pathway was less clear, but some
evidence showed a possible mechanism of action by decreasing CRP levels. Even though
several pharmacological therapies affect CRP levels and thus lower inflammation in the
body, the benefit does not come without side effects. Natural foods, on the other hand, do
not have side effects and are less expensive to acquire than medications.

The human and material loss associated with CVD warrants the further
investigation of the implication of polyphenols in the treatment of CVD. Figs have not
been studied extensively or exclusively, but according to Vinson et al., (2005) affect
inflammatory levels in the body, as well as exert an antioxidant effect. According to the
Fig Advisory Board, figs are well known for their nutritional benefit all over the world
and can be grown in a cost effective manner in most regions. Demonstrating their effect
in combating heart disease would be a great tool in aiding the fight against this disease.
CHAPTER 3

METHODS

A. Overview

Analyses were performed on data collected from participants who took part in a randomized crossover study to assess the effect of California Mission fig consumption on CRP and antioxidant levels in the body. Below are the details of the study followed by the specifics of the analyses.

B. Study Design

The study was designed to be a 12 week randomized crossover study with an intervention and a control phase, each lasting 5 weeks (Table 1). The screening period lasted 2 weeks, after which 102 adult men and women were randomly assigned to either the intervention (eating figs) or control study phase (not eating figs) for the first 5 weeks of the trial and then were crossed over into the other phase for the second 5 weeks. During the intervention phase participants were asked to incorporate a prepackaged, pre-weighed 40 gram serving of California Mission figs (3-5 figs) at each meal for a total of three meals a day in their daily diet. Participants consumed their normal everyday diet while participating in the study with the exception of incorporating figs as part of their meals during the intervention phase and abstaining from ingesting figs or prunes during the control phase. Each study participant received a $25 gift card upon completion of the study.
**Table 1. Study Design**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weeks</th>
<th>0 and 2</th>
<th>2 to 6</th>
<th>6 and 7</th>
<th>7 to 11</th>
<th>11 and 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Screening</td>
<td>Reg. Diet</td>
<td>Blood Draw/</td>
<td>Reg. Diet w/</td>
<td>Blood Draw/</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>End of Study</td>
<td>Figs</td>
<td>End of Study</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Screening</td>
<td>Reg. Diet w/ Figs</td>
<td>Blood Draw/</td>
<td>Reg. Diet</td>
<td>BloodDraw/</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Crossover Week</td>
<td></td>
<td>End of Study</td>
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</tbody>
</table>

The Gantt chart and the budget for the study are shown in Appendices C and D.

**C. Study Participants**

Approximately 141 potential subjects were recruited and screened. Eligible participants were men and women aged 30 to 75 years with LDL cholesterol concentrations in the above optimal (100-129 mg/dL) or borderline (130-159 mg/dL) level. Individuals with LDL levels of 160-189 mg/dL were only allowed to participate with the permission of a physician. Inclusion of participants with elevated levels of LDL was done purposefully to ensure that CRP levels would be elevated in the study population. Additional inclusion criteria were a body mass index (BMI) of 18.5-35 kg/m², no cigarette smoking within the past year, and a written informed consent.

Exclusion criteria were any secondary cause of hyperlipidemia (kidney or liver disease, untreated hypothyroidism); current or previous (within the past 2 months) use of any lipid-lowering drug; Type 1 diabetes or uncontrolled Type 2 diabetes (HbA1c > 7%); triacylglycerol concentrations >300 mg/dL; current or previous (within the past 3 months) treatment with estrogen or steroid therapy; stated dislike of figs; use of certain dietary supplements, e.g. Metamucil, sterol/stanol margarine and others that may influence lipid concentrations; chronic disease that may affect concentrations of lipids or
markers of inflammation (e.g. cancer other than skin cancer within the last 5 years, chronic rheumatological disease, chronic severe depression), and any condition deemed by the study investigators to limit compliance with the protocol (e.g. drug abuse).

D. Recruitment

Participants were recruited from churches and businesses in communities in and around San Bernardino, California. Presentations and flyers about the California Mission fig study were presented at churches and business establishments as well as at the Drayson Center exercise facility at Loma Linda University. Potential subjects were recruited by flyers approved by the university’s Institutional Review Board. Registration sheets were left at different recruiting sites for individuals and were the basis for later communications by the researchers. Upon arrival at the scheduled screening appointments all study procedures were explained in detail and a signed informed consent form was obtained from each eligible participant. Only subjects who signed the consent form were allowed to take part in the study.

E. Procedures

The week by week procedures are given in Table 2.
Table 2. Week by Week Procedures

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0 to 2 Screening</td>
<td><strong>Initial visit</strong>: Potential subjects were individually given a detailed explanation about the purpose of the study and the procedures they would follow during the course of the 12-week study as described above.</td>
</tr>
<tr>
<td>(fasting, if not</td>
<td>Subjects were informed that the only change required in their regular diet was the inclusion of 3-5 dried mission figs (serving size of 40 grams) eaten at each meal everyday for a period of 5 weeks during the intervention phase of the trial.</td>
</tr>
<tr>
<td>fasting, one extra</td>
<td>Consumption of figs beyond this limit or prunes was not allowed throughout the study.</td>
</tr>
<tr>
<td>visit will be required</td>
<td>Informed, written consent was obtained.</td>
</tr>
<tr>
<td>within this period)</td>
<td>A complete health questionnaire was completed during the screening period (Appendix D). Subsequent brief health questionnaires were completed at different intervals throughout the study (appendix E).</td>
</tr>
<tr>
<td></td>
<td>Baseline blood samples were drawn after a 12 hour fasting period at the screening visit, or within 10 days of enrolling in the study.</td>
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<tr>
<td></td>
<td>Vital signs and anthropometrics were measured and recorded.</td>
</tr>
<tr>
<td></td>
<td>Potential participants who met the study criteria in regard to the fasting serum concentrations were contacted by telephone and given an appointment for the week 2 randomization visits which were scheduled within 2 weeks of the screening visit.</td>
</tr>
<tr>
<td>Week 2 Randomization</td>
<td><strong>Procedure for randomization</strong>: A random numbers table was used to generate a list of participants. Even numbers designated randomization to the intervention phase followed by the control phase. Odd numbers designated randomization to the control phase followed by the intervention phase. Numbers and their assigned status were written on slips of paper and placed in consecutively numbered opaque envelopes. Subjects were consecutively assigned a randomization number corresponding to the number on the envelope randomly pulled. After assignment of the number, the envelope was opened and the group assignment noted and the subject informed.</td>
</tr>
<tr>
<td>(nonfasting)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 (continued). Week by Week Procedures

| Week 2 Randomization (nonfasting) (Continued) | Subjects randomized to the fig study phase (intervention) first received enough prepackaged fig portions for 3 weeks of consumption (1 extra week in case the follow-up visit is delayed) while those in the control phase were advised to continue their regular diet and not to eat any figs or prunes for 5 weeks. |
| Week 2 Randomization (nonfasting) | Subjects concerned about weight gain were permitted to substitute the figs for their usual desserts or sweet snacks. If the fig portions were forgotten (e.g. not taken to work) they were consumed at a later time in the day with meals or as a snack. Daily compliance with the assigned portions of figs was recorded on a compliance form provided (see Appendix F). Vital signs and anthropometrics were measured and recorded. The study procedures were reiterated and subjects were asked to complete a short health questionnaire (appendix E), to check whether any changes occurred in their health status or use of medications since the screening visit. |
| Week 4 (Follow-up visit/fig pickup for intervention group – nonfasting) | Vital signs and anthropometrics were measured and recorded. Participants in the intervention phase picked up figs for the remaining 3 weeks plus an extra week’s supply. A reminder to follow the study protocol was given to all study participants. Also participants were encouraged to ask any questions they had regarding their diet. Compliance sheets were gathered and new ones were provided. Participants who were consuming their usual diet were reminded not to consume any figs or prunes for the remainder of the study phase. Subjects were interviewed in person or by phone to obtain a 24 hour dietary recall. A telephone interview to obtain a 24-hour dietary recall was performed on a randomly selected day between weeks 4 and 6. |
Table 2 (continued). Week by Week Procedures

<table>
<thead>
<tr>
<th>Week 6 Follow-up visit (fasting)</th>
<th>Vital signs and anthropometrics were measured and recorded. All the participants in the study had blood drawn after a 12 hour fast. Compliance sheets were gathered from participants consuming figs and new ones provided.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 7 Crossover visit (fasting)</td>
<td>Vital signs and anthropometrics were measured and recorded. All participants in the study had blood drawn after a 12 hour fast. Compliance sheets will be gathered from participants consuming figs. Participants crossed over into opposite phase: Those who were not eating figs began consuming 40g prepackaged figs at each meal three times daily and those who had stopped ingesting the figs and resumed their regular diet. Compliance sheets were given to the new intervention group along with a 3 week supply of figs (1 extra week in case of delay of follow-up appointment)</td>
</tr>
<tr>
<td>Week 7 Crossover visit (fasting)</td>
<td>Instructions were given to participants not to consume any other than the prepackaged figs or prunes during the subsequent five weeks. Those who had just completed the intervention phase were instructed to abstain from eating any figs or prunes with their meals. Those concerned about weight gain were advised to alter their daily intake to account for the 330 kcalories from figs. All subjects completed a brief health questionnaire that included a question that assessed their satisfaction with eating figs (Appendix E) A telephone interview to obtain a 24-hour dietary recall was performed on a day randomly selected day between weeks 7 and 9.</td>
</tr>
<tr>
<td>Week 9 (Follow-up visit/fig pick-up for the intervention group - nonfasting)</td>
<td>Vital signs and anthropometrics were measured and recorded. Participants who were in the intervention phase picked up figs for the remaining 3 weeks plus an extra week’s supply.</td>
</tr>
</tbody>
</table>
Table 2 (continued). Week by Week Procedures

<table>
<thead>
<tr>
<th>Week 9 (Follow-up visit/fig pick-up for the intervention group - nonfasting) (Continued)</th>
<th>A reminder to follow the study protocol was given to all study participants. Also participants were encouraged to ask any questions they had regarding their diet. Compliance sheets were gathered and new ones provided. Those consuming usual diet were reminded not to consume any figs or prunes for the remainder of the study phase. Subjects were interviewed in person or by phone to obtain a 24 hour dietary recall. A telephone interview to obtain a 24-hour dietary recall was performed on a randomly selected day between weeks 4 and 6.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 11 Follow-up visit (fasting)</td>
<td>Vital signs and anthropometrics were measured and recorded. All study participants had blood drawn after a 12 hour fast. Compliance sheets were gathered from participants consuming figs and new ones provided.</td>
</tr>
<tr>
<td>Week 12 Follow-up visit (fasting)</td>
<td>Vital signs and anthropometrics were measured and recorded. All the participants in the study had blood drawn after a 12 hour fast. Compliance sheets were gathered from participants consuming figs. All subjects completed a brief health questionnaire that included a question that assessed satisfaction with eating figs (Appendix E) A thank you letter was given to each participant who completed the 12 week fig research study along with a $25 gift card. Current addresses were confirmed/obtained for later mailings of final blood results.</td>
</tr>
</tbody>
</table>

F. Procedures for Blood Draws

At each visit involving blood draws (screening, week 6, week 7, week 11, and week 12), subjects were asked to fast overnight. Water and standard allowed medication were to be taken on the morning prior to the visit. Blood samples were drawn between
7:00 to 10:00 a.m. At screening, one 10 ml red top tube was drawn and centrifuged.

