Effects of Coconut Oil Supplementation on Biomarkers of Inflammation and Lipid Peroxidation

by

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ABSTRACT

Objective: The purpose of this randomized, placebo-controlled trial was to investigate the effect a daily coconut oil supplement (2 grams) would have on a common serum marker of systemic inflammation (C-reactive protein) and an indicator of oxidative stress (TBARS) when compared to the control group receiving a placebo capsule (white flour) in healthy, sedentary adults between the ages of 18-40 in Phoenix, Arizona.

Design: This study was designed as secondary analyses of blood samples originally collected to study the effects of coconut oil supplementation on blood lipids and body composition. The original study consisted of 32 healthy, adult volunteers recruited from the Arizona State University campus in Phoenix, Arizona. Participants followed no food restrictions or special diets, exercised less than 150 minutes per week, had no diagnoses of chronic disease, were not taking statin medications, were non-smokers, and no female participants were pregnant. Participants were randomized into either the Coconut Oil group (CO) or the Placebo group (PL) at week 0, and baseline blood samples and anthropometric measurements were obtained. Each participant completed an 8-week protocol consisting of two supplement capsules daily (coconut oil or placebo). Final fasting blood samples and anthropometric measurements were taken at week 8. This study analyzed the blood samples for measurements of C-reactive protein (CRP) and thiobarbituric reactive substance (TBARS).

Results: Eight weeks of 2 grams per day coconut oil supplementation, in comparison to placebo treatment, did not significantly reduce serum CRP (-13% and +51%

respectively, p=0.183) but did significantly increase TBARS (+16% and -27% respectively, p=0.049).

Conclusions: Coconut oil supplementation (2 g/day) may impact lipid peroxidation as indicated by an increase in plasma TBARS concentration. Future trials are necessary to corroborate these results using other indices of fatty peroxide formation.

DEDICATION

This work is dedicated to my parents, John Rooyakkers, and Beverly & Dennis Wilkie, who always made me believe I can accomplish anything with motivation and diligence. I would also like to dedicate this to my son, Eric Norman, to Judy Borden, to Michael Stroup, and to my grandma, Helen Rooyakkers. Without your love, support, and encouragement, I would not be the person I am today.

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Chapter 1

INTRODUCTION

Overview

Chronic inflammation and oxidative stress are associated with several age-related conditions including weakness/frailty, dementia, diabetes, atherosclerosis, cardiovascular disease, metabolic syndrome, and cancer. It is estimated that 25% of cancer diagnoses globally may be related to chronic inflammation[28]. Recently, coconut oil and its components have been promoted as functional foods, meaning they provide benefits such as disease prevention and improved health in addition to simple nutrition. Limited studies suggest coconut oil may reduce acute and chronic inflammation, as well as prevent excessive oxidation. Because the accumulated effects over time have such damaging effects, it is of interest to investigate the possible anti-inflammatory and anti-oxidant properties of coconut oil.

Certain dietary components such as fatty acids are known to affect inflammation, while polyphenols exhibit anti-oxidant activities. Coconut oil contains a high amount of lauric acid, and has been shown to reduce markers of inflammation in animal studies[20, 24, 67]. Decreased levels of oxidation markers have also been reported in animals fed coconut oil[39, 40, 41]. Although studies are limited, evidence suggests both anti-inflammatory and anti-oxidant properties of coconut oil. It would be beneficial to investigate these effects in human studies.

No research was found that specifically studied the effect of dietary coconut oil supplementation on serum markers of inflammation or oxidative stress in humans. If coconut oil intake can reduce inflammation (biomarker: C reactive protein [CRP]) and/or

lipid peroxidation (biomarker: thiobarbituric acid reactive substance [TBARS]), replacing pro-inflammatory oils in the diet - such as soybean and corn oils - with coconut oil may reduce the incidence of cardiovascular events and age-related diseases.

Purpose of Study

The purpose of this study was to determine the effect of a daily coconut oil supplement (2 grams) on clinical markers of chronic inflammation and oxidative stress as compared to a placebo (white flour). Each experimental group consumed two one-gram capsules daily of either the coconut oil supplement or placebo. Few clinical studies have examined the relationship between coconut oil consumption and inflammation or oxidative stress.

Research Aim & Hypothesis

We hypothesized that a daily supplement of coconut oil would result in lower levels of markers of inflammation (CRP) and oxidative stress (TBARS) when compared to a placebo control in healthy adults.

Definition of Terms

Acute Inflammation: Acute inflammation is the initial process the body employs to protect itself from trauma or invading pathogens. It involves a cascade of events, which includes the activation of endothelial cells and tissue macrophages; the recruitment of leukocytes, granulocytes, and adhesion molecules; the activation of platelets and clotting systems; and other systemic responses to resist pathogens and manage injuries. The acute response increases rapidly within minutes to hours and declines gradually as the event is resolved[62]. Symptoms of acute inflammation include fever, swelling, pain, rash, and redness[25].

Chronic Inflammation: Chronic inflammation is a prolonged state of mild to moderate inflammation associated with mononuclear immune cells including lymphocytes, macrophages, and plasma cells. The response is initiated by vascular adhesion molecules, which interact with lymphocytes and monocytes that eventually migrate to extravascular spaces[62]. Chronic inflammation can lead to tissue damage and fibrous tissue accumulation[28].

Oxidative Stress: Oxidative stress is a continued state of excessive production of reactive oxygen species (ROS), which overwhelms the system of anti-oxidant defenses. Oxidative stress damages cells and leads to cell death, which can contribute to cancer, atherosclerosis, and other age-related diseases[13].

Delimitations

Participants in this study were healthy, sedentary, adults between the ages of 18-40 years. Our results may not apply to children, pregnant women, active adults, older adults, or people who are overweight or have a chronic disease condition. Also, these results may not generalize to other brands or coconut oil products, or to differing dosage levels.

Limitations

A limiting factor of this study is participant compliance. Although participants were instructed to mark their daily supplement consumption on a provided compliance calendar, investigators did not directly witness participants taking the supplement. A second limitation is lack of a controlled diet or environment. Dietary and environmental factors other than coconut oil likely affect serum markers of inflammation and lipid peroxidation. The duration of the study and low dose of coconut oil may also affect the results. A study lasting longer than eight weeks and/or a higher dose of coconut oil may

achieve different results. Finally, the method of coconut oil production could affect the results (RCO vs VCO).

Chapter 2

LITERATURE REVIEW

A. Coconut Oil - Overview

Coconut oil is a mild flavored, mostly colorless oil produced from mature coconuts. It is a clear liquid above 76°F (24C) and a white or light brown solid at lower temperatures. Refined coconut oil (RCO) is produced by pressing dried coconut kernel (copra), and then chemically refining, bleaching, and deodorizing the extracted oil. RCO can be partially hydrogenated similar to vegetable oils by a reaction using hydrogen and a catalyst. This process raises the melting point of the oil by reducing the number of double bonds in the molecule. An undesirable effect is the creation of trans-fatty acids, which increase cardiovascular risks similar to saturated fats[1, 37].

Virgin coconut oil (VCO) is produced without chemicals or high heat[15]. According to Onsaard et al (2005), the simplest method of obtaining unrefined coconut oil is from coconut milk[44]. This "wet extraction" is a three-stage process that involves creaming the coconut milk, clustering the oil globules, and allowing the globules to merge, or coalesce. This method is a simple way to make coconut oil at home. Seow and Gwee (1997) describe the process of extracting oil from coconut cream by chilling, freezing, and thawing after centrifugation[54]. The oil can also be separated by fermentation. Che Man et al (1997) successfully used Lactobacillus plantarum to extract 95% oil from a mixture of 50/50 grated coconut and water. In addition, various enzymes can be used to extract the oil by breaking down the carbohydrate components of coconut meat[10].

Coconut oil has a variety of uses ranging from food and cooking to industrial applications. Throughout history, people have used coconut oil for its nutritional and

medicinal benefits. In tropical areas, coconut oil has been the main source of dietary fat for centuries. It can be used as a shortening in baked goods, and because the smoke point is about 360°F, coconut oil is an effective oil for medium-heat frying or sautéing. The medium-chain triglyceride component of coconut oil is used in some parenteral (intravenous) and enteral (tube) feeding formulas, as well as pre-term infant formulas because these types of fatty acids are easier to digest and more rapidly metabolized[8]. Coconut oil is a safe and effective moisturizer, produces a high-lather soap due to is solubility in hard water, and can be used in cosmetics, toothpaste, lotions, sunscreens, laundry detergents, as well as several other self-care and household items[30].