Experienced certified venipuncturists were hired to perform the blood draws and samples were sent to the Loma Linda University Medical Center Clinical Laboratory for analysis of total cholesterol, LDL cholesterol, HDL cholesterol and triacylglycerol levels as well as ALT, creatinine and glucose. At follow-up visits 6, 7, 11 and 12, one red top tube of 10 ml and one 4 ml green top tube for plasma was drawn. The blood was allowed to cool for about 10-15 minutes at room temperature before being put on ice. Samples were placed in a cooler and transported to Nichol Hall Room 1112 where they were separated in a refrigerated centrifuge at 1800 x g and 4 degrees centigrade. Serum/plasma was aliquoted into vials and kept in a -70 degree Fahrenheit freezer in Nichol Hall Room 1112A, the Nutrition Department laboratory.

G. Measurement of Blood Pressure and Pulse

Blood pressure and pulse measurements were taken before blood tests were completed and after participant had rested for at least 5 minutes. Blood pressure measurement procedure is indicated in Table 3.

Table 3. Blood Pressure and Pulse Procedure

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Subject was seated with back supported and arm bare and supported.</td>
</tr>
<tr>
<td>2</td>
<td>Subject refrained from ingesting caffeine for 12 hours before measurement was taken.</td>
</tr>
<tr>
<td>3</td>
<td>Measurement began after at least 5 minutes of rest.</td>
</tr>
<tr>
<td>4</td>
<td>Appropriate cuff size and calibrated equipment was used.</td>
</tr>
<tr>
<td>5</td>
<td>Two readings were obtained for each participant and averaged.</td>
</tr>
<tr>
<td>6</td>
<td>Pulse was measured for 60 seconds before blood pressure measurement.</td>
</tr>
</tbody>
</table>
H. Measurement and Justification of Anthropometrics

Though the only dietary change that participants experienced with the study was the inclusion of figs for 5 weeks, it was important to monitor weight change throughout the process. Figs have a high content of fiber which may have lead some of them to feel full faster and thus eat less overall. This may have led to some weight loss over time and may have provided a future venue for research.

1. **Height and Weight**

   Height was measured (without shoes) using a standard and calibrated wall stadiometer only at the screening visit. Subjects were weighed using a standard scale at screening and weeks 6, 7, 11 and 12 of the study period. This scale was designed for patients up to 600 pounds and was last calibrated in November 2007.

2. **Weigh-in Procedure**

   The weigh-in procedure used is indicated in Table 4 below.

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Remove shoes</td>
</tr>
<tr>
<td>2</td>
<td>Remove jacket and other outer garments</td>
</tr>
<tr>
<td>3</td>
<td>Empty pockets</td>
</tr>
<tr>
<td>4</td>
<td>Stand on scale and wait for Assistant 2 to record measurement</td>
</tr>
<tr>
<td>5</td>
<td>Record results in the chart</td>
</tr>
</tbody>
</table>

3. **Waist Circumference**

   Waist circumference was measured to the nearest 0.1 cm at the narrowest level over light clothing, with the use of an unstretched tape measure and without any pressure to the body surface. Waist was measured at screening and at weeks 7 and 12.
I. Tracking Procedures

On the evening before appointments, participants were called as a reminder and for fasting visits, to be fasting on the morning of the visit. E-mail was used instead of a phone call when appropriate and when requested by participants. Participants who missed their scheduled appointments were contacted for a new appointment if they wished to continue in the study.

J. Dietary Assessment and Tracking Procedures

Dietary assessment was needed to monitor changes in the diet other than those attributed to an increased consumption of figs during the intervention phase compared to the control phase. This was documented by two phone interviews and one in-person interview to obtain dietary information (24 hour diet recall as described in Appendix H) on all randomized participants in each study phase for a total of 6 recalls. The phone interview was completed on a random day between weeks 2 and 4, weeks 4 and 6, weeks 7 and 9, and weeks 9 and 11. The day was chosen by pulling a weekday or weekend day from a box containing folded papers labeled Sunday through Friday, after which they were returned to the box. If the interview was not possible on the drawn day, it was conducted as soon as possible thereafter. The in-person interviews were completed on the week 4 and week 9 visits.

The Nutrition Data System for Research (NDSR), which is maintained by the Nutrition Coordinating Center at the University of Minnesota, was the software used to acquire the 24 hour dietary recall. The recall was performed by registered dietitians. A list indicating portions of sweet snacks and desserts that could be substituted by figs is provided in Appendix H.
K. Nutritional Analysis of California Mission Figs

California Mission figs were obtained from the California Fig Board. An analysis was performed on California Mission figs to determine the amount of soluble and total fiber present in a serving (40 grams) of figs. The California Fig Advisory Board has the following data showing the β-glucan content of mission figs (Table 5).

Table 5. Caloric and Nutrient Content in a 40 Gram Serving of California Mission Figs

<table>
<thead>
<tr>
<th>Nutrition Facts</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Serving Size</td>
<td>¼ cup (40 grams)</td>
</tr>
<tr>
<td>Kcalories</td>
<td>110</td>
</tr>
<tr>
<td>Total Fat</td>
<td>0g (0%)</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>0g (0%)</td>
</tr>
<tr>
<td>Trans Fat</td>
<td>0g (0)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0 mg (0%)</td>
</tr>
<tr>
<td>Sodium</td>
<td>5 mg (0%)</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>26 g (9%)</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>5 g (20%)</td>
</tr>
<tr>
<td>Sugars</td>
<td>20 g (10%)</td>
</tr>
<tr>
<td>Protein</td>
<td>1 g (1%)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>Calcium</td>
<td>6%</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>Iron</td>
<td>6%</td>
</tr>
</tbody>
</table>

L. Data Analysis of CRP and Antioxidant Levels

Data entry and analyses was conducted by the doctoral student (RA). All data was entered into the SPSS version 16 database and a 10% random sample was re-entered to check for accuracy. Variables were checked for skewedness and log transformation was done on CRP, antioxidant capacity levels and dietary recall. However, non-transformed data were presented as part of results. Antioxidant, CRP and dietary recall
data were analyzed using a paired t-test while other variables were compared using a student’s t-test. Descriptive statistics were expressed as means and standard deviations for normally distributed variables, and medians and percentiles for skewed variables.

1. **CRP Analysis**

   Frozen samples for the analysis of CRP were sent in a single group at the end of the trial for analysis at the Research Laboratory, University of California at Davis. The method employed was a high-sensitivity CRP analysis test. Testing for CRP at University California at Davis utilized a PolyChem analyzer from PolyMedCo. Coefficient of variability (CV) for runs done on three sets of controls yielded 5.72%.

2. **Antioxidant Analysis**

   Samples were analyzed for antioxidant capacity in the Nutrition laboratory at Loma Linda University. The two tests employed were the Ferric Reducing Antioxidant Power (FRAP) and the Total Antioxidant Capacity (ABTS) tests are indicated in Table 6 (refer to Appendix I and J for complete protocols).

**Table 6. Testing Protocol for Ferric Reducing Antioxidant Power (FRAP) and the Total Antioxidant Capacity (ABTS) Tests**

<table>
<thead>
<tr>
<th>Components of FRAP Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP reagents</td>
<td>A ratio of 10:1:1 of buffer (a mixture of sodium acetate and glacial acetic acid and water): Tripyridyltriazine (TPTZ): FeCl₃.</td>
</tr>
<tr>
<td>Trolox Stock Standard</td>
<td>A 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid</td>
</tr>
<tr>
<td>Plasma dilution</td>
<td>5:1 ratio of plasma to water.</td>
</tr>
<tr>
<td>Reagent</td>
<td>Reagent was mixed with plasma in a 96-well assay plate and analyzed in the Synergy apparatus.</td>
</tr>
</tbody>
</table>
Table 6 (Continued). Ferric Reducing Antioxidant Power (FRAP) and the Total Antioxidant Capacity (ABTS) Tests.

<table>
<thead>
<tr>
<th>Components of ABTS Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABTS solution</td>
<td>A mixture of ABTS and potassium persulfate in phosphate buffered saline</td>
</tr>
<tr>
<td>Trolox Stock Standard</td>
<td>A 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid</td>
</tr>
<tr>
<td>Analyses</td>
<td>Solutions were combined and placed in a spectrophotometer in a timed process for analysis</td>
</tr>
</tbody>
</table>

Baseline data for gender distribution mean values of BMI, waist circumference, blood pressure, pulse, and lipid concentrations at screening for participants were analyzed to ensure success of randomization. Participants' weights were analyzed for the two study phases and if they were found to be statistically significantly different, data would have been analyzed controlling for weight.

Compliance with fig consumption was estimated from the compliance sheets and subjects who consumed <75% of the assigned figs would have been designated as non-compliers, and analysis would be performed with and without these participants. C-reactive protein and antioxidant levels at weeks 6 and 7 and at weeks 11 and 12 were averaged to minimize day to day variability and analyzed.

Dietary data was analyzed for difference in calories, macronutrients and minerals between participants in the two study phases. Mean blood pressure, pulse and body weight measurements at the end of the intervention and control phases were analyzed and presented. All results were considered significant for a two-tailed P values <0.05. Statistical analyses were performed using SPSS for WINDOWS (SPSS, Chicago, IL).
M. Power Analysis

Power analysis was based on calculations done for a concurrent study that sought to determine if fig consumption affected lipid levels in the body. Since the population parameter was not known then \( s_1 \) and \( s_2 \) was used to calculate a weighted average that estimates \( s \)

\[
s^2 = \frac{s_1^2 n_1 + s_2^2 n_2 - 1 s_1^2 + s_2^2}{n_1 + n_2 - 2}
\]

The sample size was given a mean expected 5-6% reduction in LDL cholesterol with an SD of the change of 5-6%. For a mean difference of 5 and SD of 8 using the equation shown results in a sample size of \( (1.96+0.84)^2*2*64/25=40.1 \sim 41 \) giving a total sample size of 82. From SPSS output the total sample size requirement for the study was 84 for a power of 80%. If we allow for a dropout rate of up to 20%, the final number for the study will be \( 84 + 16 = 100 \) participants

N. Strengths and Limitations

The main strength of the study was the randomized, crossover design with subjects serving as their own controls and the adequate, calculated power. The theoretical underpinnings were excellent. The intervention was simple to administer to participants since the figs were prepackaged.

There were also several limitations. Subjects were free living and there was no way to control other food intake. Subjects concerned about weight gain may have substituted figs for desserts and sweet snacks as advised, which may have lowered lipid concentrations due to the substitution. The dietary recall analysis will result in data showing whether the composition of the diet changed between the two periods other than
in regard to fig intake. Compliance may have been affected since participants had to eat figs continuously, though this is unlikely since the intervention period was only 5 weeks.

Since antioxidants are at the highest levels immediately after a meal, frozen samples taken after a 12 hour fast might yield compromised values. C-reactive protein levels also vary greatly within individual measurements and are affected by many factors including medications, but since each participant will act as their own control, this effect should be minimized.

O. Research Ethics

In the parent study, respect and confidentiality was maintained by storing patient information in a locked room and not allowing access to anyone other than the research team. All blood samples were labeled with the participants' randomized number code, minimizing any issues with confidentiality. All data was stored separately from identifying information. In addition, computers as well as data files were password protected to further protect against any breach of confidentiality. Only the primary investigator had linking access. All data analyses were conducted on de-identified data. Only trained IRB certified health professionals were in contact with participants.

Participants were provided with an informed consent document explaining all the test procedures and possible side effects or risks, if applicable, to maximize benefits and minimize possible harms. Harms in this study were limited to hematoma, lightheadedness or other complications of venipuncture.
CHAPTER 4
PUBLISHABLE PAPER

Effect of California Mission Fig Consumption on Level of C-Reactive Protein and Antioxidant Levels

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*For Submission to the Journal of Nutrition, Metabolism and Cardiovascular diseases*

The article submitted for your review has been read and approved by all authors. This article has not been published and is not under consideration for publication elsewhere. There are no financial or other relationships between the author and any entity connected with this journal. While this study was financially supported by the California Fig Board, it was conducted independently of the funding agency.
Abstract

**Background and Aims:** Polyphenols exert both anti-inflammatory and antioxidant effects. We investigated the effect of consuming polyphenol-rich figs on high sensitivity C-reactive protein (CRP) and antioxidant levels in hyperlipidemic subjects. **Methods and Results:** Men and women with elevated LDL cholesterol levels (100-189 mg/dL) were eligible. The study had a randomized crossover design, where 141 potential participants were screened and 102 randomized to either consume 120 g/day of California figs (intervention phase) or no figs (control phase) for five weeks. Mean CRP values did not differ significantly in the intervention phase when compared to the control phase (median [interquartile range] 1[0.5, 2.1] mg/L versus 1 [0.6, 2.1]; P=0.31). Ferric reducing antioxidant power (FRAP) test results did not differ between study phases either (0.1± 0.1 mmol versus 0.1± 0.1 mmol; P = 0.96). Total antioxidant capacity (ABTS) test concentrations also did not differ during the two phases (0.2± 0.04 mmol versus 0.2± 0.04 mmol; P = 0.97). Furthermore, analysis of dietary intake revealed that participants consumed approximately 187 calories more in the intervention phase (P<0.0001), while consuming 4% less calories from fat (P<0.0001). **Conclusions:** For men and women with elevated cholesterol levels, fig consumption did not lower CRP or affect antioxidant levels.

**Keywords:** Cardiovascular disease, C-reactive protein, Antioxidants
Introduction

Cardiovascular disease (CVD) has been the leading cause of death in both women and men since the year 1900, with the exception of the 1918 influenza epidemic (American Heart Association [AHA], 2007). Cardiovascular disease is an international problem that, in 2001, was implicated in almost one third of all global deaths (AHA, 2009). Even though cardiovascular disease mortality rates have been decreasing since 2003, largely due to advances made in medicinal therapies (Capewell, Beaglehole, Seddon, & McMurray, 2000), the progress achieved is diminished by the fact that the underlying risk factors for CVD are still on the rise (AHA, 2007).

Risk factors for CVD are mostly lifestyle related, which can drastically alter risk when modified, and include high blood pressure, high cholesterol, diabetes, smoking, physical inactivity, and obesity. These risk factors, although diverse, have a common effect on the development of atherosclerosis, a chronic inflammatory disease characterized by plaque formation in medium sized arteries that can be present for years before it manifests itself in a variety of CVD forms, such as myocardial infarction (MI) or even sudden cardiac death (Ross, 1999).

Several studies have examined the effect of polyphenols on the incidence of cardiovascular disease through an antioxidant pathway. Oxidative stress has been shown to play a critical role in causing endothelial dysfunction, which leads to atherosclerosis (Davignon & Ganz, 2004). According to Steinberg, Parthasarathy, Carew, Khoo and Witztum (1989), high plasma levels of low density lipoproteins (LDL) accumulate into the arterial intima, which are then oxidized, forming fatty streaks that result in endothelial injury.
Polyphenols are natural compounds abundant in plant foods that can be ingested in greater amounts than other substances with similar effects, such as vitamins C and E. Recent epidemiologic studies supported the role of polyphenols as antioxidants through the ingestion of different foods, such as fruits, vegetables, cocoa, olive oil and soybeans or beverages, including wine and tea (Stoclet, et al., 2004). There are only a few studies to date that have utilized human subjects to examine the antioxidant effect of polyphenols on oxidative stress.

The antioxidant effect of polyphenols is not the only factor that affects the development of atherosclerosis. Inflammation is also a major part of the atherosclerotic process and has been shown to lead to more complications, such as MI and stroke (Paoletti, Gotto, & Hajjar, 2004). Oxidized LDL elicits an inflammatory response that leads to the formation of fatty streaks on the arterial wall (Berliner, et al., 1995). The inflammatory response involves the release of a group of molecules referred to as cytokines, which in turn affect the levels of different biological markers in the body, such as C-reactive protein (CRP; Berliner, et al., 1995). Of all the inflammatory biomarkers, CRP is the most studied and has been linked to cardiovascular disease in numerous studies, though the exact mechanism of action is not clear (Blake & Ridker, 2003; Ridker 2007).

In a previous study, researchers sought to determine the amount and quality of phenol antioxidants in dried fruit and compare them to fresh fruits (Vinson, Zubik, Bose, Samman, & Proch, 2005). In examining the concentrated form of the fruit, the researchers sought to investigate how the quality, as well as the quantity, of phenol antioxidants would differ between dried and fresh apricots, cranberries, dates, figs, raisins
and prunes (Vinson et al., 2005). The analysis of the data showed that dates had the highest concentration of polyphenols, while dried figs and plums had the best nutrient score among the dried fruit, as well as the most superior quality of antioxidants (Vinson et al, 2005). In conclusion, figs, with their higher quality phenols, are the most suitable fruit to use for further study to examine the effect of polyphenols on antioxidant and inflammatory levels in the human body.

Methods

The study was a 12 week randomized crossover study with an intervention and a control phase, each lasting 5 weeks. The screening period lasted 2 weeks, after which 102 eligible adult men and women were randomly assigned to either the intervention or control study phase. During the intervention phase participants were asked to incorporate a prepackaged, pre-weighed 40 gram serving of California mission figs (3-5 figs) at each meal for a total of 120 grams a day in their regular diet. Participants consumed their normal everyday diet while participating in the study, with the exception of incorporating figs as part of their meals during the intervention phase and abstaining from ingesting figs or prunes during the control phase. In addition, each study participant received a $25 gift card upon the completion of the study.

Approximately 141 potential participants were recruited and screened. Eligible participants included men and women aged 30 to 75 years with low density lipoprotein (LDL) cholesterol concentrations in the above optimal (100-129 mg/dL) or borderline (130-159 mg/dL) level. Individuals with LDL levels of 160-189 mg/dL were only allowed to participate with a physician’s permission. The recruitment of individuals with elevated LDL levels was intentional since such a condition affects CRP levels, thus
allowing for the detection of change due to the ingestion of figs. Additional inclusion criteria were a body mass index (BMI) of 18.5-35 kg/m², no cigarette smoking within the past year, and written informed consent. Exclusion criteria were any secondary cause of hyperlipidemia (kidney or liver disease, untreated hypothyroidism); current or previous (within the past 2 months) use of any lipid-lowering drug; Type 1 diabetes or uncontrolled Type 2 diabetes (HbA1c > 7%); triacylglycerol concentrations >300 mg/dL, current or previous (within the past 3 months); treatment with estrogen or steroid therapy; stated dislike of figs; use of certain dietary supplements, e.g. Metamucil, sterol/stanol margarine and others that may influence lipid concentrations; chronic disease that may affect concentrations of lipids or markers of inflammation (e.g. cancer other than skin cancer within the last 5 years; chronic rheumatological disease, chronic severe depression) and any condition deemed by the study investigators to limit compliance with the protocol (e.g. drug abuse).