B. Composition of Coconut Oil

According to the National Nutrient Database published by the United States Department of Agriculture, one tablespoon of coconut oil provides 121 kilocalories, 13.7 grams total lipid (fat), 0 grams protein, 0 grams carbohydrate, and less than significant amounts of vitamins or minerals[2].

Fatty Acids: Coconut oil contains 99.9% fatty acids. Saturated fatty acids (SFA) make up by far the most prominent component (91.9%) of coconut oil. In comparison, butter contains approximately 52% saturated fat. Although the connection between coconut oil and cardiovascular disease has recently been questioned, current dietary recommendations consider it a saturated fat, which should be consumed as less than 10% of total energy intake[22]. The remaining fatty acids consist of (6.4%) monounsaturated fatty acids (MUFA) and (1.5%) polyunsaturated fatty acids (PUFA). Because coconut oil is a plant product, it does not contain cholesterol.

Although the saturated fatty acid content is higher than other edible oils, unlike long-

chain fatty acids (LCTs) in animal fats, the saturated fatty acids in coconut oil are mainly short and medium-chain triglycerides (MCTs). Medium-chain triglycerides are metabolized more efficiently than LCTs, and are used as energy rather than stored in adipose tissue[8, 35]. Almost 50% of the fatty acid content of coconut oil is lauric acid (C12), which is much higher than canola oil, butter, or palm oil (Figure 1). After a review of the scientific literature, Fabian Dayrit (2014) concluded coconut oil should be more specifically referred to as a "medium chain triglyceride" rather than a general "saturated fat" due to distinct biochemical differences between C12 and long-chain fatty acids[14].

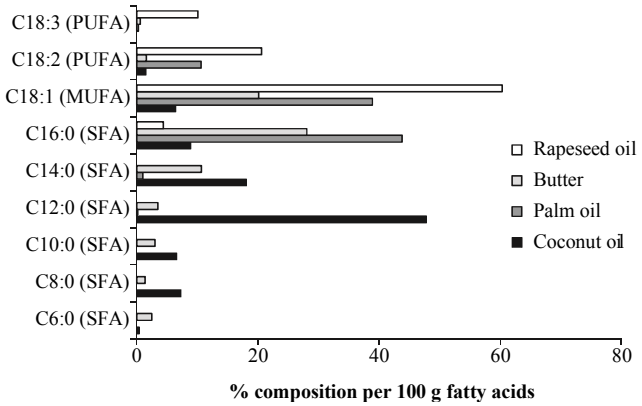


Figure 1. Comparison of the fatty acid composition of selected edible oils and fats. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Data source: McCance and Widdowson's Composition of Foods (PHE 2015)[30].

Medium-Chain Fatty Acids and Medium-Chain Triglycerides: Medium-chain fatty acids (MCFAs) are saturated fatty acid molecules comprised of 6, 8, or 10 carbons. These include capronic acid (C6, hexanoic acid); caprylic acid (C:8, octanoic acid); and capric acid (C:10, decanoic acid). Many classifications also include lauric acid (C:12, dodecanoic acid) because its biochemical actions are more similar to MCFAs than to fatty acids comprised of 14 carbons or more. In general, triglycerides are composed of three fatty acids attached to a small, three-carbon glycerol molecule. Medium-chain

triglycerides (MCT's) are specifically triglycerides in which at least two of those fatty acids are medium-chained in length[34, 38].

Although most dietary fats are long-chain fatty acids (LCFAs), natural sources of medium-chain triglycerides (MCTs) include tropical oils such as coconut and palm oils, as well as cow's milk and human mother's milk. Commercial MCT oil is synthetically produced by removing the MCFAs (mainly C:8 and C:10) from coconut or palm oil, and then recombining the fatty acid molecules with glycerol in order to create MCT oil. These oils are classified as Generally Regarded as Safe (GRAS) by the United States Food and Drug Administration, and are used clinically to support nutrition in patients requiring total parental nutrition or diagnosed with fat malabsorption, pancreatic insufficiency, and other conditions related to impaired lipid metabolism[34].

Due to their physical and chemical properties, medium-chain fatty acids are absorbed and metabolized by the body more efficiently than long-chain fatty acids, which results in differing physiological effects. While in the intestinal tract, MCTs are more easily broken down into individual fatty acids than LCTs. Similarly, MCFAs are absorbed more efficiently than LCFAs. After absorption, LCFAs are transported via chylomicrons throughout the circulatory system before reaching the liver. MCFAs, on the other hand, travel directly to the liver through the portal vein in order to be converted to energy (Figure 2).

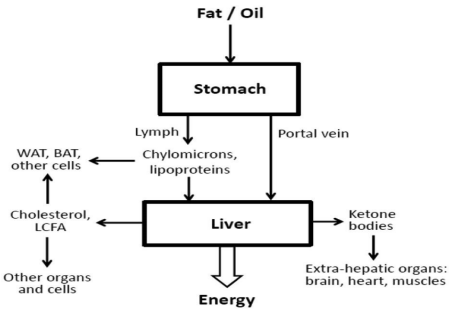


Figure 2. Overview of fatty acid metabolism. This review will follow the steps in the metabolism of lauric acid (C12) presented in this figure. (WAT = white adipose tissue; BAT = brown adipose tissue)[14].

A number of clinical studies and research reviews have been published regarding the effects of MCTs versus LCTs on human health and disease, including obesity, diabetes, and cognitive effects[34, 38].

Lauric Acid: Because lauric acid is the predominant fatty acid in coconut oil, it is of interest to consider the literature specific to this fatty acid. A review of the research reveals a controversy regarding the classification of lauric acid as either a medium-chain or long-chain fatty acid. Some researchers argue that although lauric acid is a saturated fat, it exhibits biochemical and metabolic properties more similar to medium-chain fatty acids.

In support of this argument, Fabian Dayrit (December 2014) proposed that saturated fatty acids should more specifically be classified according to carbon chain-length rather than simply "saturated fats." Since saturated fatty acids comprised of 14 carbons or more exhibit biochemical actions distinct from 6-12 carbon fatty acids, more precise classifications are warranted. After a review of the evidence specific to the 12-carbon lauric acid, Dayrit concluded lauric acid is metabolized similar to the 6-10 carbon fatty acids and should, therefore, be considered a medium-chain fatty acid. In addition, because the fatty acids and triglycerides in coconut oil are comprised of approximately

50% lauric acid, coconut oil is more correctly classified as a medium-chain triglyceride oil than a saturated fat[14].

As mentioned earlier, an important distinction between saturated MCFAs and saturated LCFAs is the difference in absorption and route of transport to the liver. Dietary fats are initially broken down into individual fatty acids in the small intestine by the pancreatic enzyme lipase. Evidence shows triglycerides that have medium-chain fatty molecules attached to the sn-1 and sn-3 positions break down more easily than those with long-chain fatty acids in those positions. Liao et al. used rat lipase to determine this step was 5-8 times faster in MCTs compared to LCTs[14]. Dayrit suggests this indicates the hydrolysis of lauric acid (C:12) occurs more rapidly than longer-chain fatty acids.

After hydrolysis, LCFAs are assembled into chylomicrons and absorbed by lymph vessels lining the intestine before being transported through the circulatory system to the liver. In contrast, MCFAs are diverted to the portal vein and transported directly to the liver from the gastrointestinal tract (Figure 2). This determination is said to be based on the solubility of the individual fatty acid as well as the number of carbons. Dayrit notes that compared to LCFAs, the solubility measurement of lauric acid is closer to C:8-C:10 MCFAs (C:14 or longer – below measurement; C:12 - 0.00077 g/100mL; C:10 - 0.0072 g/100mL; C:8 - 0.0842 g/100mL); animal experiments indicate lauric acid is more likely to be channeled through the portal vein than longer-chained fatty acids [C:12 (72%) > C:14 (58%) > C:16 (41%) > C:18 (28%)][14]. The portal vein transport of lauric acid is further supported by a clinical study with human subjects. Bragdon & Karmen (1960) reported a 2:1 distribution ratio of lauric acid between the portal vein and chylomicrons 6 hours after ingestion of 35g/d of coconut oil, while almost 100% of C:14 chains and

longer were assembled into chylomicrons[6]. From his review, Dayrit concludes the evidence shows lauric acid exhibits metabolic and physiological properties closer to medium-chain fatty acids than to long-chain fatty acids[14].