Participants were recruited from churches and businesses in communities within San Bernardino County, California. Presentations and flyers about the California Mission fig study were distributed at churches and business establishments, as well as the Drayson Center exercise facility at Loma Linda University. Potential subjects were recruited by flyers that were approved by the university’s Institutional Review Board. Registration sheets were left at different recruiting sites for individuals and were the basis for later communications by the researchers. Upon arrival at the scheduled screening appointments, all study procedures were explained in detail and a signed informed consent form was obtained from each eligible participant. Only subjects who signed the consent form were allowed to take part in the study.
Laboratory Analyses

At each visit requiring blood to be drawn (screening, week 6, week 7, week 11, and week 12), subjects were asked to fast overnight. Water and standard allowed medications were to be taken on the morning prior to the visit. Blood samples were drawn between 7:00 to 10:00 a.m. At screening, one 10 ml red top tube was drawn and centrifuged. Experienced certified venipuncturists were hired to perform the blood draws. Samples were sent to the Loma Linda University Medical Center Clinical Laboratory for analysis of total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein (HDL) cholesterol, triacylglycerol levels, alanine aminotransferase (ALT), creatinine and glucose.

At follow-up visits 6, 7, 11 and 12, one red top tube of 10 ml and one 4 ml green top tube for plasma were drawn. The blood was allowed to cool for about 10-15 minutes at room temperature before being put on ice. Samples were placed in a cooler and transported to the Loma Linda University School of Public Health, Nichol Hall Room 1112, where they were separated in a refrigerated centrifuge at 1800 x g and 4 degrees centigrade. Serum/plasma was aliquoted into vials and kept in a -70 degree Fahrenheit freezer in Nichol Hall Room 1112A. Frozen samples for CRP analysis were all sent together at the end of the trial for analysis to the University of California at Davis research laboratory.

The method employed was a high-sensitivity CRP analysis test. Testing for CRP at the UC Davis involved a Poly-Chem analyzer from PolyMedCo. Coefficient of variability (CV) for runs done on 3 sets of controls yielded 5.72%. The rest of the samples were analyzed for antioxidant capacity in the Nutrition Department laboratory at
Loma Linda University by the principal investigator. The two tests that were employed were the ferric reducing antioxidant power (FRAP) test and total antioxidant capacity (ABTS). Coefficient of variation for controls analyzed for both tests yielded values that were less than 10%. ABTS radical is 2,2'-azinobis (3-ethylbenthiazoline-6-sulfonic acid) and is reduced (decolorized) by antioxidants in the blood. In the FRAP assay, the antioxidant capacity of the plasma is evaluated as reductance of Fe3+ -> Fe2+, which is then chelated by tripyridyltriazine (TPTZ) to form Fe2+-TPTZ, which absorbs at 593 nm. Due to the varied chemistry of these two assays, the results do not match exactly.

**Dietary Analyses**

The Nutrition Data System for Research (NDSR), which is maintained by the Nutrition Coordinating Center at the University of Minnesota, was the software used to acquire the 24 hour dietary recall. The recalls were performed by research dietitians through two phone interviews and one in-person interview per study phase, for a total of six recalls. The phone interview was completed on a random day between weeks 2 and 4, weeks 4 and 6, weeks 7 and 9, and weeks 9 and 11. The day was chosen by pulling from a box containing folded papers labeled Sunday through Friday, after which they were returned to the box. If the interview was not possible on the drawn day, it was conducted as soon as possible thereafter. The in-person interviews were completed on the week 4 and 9 visits.

**Statistical Analyses**

Data entry was done by the principal investigator and a random 10% sample was re-entered to ensure accuracy. Median and interquartile ranges were presented for C-reactive protein and antioxidants, which were not normally distributed and therefore
were log transformed. However, the non-transformed variables are shown in the tables. We analyzed both the averaged CRP values for the last 2 weeks of each study phase, as well as the values obtained on the last visit. Results did not differ substantially (data not shown), therefore only the averaged values are shown. Student t tests were used to compare baseline characteristics. Analysis of CRP, antioxidants, and dietary intake was performed at the end of each study phase utilizing a paired t-test. A general linear model of repeated measures analysis was also performed to determine if there was a difference between the participants in the two study phases (group effect) or if there was a carryover effect of figs between the two study phases (period effect). Software utilized for analyses was SPSS version 15.0 (SPSS Inc., Chicago, IL, U.S).

Results

Of the 102 participants randomized, 14 dropped out (six from the intervention group and eight from the control group), six were unwilling to complete the study, two developed diarrhea, two had family emergencies, two found they disliked figs, one was placed on cholesterol medication by his physician, and one participant was diagnosed with a terminal illness. There were 36 females and 15 males who initially started the intervention phase, while 34 females and 17 males initially started the control phase. Baseline characteristics were similar between participants randomized first to the intervention versus the control phase, except for a heart pulse that was 5 bpm higher in participants starting with the intervention phase (p=0.02) as shown in Table 1. This is most likely an error caused by the number of statistical tests that were performed for all the variables.
Body weight did not change significantly during the intervention phase as compared to the control phase (p=0.08). However, female participants analyzed separately did experience a slight weight gain of 0.5 kg (p=0.01), as shown in Table 2. Other measurements such as body mass index (BMI) (p=0.06), systolic (p=0.62) and diastolic (p=0.86) blood pressure, as well as pulse (p= 0.53) were similar between the intervention and control phases.

As shown in Table 3, mean of averaged CRP values in the intervention phase did not differ significantly compared to control phase. Mean values were 1.9± 2.8mg/L versus 2.1± 3.1mg/L in the intervention and control phases, respectively (p=0.31). The FRAP antioxidant test result analysis did not show any difference between study phases (median [interquartile] 0.1 [0.1, 0.2] mmol/L; p= 0.96). The ABTS test results confirmed the lack of difference between the two phases (median [interquartile] 0.2 [0.2] mmol/L; p= 0.97), as shown in Table 3. There was also no period effect for CRP (p= 0.69), FRAP test (p= 0.36) or ABTS (p= 0.97) test, as shown in Table 4.

Analysis of dietary intake in Table 5 shows that participants consumed approximately 187 calories more during the intervention phase (p<0.0001), while calories consumed from fat were 4% lower (p<0.0001). Also, calories consumed from carbohydrates were 6% higher (p<0.0001) and calories from protein were 1% lower (p=0.01). Nutrient levels were also different for participants during the intervention phase versus the control phase. Iron levels were increased by 1.7 mg (p=0.0004) in the intervention phase, calcium levels were increased by 174 mg (p<.0001), potassium levels by 584mg (p<0.0001), magnesium by 56mg (p<0.0001), and copper by 0.29mg (p<0.0001).
Discussion

In this study, consumption of California Mission figs for 5 weeks in participants with elevated cholesterol levels did not alter CRP values or affect antioxidant levels. Analyzing CRP values for the last week of each study phase also showed similar results between the intervention and control phases. Despite eating more calories and more carbohydrates, participants ate less fat and did not have a significant change in weight.

Participants were well supported throughout the study by having direct and frequent contact with the study investigators for questions or support, which may have contributed to the high compliance rates of the study. The intervention was also easy and inexpensive to administer, which further contributed to the study's success. The crossover design allowed for the testing of each participant in both study conditions, thus increasing measurement precision and minimizing variability. Figs in particular have not been studied before in regards to evaluating their effect on inflammation and oxidative stress, while other foods with high polyphenol content have been studied. However, results have been largely inconclusive due to the lack of randomized controlled trials. Some studies on tea linked intake to an influence on the incidence and progression of cardiovascular disease and a decrease in body inflammation, but the effect was attributed largely to mechanisms other than its antioxidant content (Peters, Poole & Arab, 2001). Cocoa, on the other hand, has been shown to increase antioxidant levels in the body, as well as increasing anti-inflammatory cytokines (Schramm, Wang & Holt, 2001), but studies done on cocoa consumption were mostly in vitro and there is evidence suggesting that similar results may not occur in vivo (Spencer, Chaudry & Panna, 2000). Figs were shown to have excellent polyphenol scores among dried fruit, but they were
not compared to any of the other known foods that influence inflammation and antioxidant levels, such as wine, olive oil, and tea. This presents a difficulty in generalizing effects found in those foods to those associated with fig consumption.

Eating figs consistently for 5 weeks significantly increased mineral levels such as iron, calcium, potassium, magnesium and copper. This is an important outcome, since certain populations may be at risk for deficiency in those minerals. For example, young children, pregnant women, adolescent girls and women of childbearing age may be at risk of deficiency due to growth spurts or higher iron needs during menstruation (Centers for Disease Control [CDC], 1998).

In this current study, blood samples were drawn from participants after a 12 hour fast, which may have led to the lack of significant differences between the two study phases. According to Osakabe, Baba and Yasuda (2001), the majority of antioxidants are metabolized from the blood within 8 hours, demonstrating that the antioxidant effect of food does not last for a long period of time, but is quickly absorbed and cleared by the body. Sound nutritional recommendation would be to increase intake of fruits and vegetables that are rich sources of antioxidants and to consume them regularly.

C-reactive protein, even though more stable than other inflammatory markers, is affected by many conditions and thus predicting its change was difficult. CRP decreased by 0.13mg/L in the intervention phase which was not statistically significant. There also seemed to have been extreme values of CRP between participants, but even after controlling for these extreme values, results remained insignificant. Lack of difference in CRP values between the intervention and control phase can be attributed to the relatively
short duration of the study; perhaps in order to affect change the study phase needed to be longer.

Study Limitations

Lack of baseline values of CRP might have been a major limitation of this study. However, the crossover design helped eliminate that bias by providing the before and after picture of each participant. Participants self-selected into the study and therefore could have had similar characteristics initially, such as better diets and a more consistent exercise routine, which would decrease the heterogeneity of the sample size. Figs in this study were not analyzed for their polyphenolic content, therefore there was no polyphenol reference value to help explain the results obtained. Also figs are not a popular American fruit, which may have further narrowed the pool of potential participants.
References


Center for Disease control and Prevention (2007). CDC MMWR. Retrieved August 20, 2008, from CDC Website: http://www.cdc.gov/mmwr/preview/mmwrhtml


141 Screened

18 Low overall lipid values
6 Trig >300
8 No longer interested
4 BMI too high
1 BMI too low
1 Family emergency
1 Hormone replacement therapy

102 Randomized

51 Randomized to No Figs

5 Loss to Follow-up
1 Family emergency
1 Diarrhea
1 Disliked Figs

43 Completed Study

51 Randomized to Figs

1 Diarrhea
1 Cholesterol meds
1 Severe illness
1 Family emergency
1 Disliked figs
1 Loss to Follow-up

45 Completed Study

Figure 1. Study Flow Chart
Table 1. Study Participant Characteristics and Fasting Laboratory Variables at Screening (n=102)

<table>
<thead>
<tr>
<th></th>
<th>Intervention (n=51)</th>
<th>Control (n=51)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.2 ± 10.3</td>
<td>54.8 ± 11.4</td>
<td>0.52</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>36 (70.6)</td>
<td>34 (66.7)</td>
<td>0.67</td>
</tr>
<tr>
<td>Male</td>
<td>15 (29.4)</td>
<td>17 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>19 (37.3)</td>
<td>17 (33.3)</td>
<td>0.49</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10 (19.6)</td>
<td>13 (25.5)</td>
<td></td>
</tr>
<tr>
<td>Carribean</td>
<td>0 (0)</td>
<td>3 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Black/Hispanic</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>6 (11.8)</td>
<td>8 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>10 (19.6)</td>
<td>7 (13.7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (9.8)</td>
<td>3 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Screen lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>203±27</td>
<td>205±32</td>
<td>0.77</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>113±59</td>
<td>128±60</td>
<td>0.20</td>
</tr>
<tr>
<td>High density lipoproteins (mg/dL)</td>
<td>56±13</td>
<td>55±15</td>
<td>0.78</td>
</tr>
<tr>
<td>Low density lipoproteins (mg/dL)</td>
<td>135±22</td>
<td>136±23</td>
<td>0.77</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26±3</td>
<td>25±4</td>
<td>0.21</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.2±9.5</td>
<td>164.3±9.1</td>
<td>0.98</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.8±13.0</td>
<td>70.0±13.3</td>
<td>0.29</td>
</tr>
<tr>
<td>TSH (mIU/mL)</td>
<td>2.5±1.4</td>
<td>2.4±1.3</td>
<td>0.77</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>93±13</td>
<td>93±11</td>
<td>0.99</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.9±0.3</td>
<td>0.9±0.2</td>
<td>0.70</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>28±15</td>
<td>21±11</td>
<td>0.08</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125±22</td>
<td>125±19</td>
<td>0.98</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80±10</td>
<td>79±9</td>
<td>0.70</td>
</tr>
<tr>
<td>Pulse (bpm)</td>
<td>66±11</td>
<td>61±9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

± indicates mean ± standard deviation
n(%) indicates number of participants and their percentage
TSH: thyroid stimulating hormone, BMI: body mass index, ALT: Alanine Aminotransferase
Table 2. Descriptive Data for Participants Who Completed Study at Baseline, Intervention Phase and Control Phase*

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Baseline</th>
<th>Intervention Phase</th>
<th>Control Phase</th>
<th>Difference between means</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>84</td>
<td>26.4±37</td>
<td>26.6±3.9</td>
<td>26.4±3.7</td>
<td>-0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Body Weight (kgs) - all</td>
<td>85</td>
<td>71.0±13.4</td>
<td>71.4±14.0</td>
<td>71.0±13.5</td>
<td>-0.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td>65.5±10.4</td>
<td>65.8±10.2</td>
<td>65.3±10.4</td>
<td>-0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td>82.0±11.9</td>
<td>83.0±13.7</td>
<td>82.1±11.9</td>
<td>-0.9</td>
<td>0.31</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>84</td>
<td>125±21</td>
<td>124±17</td>
<td>126±33</td>
<td>1.7</td>
<td>0.62</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>84</td>
<td>79±10</td>
<td>77±9</td>
<td>76±9</td>
<td>-0.1</td>
<td>0.86</td>
</tr>
<tr>
<td>Pulse (bpm)</td>
<td>84</td>
<td>64±11</td>
<td>64±10</td>
<td>63±10</td>
<td>-0.6</td>
<td>0.53</td>
</tr>
</tbody>
</table>

* mean ± standard deviation

BMI, weight and blood pressure measurements were missing for 4 participants while 3 participants were missing only weight measurement.
Table 3. Mean CRP Levels of the 2 Measurements Taken at the End of Each Study Phase and Antioxidant Levels Measured at the Last Study Week According to Study Phase.

<table>
<thead>
<tr>
<th>Outcome Variables</th>
<th>Intervention Phase</th>
<th>Control Phase</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Median</td>
<td>25th %</td>
</tr>
<tr>
<td>C-reactive</td>
<td>88</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>FRAP</td>
<td>87</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>ABTS</td>
<td>88</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*indicates the p-values for the natural log transformed outcome variables
Blood sample was not available for one participant for the FRAP test.
Table 4. Dietary Intake of Participants in Each Study

<table>
<thead>
<tr>
<th></th>
<th>Intervention Phase</th>
<th>Control Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>83</td>
<td>1802±513</td>
</tr>
<tr>
<td>% Kcalories Fat</td>
<td>83</td>
<td>25±7</td>
</tr>
<tr>
<td>% Kcalories Carbohydrate</td>
<td>83</td>
<td>64.9±9</td>
</tr>
<tr>
<td>% Kcalories Protein</td>
<td>83</td>
<td>14.5±3.9</td>
</tr>
</tbody>
</table>
### Table 5. Dietary Mineral Intake of Participants

<table>
<thead>
<tr>
<th>Mineral (mg)</th>
<th>Intervention Phase</th>
<th>Control Phase</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean±SD</td>
<td>Median</td>
</tr>
<tr>
<td>Iron</td>
<td>83</td>
<td>19.8±11.6</td>
<td>16.7</td>
</tr>
<tr>
<td>Calcium</td>
<td>83</td>
<td>1194.2±523</td>
<td>1085.8</td>
</tr>
<tr>
<td>Potassium</td>
<td>83</td>
<td>3392.5±961.8</td>
<td>3346</td>
</tr>
<tr>
<td>Magnesium</td>
<td>83</td>
<td>445.7±1.51</td>
<td>425</td>
</tr>
<tr>
<td>Copper</td>
<td>83</td>
<td>2.3±1</td>
<td>2.1</td>
</tr>
</tbody>
</table>
CHAPTER 5

ADDITIONAL FINDINGS

A. Summary and Implications of Findings

Table 1 presents descriptive data results for study participants randomized to intervention and control phases. In the two study phases, results revealed similar characteristics and laboratory values at baseline, with the exception of pulse, which was 5bpm higher in the intervention group. However, this could be a random error that resulted due to the number of statistical tests performed for all the variables below.

**Table 1. Descriptive Data at Baseline, Comparing Intervention Phase to Control Phase**

<table>
<thead>
<tr>
<th></th>
<th>Figs (n=45)</th>
<th>Control (n=43)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.1± 10.5</td>
<td>55.2 ± 11.5</td>
<td>0.71</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.94</td>
</tr>
<tr>
<td>Female</td>
<td>30 (66.7)</td>
<td>29 (67.4)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (33.3)</td>
<td>14 (32.6)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>White</td>
<td>16 (37.2)</td>
<td>14 (31.1)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>7 (16.3)</td>
<td>12 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Carribbean</td>
<td>0 (.0)</td>
<td>3 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Black/Hispanic</td>
<td>1 (2.3)</td>
<td>0 (.0)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>6 (14.0)</td>
<td>7 (15.6)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>9 (20.9)</td>
<td>6 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (9.3)</td>
<td>3 (6.7)</td>
<td></td>
</tr>
<tr>
<td>Screen Lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>204± 30</td>
<td>202± 27</td>
<td>0.77</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>107± 59</td>
<td>131± 63</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Table 1 (Continued). Descriptive Data at Baseline, Comparing Intervention Phase to Control Phase

<table>
<thead>
<tr>
<th></th>
<th>Figs (n=45)</th>
<th>Control (n=43)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Density Lipoproteins (mg/dL)</td>
<td>57± 13</td>
<td>55± 14</td>
<td>0.51</td>
</tr>
<tr>
<td>Low Density Lipoprotein (mg/dL)</td>
<td>133± 22</td>
<td>135± 23</td>
<td>0.67</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.0± 3.3</td>
<td>25.7± 4.0</td>
<td>0.11</td>
</tr>
<tr>
<td>Height (ins)</td>
<td>163.9±9.5</td>
<td>163.6±9.3</td>
<td>0.88</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>72.9±13.3</td>
<td>69.0±13.3</td>
<td>0.18</td>
</tr>
<tr>
<td>TSH (mIU/mL)</td>
<td>2.3± 1.4</td>
<td>2.5± 1.4</td>
<td>0.71</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>93.1± 13.9</td>
<td>92.2± 8.9</td>
<td>0.71</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.9± 0.3</td>
<td>0.9± 0.2</td>
<td>0.71</td>
</tr>
<tr>
<td>Alanine Aminotransferase (U/L)</td>
<td>22.6± 7.6</td>
<td>22.2± 11.9</td>
<td>0.91</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>126.0± 23.1</td>
<td>127.3± 21.0</td>
<td>0.79</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>80.7± 10.9</td>
<td>79.0± 11.0</td>
<td>0.70</td>
</tr>
<tr>
<td>Pulse (bpm)</td>
<td>66.3± 11.6</td>
<td>61.7± 9.0</td>
<td>0.04</td>
</tr>
</tbody>
</table>

± indicates mean ± standard deviation
n(%) indicates number of participants and their percentage
TSH: thyroid stimulating hormone, BMI: body mass index, ALT: Alanine Aminotransferase
C-reactive protein (CRP) is affected by many factors, such as exercise, medications and obesity. It was important to identify those who had extreme values (>10mg/L) of CRP and review their chart to see if there were any known conditions that might have caused this spike. Review of the charts revealed four participants who had CRP values that were extreme in the study. Two of the participants were on medications for chronic conditions, such as hypertension and diabetes, which may explain the increased CRP levels found. The remaining two charts did not reveal any information reported in the health questionnaires that could explain the high CRP values. The lack of possible explanations in these two cases could be attributed to the health questionnaires only inquiring about changes in medical conditions or medications. Other situations, such as starting an exercise program, that affect CRP levels, might have occurred without being reported. However, as seen in Table 2, data analyzed without the four extreme values yielded non-significant differences between the intervention and control phases of the study.