A review article by Laurence Eyres et al. published in Nutrition Reviews (volume 74, 2016) maintains the classification of lauric acid as a medium-chain fatty acid is inaccurate. Therefore, coconut oil cannot be considered a medium-chain triglyceride oil, and any reported benefits of MCFAs are not applicable to coconut oil[19]. As part of their review of studies regarding dietary coconut oil and risk factors of cardiovascular disease, the authors suggest the metabolic actions of lauric acid are more like long-chain fatty acids. According to a study cited as evidence, 70-75% of lauric acid fatty acids were reportedly absorbed and transported with chylomicrons after dietary intake, rather than diverted to the portal vein. However, the test oil used was a synthetically produced high-lauric oil, which may exhibit properties distinct from natural lauric acid or coconut oil. Eyres et al. further contend the solubility and molecular weight of lauric acid are closer to longer-chain fatty acids, but no evidence is cited to indicate these factors cause lauric acid to be metabolized as a LCFA[19]. As reported by Dayrit, the solubility measurement of lauric acid is less than C:10, while C:14 is below measurable levels. In addition, 72% of lauric acid travels through the portal vein compared to 58% of C:14 myristic acid[14]. Without additional evidence to support the conclusions of Eyres et al., it is reasonable to consider the properties of lauric acid similar to those of C:8-C:10 medium-chain fatty acids. Consequently, it is logical to extrapolate the effects of lauric acid to coconut oil.

Polyphenols: Positive health effects of dietary coconut oil could also be attributed to phenolic compounds. Polyphenols are abundant dietary micronutrients obtained from

plant sources including fruits, vegetables, cocoa, tea, wine, and oils pressed from olives or coconuts. These compounds exhibit beneficial antioxidant activities, protecting cells from damage due to oxidative stress. Excessive oxidative stress is associated with age-related diseases such as cardiovascular disease, cancer, and Alzheimer's disease[31]. Several phenols have been identified in coconut oil, including protocatechuic acid, vanillic, caffeic, ferulic, and p-coumaric acids[32, 53]. Although both VCO and RCO contain these compounds, Marina, et al. (2008) found phenolic content was 7% higher in VCO versus RCO. Polyphenol amount was highest in VCO produced by fermentation and lowest in refined coconut oil (Figure 3)[32].

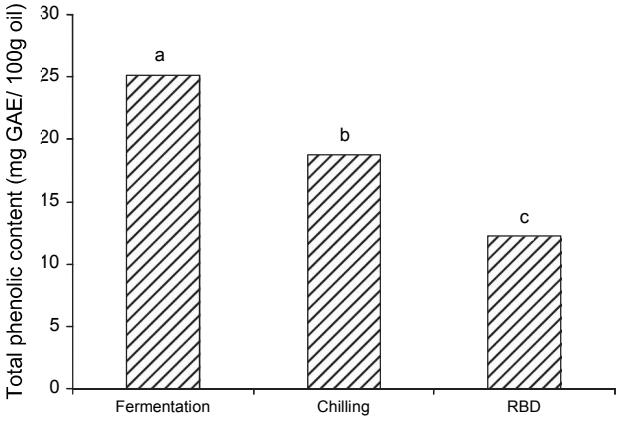


Figure 3. Mean total phenolic content of virgin coconut oil, (fermentation and chilling methods) and RBD coconut oil. Values with different lower case letters are significantly different at P< 0.05 using SAS statistical software (Duncan's multiple range test). GAE, gallic acid equivalents. Source: Marina et al. (2008)[32].

A 2009 review of published studies concluded VCO exhibits higher antioxidant potential than RCO[32]. This review cites several animal studies conducted by Nevin and Rajamahan. For example, reduced levels of thiobarbituric acid-reactive substance (TBARS) were reported in VCO-fed rats, suggesting the polyphenol fraction of VCO is more effective at preventing oxidation of LDL than RCO[39]. Their follow-up study found increased catalase (CAT) and superoxide dismutase (SOD) in rats fed VCO compared to

other oils. Rats fed VCO also exhibited significantly lower levels of lipid peroxide in tissues (heart, liver, kidneys), as well as higher total glutamine (GTN), an indicator of antioxidant status[40]. Additionally, Nevin and Rajamahan reported that when LDL was isolated from Sprague-Dawley rats fed VCO and exposed to oxidant in vitro, oxidation resistance was significantly higher than LDL isolated from rats fed RCO[32, 41].

An interesting point to add is an analysis conducted by Dayrit, et al (2008). Their study compared VCO versus RCO by phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR) using lauric acid to quantify free fatty acid content. The authors reported free fatty acids were eight times higher in VCO compared to RCO (0.127% vs 0.015%)[15]. A question to consider might be whether or not the method of producing and/or refining coconut oil influences physiological effects or markers of inflammation and oxidative stress. The production method of coconut oil should be considered when interpreting the results of studies investigating any possible effects.

C. Possible Risks and Side Effects of Coconut Oil Consumption

Although research regarding an association between coconut oil and cardiovascular disease is contradictory, the American Heart Association and the United States Dietary Guidelines classify coconut oil as a saturated fat with damaging effects on the cardiovascular system[22, 29]. A 2016 review of 8 clinical and 13 observational studies concluded the body of evidence confirms coconut oil elevates total cholesterol, LDL-C, and HDL-C when compared to unsaturated plant oils[19].

Critics of this recommendation argue many of the studies that reported increased cardiovascular risk factors used hydrogenated coconut oil as the test oil, rather than RCO or VCO. It is possible the negative results on cardiovascular indicators were due to a

deficiency of essential fatty acids (EFA), as well as the *trans*-fatty acid component resulting from the hydrogenation process[18]. Diets high in hydrogenated oil are consequently low in essential polyunsaturated fatty acids (omega-6, linoleic acid (LA); omega-3, a-linoleic acid (ALA)). These fatty acids are considered "essential" dietary components because they are required for biochemical reactions but cannot be synthesized by the human body. Animal and human studies indicate an increase in serum cholesterol related to diets which replace these essential fatty acids with partially hydrogenated oils[18, 57]. In addition, the process of chemical "partial hydrogenation" of unsaturated plant oils produces fatty acids with a *trans* isomer in the carbon chain, as opposed to a *cis* isomer. This configuration results in a more solid oil with a longer shelf-life. A large body of evidence, including clinical and epidemiological studies, correlates dietary *trans*-fat intake to increased plasma lipids and coronary heart disease[1, 36, 37]. These factors should certainly be taken into consideration when reviewing studies that use hydrogenated coconut oil rather than RCO or VCO.

On the other hand, many of the studies indicating a positive effect of coconut oil on lipid parameters were limited in duration and/or number of participants, or failed to show a strong enough correlation to establish a benefit[63]. Future studies are required in order to resolve these discrepancies.

Possible side effects resulting from an excessive intake of coconut oil might be expected due to its high medium-chain triglyceride content. Although a review of the evidence conducted by Traul et al. (2000) reported no toxic effects resulting from an MCT intake up to 15% of total kilocalories (either orally or parenterally), Jeukendrup & Aldred (2004) found that more than 25-30 grams of MCTs ingested during a single meal can

cause intestinal symptoms such as abdominal cramping, bloating, nausea, vomiting, and diarrhea[26, 34, 59].

D. Reported Health Benefits of Coconut Oil

Sales of coconut oil have increased worldwide in recent years as a variety of health benefits have been reported by the media and on the internet. In 2010, Americans consumed approximately 1.28 kg per person[30]. Some coconut oil distributers market VCO as a functional food with health benefits related to weight loss, diabetes, wound healing, gastrointestinal disorders, Parkinson's disease, and dementia[32, 30]. Although research is limited and sometimes contradictory, studies indicate a positive effect on cardiovascular disease risk factors, diabetes, and weight loss[15, 38].

Cardiovascular Disease: A controversy exists regarding the effect of coconut oil on cardiovascular risk factors. Current dietary recommendations are based on the classification of coconut oil as a saturated fat, along with the belief that all saturated fats increase the risk of cardiovascular disease. Serum cholesterol is a common clinical marker used to predict cardiovascular disease. Several researchers have investigated the effects of dietary coconut oil on blood lipids including cholesterol and triglycerides.

An early study by Reiser et al. reported a significantly higher increase in total cholesterol and HDL-C, as well as reduced triglycerides, from a diet high in coconut oil compared to beef fat. Although LDL-C increased significantly more than safflower oil, there was no difference between coconut oil and beef fat. This small, randomized, crossover designed trial included 19 male medical students, consuming each diet for 5 weeks. Although the intent was to investigate the effects of beef fat, the outcome indicated coconut oil

increased LDL-C to a greater extent than safflower oil, but increased HDL-C significantly more than both safflower oil and beef fat[47].

Cox et al. conducted two trials comparing the effects of coconut oil, butter, and safflower oil on serum lipids and lipoproteins. Their initial study was a randomized, control trial which included 28 participants (men and women ages 29-67 years)[11]. Each 6-week trial diet consisted of 36% total energy from fat, with 50% from the test oil. Although both coconut oil and butter increased total cholesterol and LDL-C more than safflower oil, coconut oil raised these levels significantly less than butter. No significant difference in HDL-C was reported. Triglycerides were significantly reduced after the coconut oil and safflower interventions[11]. These results suggest dietary coconut oil increases serum lipids and lipoproteins to a lesser extent than butter. The researchers confirmed their results in a follow-up study of 41 Pacific Island adults (19-72 years of age), in a sequential, non-randomized trial. Using the same test oils during 6-week trials, results showed a decrease in triglycerides, but no significant difference between the three groups. Total cholesterol and LDL-C increased significantly after butter and coconut oil, while coconut oil increased HDL-C by a greater amount than safflower oil[12]. Since this study confirmed the results of the previous study, researchers suggest dietary coconut oil may have a less detrimental effect on serum lipids than butter.

A 2004 Harvard Medical School study reported a significant improvement in the ratio of total cholesterol to HDL-C after 6 weeks of coconut oil. This randomized, double-blind crossover experiment compared serum lipoproteins after 6-week interventions of coconut oil, soybean oil, and hydrogenated soybean oil in 22 healthy, young men (mean age: 32). Researchers concluded 50% of dietary fat intake from coconut oil produced no adverse

effects, and may actually indicate a benefit due to increased HDL-C and improved ratio compared to soybean oil[42]. Since this study is limited by a small sample of healthy males, a study with a larger sample size and increased population diversity would be of interest. More recently, Voon et al. conducted a randomized, crossover designed trial including 45 Malaysian adults. After 5 weeks, diets containing 67% test oils of coconut oil, virgin olive oil, and palm oil were compared for their effects on cardiovascular risk factors. No difference in CRP or homocysteine was reported. Interestingly, HDL-C was significantly higher after the coconut oil phase than olive oil, and there was no significant difference in total cholesterol to HDL-C ratio between the three test oils[61]. Since the ratio is a more reliable predictor of cardiovascular event than either total cholesterol of LDL-C alone, coconut oil may not increase atherosclerosis or cardiovascular disease.

Obesity: Because abdominal obesity is one factor included in the group of clinical conditions known together as "metabolic syndrome," investigating possible benefits of dietary MCTs is of interest. Results of clinical studies conducted on animals and humans indicate MCT consumption may enhance weight loss. According to a review of the evidence conducted by Marten et al. (2006), a number of studies report decreased fat accumulation and less weight gain in rats fed a diet high in MCTs versus LCTs. The researchers attributed these results to enhanced thermogenesis, likely related to oxidation of MCFAs in the liver[34]. Clinical interventions on human participants also report a positive effect on weight loss, especially in obese men and women. For example, St-Onge and Bosarge (2008) compared the effects of dietary MCTs to olive oil on body weight and fat mass in overweight participants. After a 16-week program, participants who consumed 18-24 grams of MCT oil per day had lower body weight and fat mass than

those consuming olive oil[56.] Clinical studies conducted by Seaton et al. (1986) and Scalfi et al. (1991) indicated greater postingestion energy expenditure after a meal supplemented with MCT (48g and 30g, respectively) versus LCT[51, 52]. Additionally, researchers in Japan conducted a series of 12-week intervention studies on obese subjects comparing moderate doses of MCT consumption (10g, 5g, or 1.7g per day) to a control mixture of soybean and rapeseed oils. Interestingly, greater weight loss and increased fat loss was reported in all groups receiving the MCT intervention[34]. These results suggest even low to moderate doses of MCTs could have beneficial effects on weight loss. Future studies might investigate variations in dosage, possible interactions between other dietary components, and effects on various population groups. If evidence supports a positive effect of dietary MCTs on weight management, replacing long-chain fatty acids with MCT oils in cooking could be beneficial to maintaining body weight and reducing fat accumulation.

Research indicates MCT consumption may enhance thermogenesis, increase fat oxidation, and improve postprandial energy expenditure. Several clinical studies reported a significantly higher thermic effect of MCT versus LCT. For example, Seaton et al. (1986) measured the metabolic rate of seven healthy male volunteers by indirect calorimetry before and after ingestion of a 400-kcal meal containing either MCT or LCT. According to their results, oxygen consumption was 12% higher six hours after ingestion of MCT compared to 4% after LCT[52]. Scalfi et al. reported greater postprandial thermogenesis (PPT) in both lean and obese subjects after a meal with 30g MCT plus 8g LCT than a meal with 38g LCT (Scalfi et al. 1991)[51]. In addition, a double-blind, cross-over study conducted by Hill et al. reported a greater degree of thermogenesis after intake

of 40% fat of MCT versus LCT. During an inpatient trial held at the Vanderbilt University Clinical Research Center, ten male participants were fed a liquid diet with MCT for seven days and LCT for seven days, with a week washout period between trials. Results on day one indicted a greater thermic effect of food (TEF) after an MCT meal (8% ingested energy) than an LCT meal (5.8%). After five days, the effect of MCT was even greater (15.7% vs 7.3%)[23]. Compelling evidence supports the thermogenic effect of medium-chain triglycerides. Theoretically, this effect could contribute to enhanced weight loss from an increase in coconut oil consumption.

Diabetes: Insulin resistance is an additional contributor to metabolic syndrome, which may be mediated by MCFAs. Animal and human studies suggest an antidiabetic effect of MCTs. High dietary intakes of long-chain fatty acids have been related to insulin resistance. In contrast, some research suggests dietary MCTs may exhibit an antidiabetic effect. A 2009 study by Wein et al. reported a protective effect of MCTs on insulin resistance in rats fed a high fat diet[64]. In addition, a recent study of diabetic patients who consumed a diet consisting of 40% of calories from either MCTs or LCTs found a 30% increase of insulin-mediated glucose metabolism in MCT consumption compared to LCTs[38]. A 2002 review by Pfeuffer & Schrezenmeir, however, reported most studies at that time failed to show a decrease in glucose or insulin. Although the increase in serum glucose measured shortly after intake of MCT was lower, a 30-day intervention in type 2 diabetics receiving an MCT-rich diet produced no change in fasting glucose or insulin[66]. More recently, Tholstrup et al. (2004) reported an increase in fasting glucose after a 3-week trial of 70 grams MCT compared an equal amount of high-oleic sunflower oil[58]. Due to contradictions observed in the current evidence, further research investigating the

metabolic effects of dietary MCFAs on glucose production and insulin resistance is certainly encouraged.