Table 2. CRP Analysis without Outliers

<table>
<thead>
<tr>
<th>Outcome Variables</th>
<th>N</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>25th %</th>
<th>75th %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q1</td>
<td>Q3</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (intervention phase)</td>
<td>86</td>
<td>1.70±1.79</td>
<td>0.98</td>
<td>0.30</td>
<td>8.68</td>
<td>0.56</td>
<td>2.0</td>
<td>0.67</td>
</tr>
<tr>
<td>C-reactive protein (control phase)</td>
<td>85</td>
<td>1.50±1.61</td>
<td>0.92</td>
<td>0.30</td>
<td>8.94</td>
<td>0.51</td>
<td>1.97</td>
<td></td>
</tr>
</tbody>
</table>

Outliers are values 10 and greater
± indicates mean ± standard deviation
Since analysis of the averaged values for CRP from weeks 4 and 5 did not yield any significant differences between the study phases, analysis of week 5 values were carried out. Analyzing the CRP values for week 5 only did not yield any significant differences between the two study phases either, as is shown in Table 3.

**Table 3. CRP Analysis Comparing Week 5 of each Study Period**

<table>
<thead>
<tr>
<th>Outcome Variables</th>
<th>N</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>25th %</th>
<th>75th %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive Protein</td>
<td>82</td>
<td>1.88±3.06</td>
<td>0.94</td>
<td>0.30</td>
<td>22.14</td>
<td>0.52</td>
<td>2.08</td>
<td>0.85</td>
</tr>
<tr>
<td>Week 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive Protein</td>
<td>84</td>
<td>1.97±3.10</td>
<td>0.98</td>
<td>0.30</td>
<td>17.17</td>
<td>0.52</td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Blood samples were not available for 6 in the first study phase while 4 were missing in the last week of the end of the study.

Table 4 shows that there was no significant change in CRP and antioxidant levels between the intervention and control phases that can be attributed to time. Also there was no significant difference in CRP and antioxidant levels (using the ABTS test) between those randomized to the intervention or control phase. Group effect for the FRAP test indicated that there may have been a difference between the groups’ antioxidant level at randomization since the p-value was borderline significant.

**Table 4. General Linear Model Repeated Measures Analysis**

<table>
<thead>
<tr>
<th></th>
<th>CRP</th>
<th>FRAP</th>
<th>ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-value</td>
<td>P-value</td>
<td>F-value</td>
</tr>
<tr>
<td>Period effect (Pre- Post)</td>
<td>0.2</td>
<td>0.69</td>
<td>0.8</td>
</tr>
<tr>
<td>Group effect</td>
<td>0.2</td>
<td>0.66</td>
<td>3.7</td>
</tr>
<tr>
<td>Group*Period Interaction</td>
<td>0.2</td>
<td>0.70</td>
<td>0.2</td>
</tr>
</tbody>
</table>
In this study participants were required to consume a total of 120g of figs everyday during the five weeks of the intervention phase. Table 5 demonstrates that the average grams of figs consumed by participants during the intervention phase were 126.34g and 0.19g during the control phase per 24 hour period. This reveals high compliance rates with study protocol. This successful adherence of the participants to the study protocol can serve as a positive model for other studies of whole food consumption requiring similar procedures.

**Table 5. Average Grams of Figs per Recall**

<table>
<thead>
<tr>
<th>Phase</th>
<th>No. of Observations</th>
<th>Mean±SD</th>
<th>Median</th>
<th>25% Q1</th>
<th>75% Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>235</td>
<td>126.3±40.8</td>
<td>134.40</td>
<td>109.20</td>
<td>151.20</td>
</tr>
<tr>
<td>Control</td>
<td>243</td>
<td>0.19±2.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The study protocol called for obtaining 3 dietary recalls per study phase, for a total of 6 recalls for the study. Table 6 demonstrates that of the 83 participants who completed 2 or more dietary recalls, 71 completed all 6 and 1 person completed only two. The maximum number of recalls required was successfully obtained in 85% of participants.

**Table 6. Number of Recalls Completed**

<table>
<thead>
<tr>
<th>Number of recalls</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Frequency</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>1.2</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1.2</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>3.6</td>
<td>5</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>8.4</td>
<td>12</td>
<td>14.5</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
<td>85.5</td>
<td>83</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 7 shows the number of recalls completed per study phase. As indicated below, more participants successfully completed the 3 required dietary recalls in the control phase than in the intervention phase (78 versus 73 participants).

**Table 7. Number of Recalls per Phase**

<table>
<thead>
<tr>
<th>Study Phase</th>
<th>Number of Recalls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Intervention</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Frequency</td>
<td>2.41</td>
<td>3.61</td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Frequency</td>
<td>.60</td>
<td>2.41</td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Frequency</td>
<td>3.01</td>
<td>6.02</td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8 shows the frequency of recall according to the day of intake. Results show that there were almost twice as many recalls done during the weekdays than there were on the weekend. This confirms high adherence to the study protocol that called for obtaining two weekdays and one weekend day recalls per study phase, for a total of 6 for the study.
Table 8. Distribution According to Day of Intake

<table>
<thead>
<tr>
<th>Study Condition</th>
<th>Weekday</th>
<th>Weekend</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>157</td>
<td>78</td>
<td>235</td>
</tr>
<tr>
<td>Percent</td>
<td>32.85</td>
<td>16.32</td>
<td>49.16</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>161</td>
<td>82</td>
<td>243</td>
</tr>
<tr>
<td>Percent</td>
<td>33.68</td>
<td>17.15</td>
<td>50.84</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>318</td>
<td>160</td>
<td>478</td>
</tr>
<tr>
<td>Percent</td>
<td>66.53</td>
<td>33.47</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 9 shows that fig intake did not differ significantly by kcalories or grams of figs consumed between participants in the intervention or control phase.

Table 9. T-test for Difference in Intake Between the Two Study Phases

<table>
<thead>
<tr>
<th>T-Tests</th>
<th>DF</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gramweekend - gramweekday</td>
<td>80</td>
<td>0.15</td>
<td>0.8823</td>
</tr>
<tr>
<td>kcalweekend - kcalweekday</td>
<td>80</td>
<td>0.88</td>
<td>0.3811</td>
</tr>
</tbody>
</table>
CHAPTER 6
CONCLUSIONS

A. Summary and Limitations

Despite being an excellent source of polyphenols, figs did not affect CRP or antioxidant levels in the body in participants with elevated cholesterol values. Mineral values for iron, magnesium, potassium, calcium and copper were significantly higher during the intervention phase than the control phase. Also analysis of systolic blood pressure values showed a decrease of 2 mmHg between the two study phases that, although not statistically significant, might have a clinical significance that warrants further investigations. During the intervention phase, participants were asked to incorporate a package of figs in their habitual diet at each meal every day for 5 weeks. Since each package was 330 kcals, weight gain was a concern.

Despite consuming more calories overall, there was no significant overall weight gain between the participants in the two phases. This finding can be attributed to the fact that participants mostly consumed the additional calories from carbohydrates and not from fat and protein. Carbohydrate kcals are more readily available to be used as fuel and thus are utilized by the body for energy first while fat consumed is usually stored. Weight analysis for females showed a slight but significant gain of 0.5kg during the intervention phase. Since weight gain is one of the factors affecting CRP levels, it might have counteracted the effects of the figs. However, further studies are needed to support this conclusion.
This study had several limitations. Participants self-selected into the study, and might have had better diets and exercise routines, thus somewhat limiting our ability to generalize. However, randomization would have addressed any systematic differences between the two study conditions thereby at least assuring internal validity. In addition, polyphenol content of the California mission figs consumed in this study was not evaluated. This introduces the possibility that the quality or quantity of the polyphenols in the figs themselves might have been the reason the study phases did not show significant differences. In addition, the absence of baseline data for CRP and antioxidant levels might have been considered a limitation had the design not been a crossover, providing the before and after effect of the intervention variable.

B. Future Studies

The knowledge gained through the process of this study necessitates its replication using different measurement methodology. In this current study, blood samples were drawn from participants after a 12 hour fast, which may have led to the lack of significant differences in antioxidant levels between the two study phases. According to Osakabe, Baba and Yasuda (2001), the majority of antioxidants are metabolized from the blood within 8 hours of consumption. Therefore, direct antioxidant capacity measurements might not have been the optimum measurement method. Urine analysis or direct oxidized LDL measurements, though more costly, might have yielded more reliable results.

While antioxidant levels needed to be assessed within a shorter period of time after ingestion, more time might have been needed to allow for change to occur. Perhaps future studies assessing the effects of fig consumption on CRP should examine
changes within different durations. Furthermore, to offset the issue of weight gain that might occur due to the long term consumption of higher calories, future studies could decrease the grams of figs consumed daily.

As mentioned earlier, we observed that there was a 2 mmHg difference in blood pressure observed between the two study phases that should be taken into consideration. Although this difference was not statistically significant, it might have some clinical implications. Thus further studies are warranted in this area to determine the effects of figs on systolic blood pressure through the actions of polyphenols, fiber, both or another factor altogether. This study analyzed quantitative data only, therefore, to gain additional information and a wider perspective, future studies should include qualitative data as well. For instance, health questionnaires completed by participants can be used to evaluate levels of satiety during the intervention phase and the control phase, as well as the effect of fig consumption on bowel movements. Analysis of fig intake showed that it was possible for participants to consistently eat the same food with each meal successfully for 5 weeks (Table 4), allowing for the possibility that future studies can conduct similar protocols. Also, the relationship between cholesterol levels and CRP values was not explored in this study due to time and financial restrictions. However, there is enough evidence to suggest that elevated cholesterol levels might raise CRP levels and thus a correlation between the two variables should be investigated.

C. Conclusions and Implications

Figs consumed for 5 weeks did not affect CRP or antioxidant levels in participants with elevated cholesterol levels. Despite this finding, figs significantly increased mineral values, including iron, calcium, magnesium, potassium and copper.
This is important because these minerals are essential to healthy lifestyles and disease prevention. Finally, this study also showed that studies with foods that increase caloric intake as well as carbohydrate intake can be done without significant weight gain and with high compliance. This holds implications for future studies with figs and other foods.
References


California Fig Advisory Board (2008). Retrieved January 3, 2008 from Fig Advisory Board Website: http://www.calfreshfigs.com/index-5.html


Centers for Disease Control and Prevention (1998). Retrieved May 15, 2009, from CDC Website:

http://www.cdc.gov/nccdphp/dnqa/nutrition/nutrition_for_everybody/iron_deficiency/index.htm#Risk

Centers for Disease Control and Prevention (2007). CDC MMWR. Retrieved August 20, 2008, from CDC Website:

http://www.cdc.gov/mmwr/preview/mmwrhtml

Centers for Disease Control and Prevention (2009). Retrieved May 20, 2009, from CDC Website:

http://www.cdc.gov/healthyweight/assessing/bmi/childrens_BMI/about_childrens_BMI


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United States Department of Agriculture (2005). Retrieved November 12, 2008, from United States Department of Agriculture Website:
http://www.cnpp.usda.gov/DietaryGuidelines


APPENDIX A: IRB APPROVAL

INSTITUTIONAL REVIEW BOARD
Initial Approval Notice - Expedited Review
OFFICE OF SPONSORED RESEARCH • 11188 Anderson Street • Loma Linda, CA 92350
(909) 558-4331 (voice) • (909) 558-0131 (fax)

To: Tonstad, Serena
Department: Health Promotion & Education
Protocol: The effects of consumption of California dried mission figs on serum lipid concentrations in hyperlipidemic adults

This study was reviewed and approved administratively on behalf of the IRB. This decision includes the following determinations:

Risk to research subjects: Minimal
Approval period begins 20-Aug-2008 and ends 19-Aug-2009
Stipulations of approval:

Consent Form
Unless IRB has given a specific waiver of informed consent (as documented in the approval stipulations above) the IRB-approved and stamped consent form accompanies this letter. This now becomes the official master consent form for making copies to provide to study participants.

Adverse Events / Protocol Changes
The IRB should be notified in writing of any modifications to the approved research protocol. Adverse effects must be reported to the IRB in accordance with institutional policy. If sponsor or contractual adverse event reporting requirements differ from requirements for reporting to IRB, all reporting requirements must still be met.

Protocol Review
Your protocol is tentatively scheduled for review and renewal at least two weeks prior to the approval end-date indicated above. To assure uninterrupted approval of this project, you will be sent a report form to request renewal by completing and timely returning to Office of Sponsored Research. Anticipate the approval expiration so your study does not lapse; contact IRB for assistance if necessary. In addition to reporting the requested renewal status information, you may also use the form to close the study at that time, if applicable.

Records
All records relating to this project, including signed consent forms, must be kept on file for three years following completion of the study. Please note the PI's name and the IRB number assigned to this IRB protocol (as indicated above) on any future communications with the IRB. Direct all communications to the IRB c/o the Office of Sponsored Research. Thank you for your cooperation in LLU's shared responsibility for the ethical use of human subjects in research.

Signature of IRB Chair/Designee: [Signature]

Loma Linda University Adventist Health Sciences Center holds Federalwide Assurance (FWA) No. 6447 with the U.S. Office for Human Research Protections, and the IRB registration no. is IOR6228. This Assurance applies to the following institutions: Loma Linda University, Loma Linda University Medical Center (including Loma Linda University Children's Hospital, LLU Community Medical Center), Loma Linda University Behavioral Medicine, and affiliated medical practices groups.

IRB Chair:
Rhodes L. Rigby, M.D.
Department of Medicine
(909) 558-2341, rigby@ahs.llumc.edu

IRB Administrator:
Linda G. Halstead, M.A., Director
Office of Sponsored Research
Ext 43570, Fax 80131, halstead@univ.llu.edu

IRB Specialist:
Mark Testerman
Office of Sponsored Research
Ext 43042, Fax 80131, mtesterman@llu.edu

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APPENDIX B: INFORMED CONSENT

The Effects of Consumption of California Dried Mission Figs on Serum Lipid Concentrations in Hyperlipidemic Adults

Purpose and Procedures
You are invited to participate in a research study because your blood cholesterol is not optimal. The purpose of this study is to identify whether eating California dried mission figs three times daily with meals for five weeks lowers total cholesterol and low density lipoprotein (LDL) cholesterol in adults with above optimal, borderline or high cholesterol levels.

At the first visit you need to be fasting. You will be asked questions about your health history and your blood lipids, glucose and liver function will be measured. We will check if you meet the requirements for participation. If you do, we will call you up to arrange the next visit. At this visit you will be assigned through chance to start eating 1 portion (1/4 cup) of prepackaged California dried mission figs with your meals three times daily for 5 weeks OR to continue your usual diet. If you start with the figs, after five weeks you will return to your usual diet. If you start with your usual diet, after five weeks you will start eating figs as described above. Figs will be provided at no charge.

The study involves 8 visits in the course of the 12 weeks. The purpose of the visits is to deliver the figs and take blood tests. All the study visits are required. There will be blood drawings after 4 and 5 weeks and again after 11 and 12 weeks of the study. This adds up to a total of 5 blood drawings. At each blood drawing two tubes of blood totaling 5 tablespoons will be obtained. The results of the blood tests after the first one will not be available until after the end of the study.

We will measure your body weight, waistline and blood pressure at 6 of the 7 study visits. You will be asked to report your food intake during the last 24 hours on 6 occasions. On 4 of these occasions you will be called up at home by a dietitian for a telephone report. The other two reports will be done during site visits.

Who can take part
Men and women aged 25 to 75 years with LDL cholesterol that are in the above optimal (100-129 mg/dL), borderline (130-159 mg/dL) or high (160-189 mg/dL) range can take part. If your cholesterol is treated with medications you cannot take part. If your cholesterol is in the high range, we need the permission of your physician. If you have other diseases that are the cause of your high cholesterol you cannot take part. If you have type 1 diabetes, type 2 diabetes that is not well controlled or use certain medications you cannot take part.

Initials and date: ____________________

Page 1 of 3
Risk

Pain and minor bruising at the site of the needle insertion and possible lightheadedness upon arising after the blood is drawn are possible complications of blood drawing. The committee at Loma Linda University that reviews human studies (Institutional Review Board) has determined that participating in this study exposes you to minimal risk.

Benefits

You may not receive any direct benefit from participating in this study. You will be told your cholesterol levels and receive dietary advice about lowering blood cholesterol. The dietary changes including figs may lead to lower blood cholesterol. The benefit to humanity is an understanding of whether a simple dietary change (eating dried figs) can lower blood cholesterol.

Participants’ Rights

Your participation in this study is voluntary. Your decision whether or not to participate or terminate at any time will not affect your present or future medical care. If you decide to stop, please inform the study investigator.

Confidentiality

All of the information that is collected in this study will be kept strictly confidential. Information that we collect will be assigned linking codes. All this information will be stored separately from your name and other personal data. Only the primary investigator will have linking access. Any publication resulting from this study will refer to the participants as a group.

Additional Costs/Reimbursement

You will be given free figs for the duration of the study. There is no cost to you for participating in this study. The blood tests will be performed at no cost. There is a modest monetary compensation of $25.00 for your effort upon completion of the study.

Impartial Third Party Contact

If you wish to contact an impartial third party not associated with this study regarding any question or complaint you may have about the study, you may contact the Office of Patient Relations, Loma Linda University Medical Center, Loma Linda, CA 92354, or call the Office of Patient Relations at (909) 559-4647 for information and assistance.

Informed Consent Statement

I have read the contents of this consent form, and have listened to the verbal explanation given by the investigator. My questions concerning this study have been answered to my satisfaction. I hereby give voluntary consent to participate in this study. This consent does not waive my rights, nor does it release the investigators, institution, or sponsors from their responsibilities. I may call the graduate student investigator, Joycelyn M. Peterson or the faculty advisor, Serena Tonstad, MD PhD at Loma Linda University, Department of Health Education & Promotion during routine office hours at (909) 558-4741 if I have additional questions or concerns. I have been given a copy of this letter for future reference.

Initials and date: _______________
I have reviewed the contents of the consent form with the person signing above. I have explained potential study risks and benefits.

Joycelyn M., Peterson MPH, RD  
Department of Health  
Education & Promotion  
Loma Linda University  
(909) 558-8577  

Serena Tonstad, MD PhD  
Department of Health  
Education & Promotion  
Loma Linda University  
(909) 558-4575

Rasha Abdrabou, MPH  
Department of Health  
Education & Promotion  
Loma Linda University  
(909) 558-8577
## APPENDIX C: GANNT CHART

<table>
<thead>
<tr>
<th>Activity</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aug</td>
<td>Sept</td>
</tr>
<tr>
<td>Literature Review</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Concept Paper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Write proposal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defend proposal</td>
<td></td>
<td></td>
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<tr>
<td>IRB approval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recruit for fig study data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline blood sample data entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Review data analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Committee review</td>
<td></td>
<td></td>
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<tr>
<td>Write publishable papers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral defense</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Submit final copy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX D: STUDY BUDGET

Fig concentrate
(4oz x 90 days = 360)

100 subjects = 72,000 oz (4,500lbs)

Fig cost and freight by CAL. Fig Advisory Board.

Blood samples (screening period)
$27.00 each X125 participants

Fig Advisory Board

Fig cost and freight by CAL. Fig Advisory Board.

Four blood samples
$15.00 each x 4 = $60.00 x 100 subjects

Blood samples (screening period)

$3,375.00

2Phlebotomist @$17.00 per hr for 36 hrs

$1,224.00

Computer software to analyze diets (2)

Statistician

UC Davis Laboratory fees for CRP analysis

$14,650.00

2 Dietitians at $17.00 per hour x 300 hrs

$10,000.00

Statistician

$2,830.00

Compensation for each subject ($25.00 x 100)

$2,500.00

Estimated University cost for use of facility and equipment

at Drayson Center 6 sessions by $100.00 per session

$600.00

Recruitment for 100 participants 500 flyers

$500.00

Misc.

$600.00

Grand total

$42,279.00
APPENDIX E: PARTICIPANT QUESTIONNAIRE (BASELINE)

LOMA LINDA UNIVERSITY
FIG RESEARCH STUDY

Welcome to the fig research study! Please take a few minutes to complete the following questionnaire. It will be used to facilitate information only and does not represent a diagnostic evaluation. If you are uncomfortable answering any of these questions, leave them blank. Thank you for taking the time to fill out this questionnaire.