Alzheimer's disease: Animal and human studies report a possible link between MCFAs and improved cognitive function in Alzheimer's patients. The suggested mechanism is related to mild ketogenesis[30]. It is believed Alzheimer's disease and age-related cognitive decline is associated with decreased glucose metabolism in the brain[45]. A limited amount of research suggest ketone bodies, especially –hydroxybutarate (-OHB) produced in the liver from MCT oxidation, can be utilized as an alternative energy source by the brain. For example, a study examined cognitive function and ketone body levels in older dogs after 8 months of a diet containing 5.5% MCT supplement compared to a control diet. The results indicated improved cognitive abilities and significantly increased levels of –OHB in the MCT oil fed dogs[45]. A previous, double-blind, placebo controlled trial of 20 patients with Alzheimer's disease or mild cognitive impairment reported improved cognitive function in a genetic subset of patients after a 40ml oral intake of MCTs compared to a placebo[46]. To follow this study, Henderson et al. conducted a much larger study including 140 patients diagnosed with mild to moderate Alzheimer's disease. Subjects received either 20mg MCT powder or an isocaloric placebo for 90 days. Results of their multi-centered, double-blind, randomized, placebo controlled parallel trial reported improvement in cognition tests after the 90-day trial period. However, similar to the previous pilot study, only a genetic subset showed significant improvement[55]. These results are intriguing and future research into possible cognitive benefits of MCT is certainly encouraged.

Infection. Another intriguing benefit may be the reported microbial action of coconut oil. Although most existing research has been conducted *in vitro*, studies indicate an adverse effect of the monoglyceride lauric acid on microorganisms such as yeast, fungi, bacteria, and viruses[18, 27]. Lauric acid is a component in human breastmilk, which provides newborns protection from pathogens until their immune systems develop[30]. It is hypothesized that monolaurin destroys viruses, bacteria, and other pathogens by disintegrating the plasma membranes, which effectively kills the microorganisms[18]. A 2004 *in vitro* study examined the sensitivity of various *Candida* species to coconut oil versus fluconazole. Results indicated a 100% sensitivity of *Candida* species exposed to a minimum inhibitory concentration (MIC) of 25%[43]. Recently, researchers investigated a possible effect of dietary coconut oil on *Candida albicans*, a fungus common in the human GI tract that is normally harmless but can lead to infection if colonization becomes excessive. In an experiment using mice, Gunsalus, et al. (2015) reported a diet high in coconut oil resulted in less intestinal colonization of *Candida albicans* than either soybean oil or beef tallow[21]. Since approximately 50% of the fatty acid content of coconut oil is lauric acid, it is possible coconut oil could be used as a non-toxic replacement for, or in conjunction with, pharmaceuticals commonly used to treat fungal infections, such as fluconazole[14].

E. Inflammation

Inflammation is a biochemical process meant to protect tissues against internal and external toxins including viruses, bacteria, pollen, and chemicals. Symptoms of acute inflammation include redness, swelling, pain, and fever[25]. Dietary fats are of interest, as numerous studies confirm both pro-inflammatory and anti-inflammatory effects related to

fat intake. For example, safflower oil, which is rich in the polyunsaturated omega-6 arachidonic acid, promotes inflammation, while omega-3 fatty acids found in fish oils are anti-inflammatory[22]. Although little research exists regarding coconut oil and inflammation, two experimental studies using rats reported an anti-inflammatory effect. Both studies induced acute and chronic inflammation in male Sprague-Dawley rats, and then administered coconut oil either topically or orally. While both experiments resulted in decreased acute inflammation in the paw edema test, only Intahphuak, et al. (2010) reported a reduction of chronic inflammation[24, 67]. Further research should be conducted in order to investigate this discrepancy.

Chronic inflammation: Inflammation is a causative factor in atherosclerosis and cardiovascular events[24, 48, 49]. Chronic inflammation is a continued state of inflammation associated with mononuclear immune cells (monocytes, lymphocytes, macrophages, and plasma cells), tissue damage, and accumulation of fibrous tissue[28]. Abundant research indicates this continued state of inflammation contributes to age-related diseases including type 2 diabetes, atherosclerosis and cardiovascular events, Parkinson's disease, Alzheimer's disease, weakness, and frailty[16]. A relationship between chronic inflammation and tumor progression is also reported. Researchers estimate 25% of cancer diagnoses are related to chronic inflammation[28]. Because some dietary fats contribute to inflammation and others support anti-inflammatory processes, it is of interest to investigate the effect of coconut oil intake on markers of systemic inflammation[20].

A system of biochemical molecules work together in order to regulate inflammation. Fatty acid molecules are a critical component of both the inflammatory response to destroy pathogens and the modulating anti-inflammatory process[20]. When an invading

toxin or injury is detected, pro-inflammatory molecules such as cytokines [tumor necrosis factor (TNF); interleukins (IL)-1, -6] and eicosanoids [prostaglandins (PG); leukotrienes (LT)] stimulate a range of effects including fever, increased glucose, reactive oxygen species (ROS), adhesion molecules, increased triglycerides, muscle protein breakdown, and anorexia[20]. Arachidonic acid (AA) is an omega-6 polyunsaturated fatty acid (n26) involved the production of these pro-inflammatory molecules[25]. An overabundance of these molecules for an extended period of time causes cell damage and promotes the age-related diseases mentioned previously[28]. Because AA is synthesized from linoleic acid, Americans typically obtain significantly more than required due to the large quantity of soybean oil, corn oil, safflower, and sunflower oil in Western diets[25]. This illustrates a possible mechanism for the relationship between dietary pattern and progressive diseases associated with chronic inflammation. It is worth noting coconut oil contains very little linoleic acid[20].

In contrast, the polyunsaturated omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), mainly found in fish oils, mediate the inflammatory process by disrupting the production and activity of pro-inflammatory cytokines and eicosanoids. For example, EPA prevents the synthesis of pro-inflammatory eicosanoids PG and LT by competing with AA molecules[25]. Extensive research reports a significant reduction in PGs and LTs after dietary supplementation with fish oils[7]. Clinically, fish oil appears to provide a therapeutic benefit on rheumatoid arthritis, as evidenced by more than 10 double-blind, placebo-controlled experimental studies[7, 25].

Interestingly, studies also indicate anti-inflammatory effects of oils and fats low in linoleic acid. An 8-week experiment conducted by Mulrooney & Grimble (1993) found a

reduction of inflammatory responses triggered by IL-1 and TNF in rats fed coconut oil. An earlier study by Grimble (1990) reported rats fed coconut oil produced less PG than those fed corn oil. This raises the question of what effect, if any, coconut oil supplementation might have on inflammation markers in humans.

C-reactive protein (CRP): Because systemic inflammation is associated with so many age-related diseases, simple tests to determine the presence of inflammation can be valuable clinical tools. One of the most widely-used and available tests for systemic inflammation is high-sensitivity CRP[65]. C-reactive protein is one of 40 acute phase proteins activated during the immune system's response to pathogens or cell damage. It is produced in the liver and released rapidly when injury or infection is detected. Plasma CRP level can increase 1000 times after the inflammatory response is initiated[5]. Although normal CRP levels are less than 1g/ml, any measurement higher than 10g/ml has historically been attributed to active inflammation and immune response[5, 65]. Because this protein is easily measured from a blood sample, is not affected by gender, age, or diurnal changes, and has a long half-life, serum CRP is a common laboratory test used clinically to determine the presence of systemic inflammation[65].

A large body of research confirms elevated CRP levels are seen in patients with conditions related to chronic inflammation. According to a 2006 review conducted by De Martinas et al, age-related diseases associated with increased serum levels of CRP include: insulin resistance in nondiabetics, type 2 diabetes, metabolic syndrome, cancer, weakness, and frailty[16]. Especially interesting is compelling recent research which indicates C-reactive protein is a more reliable indicator of cardiovascular damage than the standard lipid profile[65]. Most notably, a 3-year, prospective, nested case control study

with a cohort of over 28,000 healthy, post-menopausal women participating in the Women's Health Study reported cardiovascular events were more reliably predicted by CRP level than serum low-density lipoprotein (LDL) cholesterol[50]. The strongest association was seen at a CRP level between 1 and 5 g/ml. Since over two-thirds of cardiovascular events in women occur in patients with LDL-C levels related to low risk, CRP is increasingly used in conjunction with cholesterol levels, as an additional risk factor of cardiovascular disease[65].

In addition to its pro-inflammation response to pathogens, CRP is thought to directly contribute to atherosclerosis. Research conducted by Verma et al. (2004) found CRP reduces the expression of nitric-oxide synthase, which effects the stability of cells and develops plaques in the lining of blood and lymph vessels[16, 60]. As evidence, Reynolds & Vance (1987) discovered plaque deposits collected from atherosclerotic human aortas contain CRP[48, 65]. Theoretically, strategies intended to reduce serum CRP should decrease the risk of cardiovascular disease. Pharmaceutical interventions such as statins and aspirin can reduce serum CRP as much as 25-50%. Some studies show lower CRP levels after weight loss and diabetes management, as well[65]. This suggests patients with elevated CRP may benefit from dietary/lifestyle and pharmaceutical therapies. Additionally, future studies investigating possible effects of specific dietary components on CRP levels may be of interest.

Reactive Oxygen Species (ROS)/Oxidative Stress: As mentioned earlier in this review, polyphenols in coconut oil may provide antioxidant protection against oxidative stress. Oxidative stress is a detrimental effect of inflammation caused by reactive oxygen species (ROS), which are a byproduct of the inflammation process. These damaging

effects occur when the production of ROS overwhelms the defensive ability of antioxidants. A general consensus of the relevant literature indicates oxidative stress resulting from chronic inflammation is associated atherosclerosis, cancer, and other age-related diseases[28, 65]. Since antioxidant polyphenols are ubiquitous in dietary sources, many foods are marketed for their potential protective antioxidant properties.

The production of reactive oxygen species (ROS) is one of the many effects of the process of inflammation. Reactive oxygen species are "free radical" atoms or molecules derived from oxygen. Free radicals are highly reactive due to unpaired electrons in their orbits. Oxygen has two unpaired electrons, and is the most prominent molecule susceptible to free radical formation. A defensive system of antioxidants works to neutralize the toxic effects of free radicals. Although a low concentration of ROS acts as a defense against pathogens, an overabundance of ROS can damage the fatty acid, protein, and DNA components of cells and tissues[13, 28].

The fatty acids located in cell walls are especially vulnerable to ROS damage. Oxygen-derived free radicals produce lipid peroxides when they react with fatty acids of the cell membrane. Lipid peroxides make the cell walls rigid and less flexible, which leads to cell damage and death. Increased lipid peroxidation not associated with normal aging is seen in the brains of patients diagnosed with Alzheimer's disease. The accumulation of excessive lipid peroxides generates additional, potentially damaging end-products such as the reactive aldehyde malondialdehyde (MDA), which is considered a carcinogen. MDA is an end-product of arachidonic acid (AA) metabolism, which is obtained in large amounts as part of the typical Western diet. Since MDA is much more stable than free

radicals, it is often used to indirectly measure lipid peroxidation as an indicator of oxidative stress[13, 28].

Thiobarbituric acid-reactive substance (TBARS): In order to investigate the relationship between oxidative stress and age-related diseases or antioxidant therapies, it is necessary to measure serum or tissue levels of free radicals or their byproducts. The most common laboratory analysis used to estimate systemic oxidative stress is the thiobarbituric acid-reactive substance (TBARS) assay, which measures MDA in serum or tissues in order to determine lipid peroxidation. Although the assay can be performed by HPLC or spectrophotometry, HPLC is preferred due to its higher reproducibility, sensitivity, and specificity.

In order to investigate the antioxidant properties of virgin coconut oil (VCO) versus refined coconut oil (RCO), Nevin and Rajamahan (2004, 2006, 2008) used TBARS to measure lipid oxidation in rats fed coconut oil. In 2004, the research reported reduced TBARS in the VCO-fed animals, suggesting the polyphenol fraction of VCO is more effective in preventing lipid oxidation than RCO[39]. Their follow-up study found rats fed VCO had significantly lower levels of lipid peroxide in tissues (heart liver, kidneys), as well as higher total glutamine (GTN), an additional indicator of antioxidant status[40]. Additional research comparing coconut oil to sunflower oil determined TBARS level was significantly lower in the VCO group and highest in the sunflower oil group[41]. Nevin and Rajamahan proposed that the lower TBARS level may be a result of a higher antioxidant amount and lower polyunsaturated fat content as compared to sunflower oil, which is more vulnerable to oxidation[41]. Based on the results of these previous animal experiments, it would be of interest to conduct similar studies on the effects of

antioxidant activity of dietary coconut oil in human participants. If phenolic compounds or dietary fats such as lauric acid in coconut oil can reduce inflammation (serum CRP) and lipid oxidation (TBARS), increased consumption could be used in conjunction with pharmaceutical therapies such as aspirin, NSAIDs, or statins.

Chapter 3

METHODS

Participants

Subject selection

Blood samples obtained from thirty-two, healthy, non-smoking adults between the ages of 18 and 40 were analyzed for this study. Eligible participants reported no food restrictions or special diets, had a body mass index (BMI) between 22 and 35, and reported less than 150 minutes of moderate exercise per week. Subjects were selected if they had no diagnoses of active disease, were not taking statin medications, and females were not pregnant or lactating. Volunteers who were unwilling or unable to take a daily supplement capsule or to continue the study protocol for the duration of the study were excluded. These criteria were approved by the Arizona State University Institutional Review Board (IRB), and all participants provided written informed consent.

Recruitment

Participants were recruited via Arizona State University email ListServs, announcements, and flyers posted on or near the Arizona State University campus. Volunteers were offered an incentive of $10.00 and $25.00 Target gift cards to participate. An online survey through the www.surveymonkey.com website was used to pre-screen individuals who expressed interest. Selected individuals were scheduled for an initial visit in order to finalize eligibility, inform subjects of study details, and obtain signed inform consent and anthropometric measurements.

Study Design

This eight-week study was primarily designed as a randomized, double-blind, parallel, two-arm control trial to test the impact of coconut oil on blood lipids and body composition. This report represents the secondary analysis of the blood samples to determine the impact of coconut oil on common markers of inflammation (CRP) and oxidative stress (TBARS), as research indicates these conditions are associated with cardiovascular disease, cancer, and other age-related diseases[26, 34, 59, 49, 65]. Thirty-nine subjects were enrolled and randomly assigned to one group receiving the coconut oil supplement (2 grams/day) or a second group receiving the placebo control (white flour); 32 subjects completed the 8-week protocol. The study included three visits to the Arizona Biomedical Collaborative building at the Arizona State University downtown campus. At the initial visit, each volunteer completed a health history questionnaire and a mood questionnaire. A dexa scan was performed to assess body composition. Height, weight, and finger stick blood cholesterol samples were obtained in order to stratify participants prior to randomization. Selected participants were instructed to complete a three-day diet record and consume only water for at least eight hours prior to the time of their second visit. During the second visit, subjects were randomly assigned to either the coconut oil or placebo group. Each participant was provided with an eight-week supply of supplement capsules and directions regarding consumption. Participants were asked to follow their usual dietary patterns and physical activity levels during the eight weeks of the trial. A final fasting blood sample was collected and a dexa scan was performed. Participants provided investigators with their completed compliance calendars, and three-day food records.

Independent variable: The variable expected to have an effect was a daily, low-dose supplement of coconut oil. Subjects in the coconut oil group consumed two, 1000 mg softgel capsules of Puritan's Pride brand coconut oil (Cocos nucifera) daily. The serving size listed on the label is two (2) softgels, recommended twice daily, taken with food. Subjects in this study were instructed to take two capsules daily (half the recommended dose). Additional ingredients listed include gelatin, medium chain triglycerides, vegetable glycerin, and titanium dioxide color. Two softgel capsules provide 25 calories; 2.5 g (4% Daily Value (DV)) total fat; 2.5 g (12% DV) saturated fat, and less than 1 gram of protein. According to the product label, 2000 mg coconut oil typically contains: 880 mg lauric acid; 280 mg myristic acid; 92 mg caprylic acid; 120 mg palmitic acid; 90 mg capric acid; 100 mg oleic acid; 16 mg stearic acid; and 16 mg linoleic acid.

Dependent variables. Outcome measurements included weight, visceral fat, blood cholesterol, blood triglycerides, and blood markers of inflammation and oxidative stress. This study specifically examined changes in common biomarkers of chronic inflammation (CRP) and oxidative stress (TBARS).

Statistical analyses: The Statistical Package for Social Sciences (SPSS version 23) was used to perform statistical analyses. Data were reported as the mean ± SD; significance was set at $P \leq 0.05$. Repeated measures ANOVA was used to assess the differences between means, while correlation analyses evaluated the strength of the relationship between variables. Data was tested for normality and log transformed if needed in order to normalize data.