Name: ____________________________ Date of Birth: ____________________

Gender (please circle): Male or Female

Ethnicity (please circle): Asian, Black (African American or Caribbean), Hispanic, White

Telephone number:
(Home): ____________ (Cell): ____________ (Work): ____________

Mailing Address: ________________________________________________________

______________________________________________________________

Email address and fax: _________________________________________________

I. Are you under medical care for any of the following conditions or diseases? If yes, please circle

High blood pressure arteries) Angina pectoris (chest pain due to blocked

Stroke Blocked arteries in legs

Diabetes type 2 arteries Heart attack or surgery for blocked coronary

Gallbladder disease Diverticulosis

Irritable bowel syndrome Heartburn or esophageal reflux

Cancer (write in what type ________) Thyroid disease

Family history of high cholesterol High blood cholesterol

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Food allergies or intolerances (please write in) ____________________________________________

__________________________________________

Other: ______________________________________

__________________________________________

B. Please list all current medications, pain meds, vitamin or other supplements, and over the counter meds that you are taking at present

__________________________________________

__________________________________________

__________________________________________

__________________________________________

C. What has been your highest cholesterol reading? __________________________

Approximate date: __________________________

What was your most recent cholesterol reading? __________________________

Approximate date: __________________________

Are you taking medication to lower cholesterol (within the last two months) Circle Yes or No

D. Do you engage in any regular physical activity? Circle Yes or No

What type and how often? __________________________________________

__________________________________________

__________________________________________

E. Do you smoke? Circle Yes or No

If yes, for how long and how much __________________________

If you quit smoking within the past year, please write the date: __________________________

Signature ______________________________________
APPENDIX F: SHORT HEALTH HISTORY QUESTIONNAIRE

Have there been any changes in your medical condition since the start of the study?

_____ No

_____ Yes

If yes, describe.

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

Have there been changes in your use of medication since the start of the study?

_____ No

_____ Yes

If yes, describe.

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

At crossover or final visit:

On a scale of 1 to 10, with 1 indicating not at all, and 10 indicating very much, how well do you like eating the provided figs three times a day?

Not at all 1 2 3 4 5 6 7 8 9 10 Very much

Have you noticed any effects of figs on your overall health?

________________________________________________________________________

________________________________________________________________________
APPENDIX G: COMPLIANCE SHEET

LOMA LINDA UNIVERSITY SCHOOL OF PUBLIC HEALTH

FIG COMPLIANCE SHEET

Dear Participant,
Please take the time at the end of each day to circle Yes or No for each meal in the boxes provided below, to indicate whether or not you ate the required amount of figs. Begin at the first week (one) to the fifth week (five)
Thank you for taking the time to fill out the compliance sheet.

Any questions please call your research interventionist Joy Peterson at 909-261-0359, or email to joysfigresearch@yahoo.com

Circle in the box at the end of each day: (Yes) or (No)

<table>
<thead>
<tr>
<th>Week</th>
<th>Sunday</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Lunch</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Dinner</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Two</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Lunch</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Dinner</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Three</td>
<td></td>
<td></td>
<td></td>
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APPENDIX H: 24 HOUR DIET RECALL

24-Hour Dietary Recall Collection

The NCC Service Center has extensive expertise and experience in conducting 24-hour dietary recall interviews via telephone with a variety of populations, including children. Trained and certified NCC staff will train the study dietitian to conduct the 24-hour dietary recall interviews. Interviews take place over the telephone using NDSR, and are conducted by Joycelyn Peterson. Because dietary practices have been found to vary by time of week, interviews are scheduled to capture both weekend and weekday intakes. The NDSR multiple-pass interview methodology allows the respondent repeated opportunities to recall their intake within the past 24 hours and to provide detailed food descriptions. Food portion estimation visual aids are provided to respondents to assist in portion size estimation.

Contact person is Mary Stevens at 612.626.9428 or steve004@umn.edu for more information.
APPENDIX I: COMMON SWEET SNACKS AND DESSERTS CONTAINING ≈ 330 KCALORIES.

Three servings of figs = 330 kcalories

<table>
<thead>
<tr>
<th>Type</th>
<th>Serving size</th>
<th>kcalories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate chip</td>
<td>4 oz</td>
<td>350</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>4 oz</td>
<td>360</td>
</tr>
<tr>
<td>Oreo</td>
<td>4 oz</td>
<td>320</td>
</tr>
<tr>
<td>Pound cake</td>
<td>3 oz</td>
<td>330</td>
</tr>
<tr>
<td>Brownie</td>
<td>3 oz</td>
<td>350</td>
</tr>
<tr>
<td>Two fig bars</td>
<td>4 oz</td>
<td>320</td>
</tr>
<tr>
<td>Apple pie</td>
<td>4 oz</td>
<td>340</td>
</tr>
<tr>
<td>Carrot cake</td>
<td>3 oz</td>
<td>350</td>
</tr>
<tr>
<td>Granola bar</td>
<td>6 oz</td>
<td>360</td>
</tr>
<tr>
<td>Three Musketeers candy bar</td>
<td>3 oz</td>
<td>360</td>
</tr>
<tr>
<td>Ice cream</td>
<td>4 oz</td>
<td>420</td>
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</table>
**APPENDIX J: TOTAL ANTIOXIDANT CAPACITY (ABTS) TEST PROTOCOL**

**Total Antioxidant Capacity (ABTS)**

**Procedure**
1. Prepare ABTS solution (see below)
2. Dilute plasma samples 1:5
   
   *(Vortex thawed samples and centrifuge for 10 min. at 3500 rpm)*
   
   50 μl plasma + 200 μl PBS (saline)
3. Prepare plate with 20 μl blank (PBS), standard, or sample
4. Set plate reader as follows:
   
   Temperature: 37°C
   
   Dispense 180 μl ABTS solution
   
   Delay for 3:30 minutes
   
   Shake: Medium for 0:30 seconds
   
   Read: 734 nm

**Solutions**
1. Trolox Stock Standard – 2.0 mmolar in ethanol
   
   \[ \text{FW} = 250.29 \]
   
   \[ g = 0.002 \times 250.29 \times 0.1 = 0.050 \]
   
   **To make 100 ml:**
   
   Weigh 0.050 g (50 mg), add to 100 ml ethanol
   
   Portion into screw-cap vials and keep in -80°C freezer

   **Working standards:**

<table>
<thead>
<tr>
<th>Stock standard</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD 1 (0.4)</td>
<td>2 mℓ</td>
</tr>
<tr>
<td>STD 2 (0.2)</td>
<td>1 mℓ</td>
</tr>
<tr>
<td>STD 3 (0.1)</td>
<td>1 mℓ</td>
</tr>
<tr>
<td>STD 4 (0.05)</td>
<td>1 mℓ</td>
</tr>
</tbody>
</table>

2. ABTS solution (50 ml)
   
   ABTS (FW = 548.68)
   
   \[ g = 0.23 \times 548.68 \times 0.1 = 0.0126 \text{ g} \]

   AAPH (FW = 271.2)
   
   \[ g = 0.23 \times 271.2 \times 0.1 = 0.062 \text{ g} \]

   **Add ABTS and AAPH to 50 ml buffer solution. Heat to between 60 and 70°C and hold for 20 minutes** (solution should turn a dark blue color). Allow reagent to cool to room temperature before using in assay. The solution should be used the day it is prepared; 50 ml is sufficient for 2 plates.

3. Sodium phosphate buffer
   
   Add 3.1 g NaH₂PO₄·H₂O and 10.9 g Na₂HPO₄ (anhydrous) to boiling distilled water to make up **1 L of solution**. The pH of the final solution should be 7.4.

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APPENDIX K: FERRIC REDUCING ANTIOXIDANT POWER (FRAP) TEST PROTOCOL

Synergy- Ferric Reducing Antioxidant Power Assay (FRAP)  
11-12-08  

Reagent Preparation
Solutions for the FRAP reagent
1) Prepare 100 ml of 10mM Tripyridyltriazine (TPTZ) in 40 mM HCl:  
   Mix 0.312g of TPTZ  
   0.324ml of HCl (325μl)  
   and distilled H₂O in an Erlenmeyer flask to make up 100ml of solution.

2) Prepare 100ml solution of 20mM FeCl₃·6H₂O:  
   Mix 0.54g of 20mM FeCl₃·6H₂O  
   and distilled H₂O in an Erlenmeyer flask to make up 100ml of solution.

3) Prepare 1000ml solution of 300mM acetate buffer, pH 3.6:  
   Mix 3.1g of sodium acetate 3 H₂O  
   16ml glacial acetic acid  
   and distilled H₂O in an Erlenmeyer flask to make up 1000ml of solution.

FRAP Reagent: 
Mix solutions 1, 2, & 3 together (Ratio: 10:1:1 of buffer:TPTZ:FeCl₃)  
Wrap final solution in foil and store in below room temperature conditions until actual use.

Standards
1) Trolox Stock Standard (0.002M)  
   Trolox is 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (FW 250.29)  
   To make 100ml of Stock Standard,  
   \[ G = 0.002 \times 250.29 \times 0.100 = 0.050g \]
   Weigh out 0.050g Trolox and mix with 2ml of ethyl alcohol. Add distilled H₂O to make up 100ml in volumetric flask. Portion into 2ml vials and store in freezer at -70°C until use.

2) Trolox Working Standards

<table>
<thead>
<tr>
<th>Dilution 5:1</th>
<th>Dilution 10:1</th>
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<tbody>
<tr>
<td><strong>Level 4</strong></td>
<td><strong>Level 2</strong></td>
</tr>
<tr>
<td>2ml Trolox + 8ml distilled H₂O (10ml total)</td>
<td>1ml level 3 + 1ml distilled H₂O</td>
</tr>
<tr>
<td>500μl Trolox + 9.5ml distilled H₂O (10ml total)</td>
<td>1ml level 3 + 1ml distilled H₂O</td>
</tr>
<tr>
<td>1ml level 4 + 1ml distilled H₂O</td>
<td>1ml level 4 + 1ml distilled H₂O</td>
</tr>
</tbody>
</table>
Plasma dilution

<table>
<thead>
<tr>
<th>Dilution 5:1</th>
<th>Dilution 10:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>100µl plasma + 400µl distilled H2O</td>
<td>100µl plasma + 900µl distilled H2O</td>
</tr>
</tbody>
</table>

Synergy set-up
Measurement mode: Abs
Reaction mode: End-point
Blank: Reagent
Wavelength: 593nm
Temperature: 37°C
Standard/sample volume: 20µl
Reagent volume: 200µl
Shake: 5 minutes and read at 5 minutes