Chapter 4

DATA & RESULTS

Data and results of this study are based on secondary analyses of blood samples originally collected to investigate the effects a daily supplement of 2 grams coconut oil would have on common serum lipid and lipoproteins associated with cardiovascular disease risk factors (HDL-C, LDL-C, Total Cholesterol, Triglycerides, and Total Chol/HDL-C ratio). The secondary analyses measured the blood samples obtained from the original study for serum levels of CRP and TBARS.

Volunteers for this randomized, double-blind, parallel-arm control trial were recruited via email ListServs, flyers, and announcements on and near the Arizona State University campus in Phoenix, Arizona, during October 2015. A total of 154 people completed an online survey to determine initial eligibility. Eighty of these respondents met the pre-screen requirements. Written consent was obtained from 42 volunteers; however, 3 of these withdrew prior to initial data collection. Thirty-nine participants were randomized into either the coconut oil (CO) group (n=19) or the placebo (PL) group (n=20) after stratification by gender, age, and BMI. Seven volunteers dropped out prior to the end of the study. Final analyses include samples collected from 32 subjects completing the 8-week protocol.

Ultimately, analyses of the experiment group (CO) consisted of 10 females and 4 males; 15 females and 3 males were included in analyses of the PL group. Mean ages of each group were 25.1±5.7 and 24.2±5.3 years, respectively, with a range of 18 to 38 years. Mean BMI for both groups was in the normal/healthy range (CO: 23.6±4.4 kg/m^2; PL: 24.7±4.0 kg/m^2). Heights ranged from 146.1cm to 182.9cm. The lowest baseline weight

was 47.5kg and the highest 96.5kg with a mean weight of 65.2±14.3kg in the CO group and 68.6±13.6kg in the PL group. Waist circumference ranged from 67.31cm to 106.7cm; percent fat measured between 13.3% and 45.7% in both groups. The range of metabolic equivalents (METS) was from 26 to 119 kcal/kg/week. Thirteen of 14 participants in the CO group completed their compliance calendars, with a calculated 89.8% adherence; the PL group indicated 87.9% compliance with all 18 completed calendars (Table 1).

Table 1. Baseline Characteristics of each study group: coconut oil (CO) and placebo (PL).

	CO	**PL**	**P value[ab]**
N (M/F)	4/10	3/15	
Age (years)	25.1±5.7	24.2±5.3	0.643
Weight1 (kg)	65.2±14.3	68.6±13.6	0.492
Height (cm)	165.6±8.7	166.4±9.2	0.801
BMI (kg/m^2)	23.6±4.4	24.7±4.0	0.499
Waist (cm)	78.2±10.0	82.0±10.3	0.298
Body Fat (%)	23.7±8.8	27.5±7.2	0.187
METS[c] (kcal/kg/week)	59.6±15.8	47.9±22.8	0.113
Adherence (%)	89.8±11.8	87.9±8.22	0.595

Statistical analyses performed using SPSS Statistical Analysis system 23.0.
Data expressed as mean ± standard deviation. Adherence represents percent of days pills consumed. [a]Independent t-test analysis. [b]Significance is >0.05. [c]Metabolic Equivalents is a measure of physical activity.

Baseline measurements of CRP related to body fat and BMI. Using a 2-tailed t-test, the correlation between CRP and body fat (p=0.000) is significant at the 0.01 level. Correlation between CRP and BMI (p=0.030) is significant at the 0.05 level.

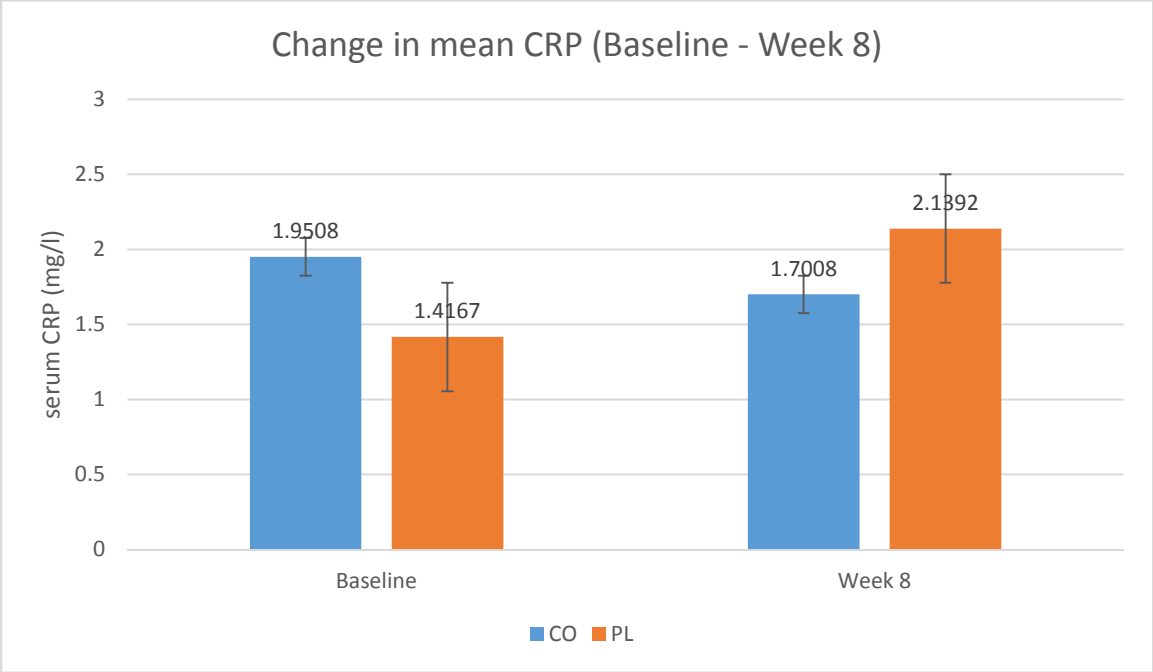
The CRP analysis of the experimental (CO) group included 13 samples. Although the mean serum CRP level of the coconut oil group decreased from baseline (1.95±3.35) to 8-weeks (1.70±3.53), the reduction is not significant (p=0.183) (Table 2) (Figure 4). The NPAR test was used due to lack of normality. When the change in CRP is transformed to achieve normality and control for age and percent fat, the intervention is weakened to an even greater extent (p=0.289).

Table 2. Pre- and post-intervention (8-weeks) serum CRP measurements.

	CO n=13	**PL** n=18	**P value**[ab]
Baseline (mg/l)	1.95±3.35	1.42±2.06	
Week 8 (mg/l)	1.70±3.53	2.14±3.49	
Change (0-weeks 8) (mg/l)	-0.250±0.734	0.723±2.91	0.183

Data expressed as the mean ± SD. [a] NPAR test used for change. [b]Significance is set at $p < 0.05$. The change in CRP was not significant.

Figure 4. Comparison of serum CRP between test and placebo groups at baseline and week 8.



Fourteen subjects included in the analysis of TBARS received the coconut oil intervention; 18 received the placebo. The NPAR test was used as data was not normalized. In contrast to the hypothesis, TBARS in the test group increased significantly. Mean baseline TBARS in the CO group was 2.45±0.651 compared to 2.85± 0.973 after 8 weeks (p=0.049). Although this is a significant increase, the significance is borderline. TBARS in the PL group decreased from baseline (3.54±3.50) to 8-weeks (2.58±0.894) (Table 3) (Figure 2).

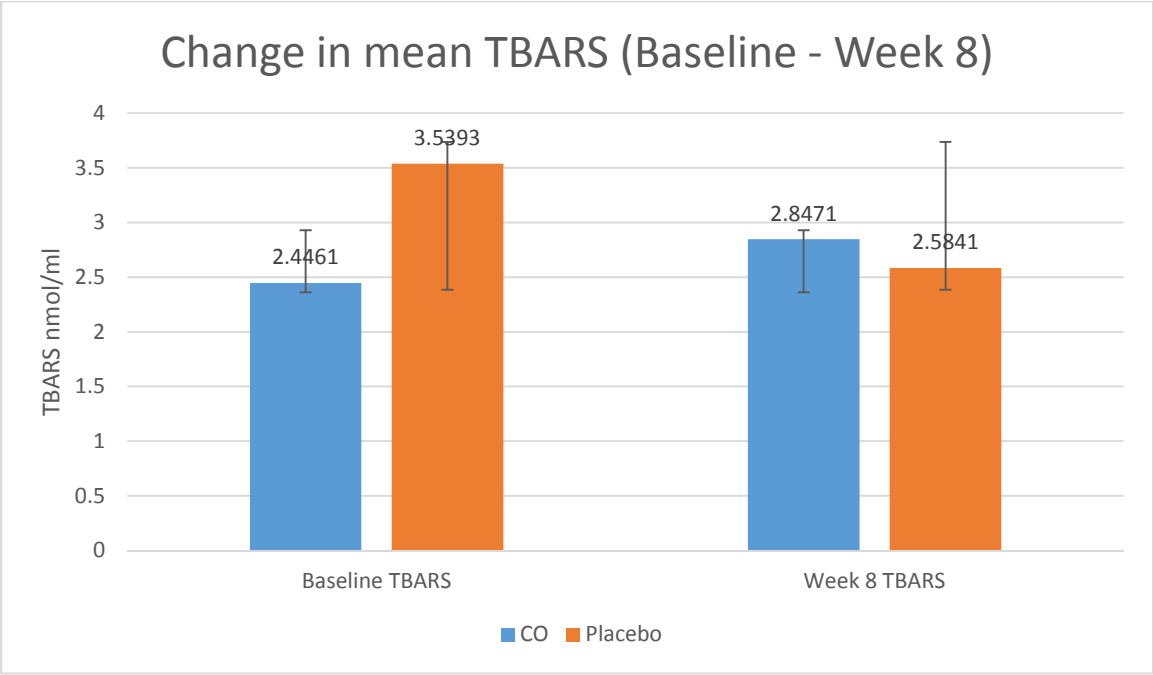
Table 3. Pre- and post-intervention (8-weeks) serum TBARS measurements

	CO n=14	**PL** n=18	**P value**[ab]
Baseline (nmol/ml)	2.45±0.651	3.54±3.50	
Week 8 (nmol/ml)	2.85±0.973	2.58±0.893	
Change (0-weeks 8)	0.401±1.04	-0.955±3.436	0.049

(mg/l)

Data expressed as the mean ± SD. [a] NPAR test used for change. [b]Significance is set at p = < 0.05.

Figure 5. Comparison of serum TBARS between test and placebo groups at baseline and week 8.



Chapter 5

DISCUSSION

In this randomized, parallel two-arm, placebo controlled trial, 8 weeks of 2 grams per day of a coconut oil supplement in the form of 2 capsules did not significantly reduce serum CRP levels compared to the placebo (white flour). In contrast to the hypothesis, TBARS levels significantly increased after 8 weeks of coconut oil supplementation.

Although the decrease in CRP was not statistically significant, it may be notable that the coconut oil intervention did not result in an increase of CRP. A large body of evidence indicates dietary fats affect the inflammation process. For example, dietary oils rich in linoleic acid, such as soybean, corn, and safflower oils, promote inflammation, while omega-3 fatty acids, especially those found in fish oils, interfere with the production of pro-inflammatory cytokines[20]. Dietary patterns high in saturated fats, particularly red meats and high-fat dairy products, are associated with increased levels of CRP. Since coconut oil is high in saturated fat, it might be expected to raise CRP. However, coconut oil contains very little pro-inflammatory linoleic acid[20]. Results of this study suggest coconut oil consumption may be not associated with the same pro-inflammatory effects as saturated fats or polyunsaturated omega-6 dietary oils.

According to a review by Yeh and Willerson (2003), a CRP level between 1 and 5 g/ml is associated with an increased risk of a cardiovascular event[65]. Ridker (2003) reported a CRP measure between 1 and 3 g/ml indicates a moderate risk, while greater than 3 g/ml is associated with a high risk[49]. In the current study, the mean baseline CRP was between 1 and 2 g/ml in both the coconut oil and placebo groups (CO: 1.95±3.35 g/ml; PL: 1.42±2.06 g/ml). Considering the study population was mainly young and healthy, it may

be of interest to investigate the effects on a population of subjects with a CRP in the moderate or high-risk groups.

Although no previous human studies were found, a limited number of animal experiments suggest coconut oil may have an anti-oxidant effect, possibly related to polyphenol content. Nevin and Rajamahan conducted several studies which reported reduced TBARS in rats fed diets rich in virgin coconut oil[39, 40, 41]. Compared to refined coconut oil, their results showed lower serum TBARS and lipid peroxide in tissues of rats fed VCO[39, 40]. Additional research indicated significantly higher TBARS in rats fed sunflower oil compared to VCO. The authors suggested the higher antioxidant amount and lower polyunsaturated content may have contributed to the lower TBARS in the VCO-fed groups[41].

Based on these previous experiments, it was expected that a coconut oil supplement in a human trial would result in decreased TBARS. To the contrary, this study indicated an increase in mean serum TBARS from 2.45±0.651 to 2.85±0.973, with a mean change of +0.16 after 8 weeks of a coconut oil supplement. Although this increase is statistically significant ($p=0.049$) the result is borderline. A possible confounding factor may be related to the dietary intake of each participant. Since MDA, which is the derivative of lipid peroxidation measured by the TBARS assay, can be influenced by dietary components, diet should be controlled when using TBARS to determine lipid peroxidation[13].

An additional consideration regarding the TBARS results reported in this study may be related to the method of production of the test oil. A review of available studies indicates VCO has a more powerful antioxidant effect than RCO[33]. Additional research reports free

fatty acids may be as much as 8 times higher in VCO compared to RCO[15]. Results of the animal experiments mentioned previously were based on test oils of VCO, RCO, and sunflower oil. Since the test oil used in this study consisted of capsules of RCO, it is possible trials using VCO may produce different results.

Strengths: This 8-week study was a designed as a double-blind, parallel, two-armed trial. Participants were randomized into either the experiment or control groups after stratification by gender, age, and BMI.

Limitations: Limitations include lack of controlled diet, inability to confirm compliance, and a healthy subject population. Although the protocol of this study considered participants would follow their usual diet, the study is limited by the lack of a controlled diet. Since TBARS, especially, may be affected by changes in diet, it is difficult to conclude the final results were related to the coconut oil supplement. Although subject compliance of daily intake was recorded on a compliance calendar, study investigators did not directly observe daily consumption of the capsules. Finally, since inflammation and oxidatitive stress are affected by age and health status, it is possible different results may be seen in an older, higher-risk subject pool.

Conclusion: In conclusion, this randomized, placebo-controlled trial found that a daily supplement of 2-grams refined coconut oil for eight weeks did not significantly affect serum levels of CRP. Lipid peroxidation was negatively affected by the coconut oil supplement as evidenced by a significant increase in serum TBARS. Future studies might include participants with a moderate or high risk of cardiocascular event as evidenced by serum CRP. Additional human studies investigating the effects of virgin or refined coconut oil on measures of lipid peroxidation are also of interest.

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APPENDIX A

IRB APPROVAL

APPENDIX A

APPROVAL:CONTINUATION

Carol Johnston

SNHP: Nutrition

602/827-2265

CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 8/15/2016 the ASU IRB reviewed the following protocol:

Type of Review:	Continuing Review
Title:	Dietary Supplementation and Health
Investigator:	Carol Johnston
IRB ID:	STUDY00003159
Category of review:	(2)(a) Blood samples from healthy, non-pregnant adults, (4) Noninvasive procedures, (9) Convened IRB determined minimal risk
Funding:	Name: Graduate College
Grant Title:	None
Grant ID:	None
Documents Reviewed:	

The IRB approved the protocol from 8/15/2016 to 9/8/2017 inclusive. Three weeks before 9/8/2017 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 9/8/2017 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the “Documents” tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:

Rachel Shedden

Claudia Thompson-Felty

APPENDIX B

TBARS ASSAY

APPENDIX B

TBARS Assay (Zeptometrix kit)

1. TBA/Buffer Reagent:
    a. Mix 212mg TBA powder + 20ml TBARS Diluent 1 in a small beaker on hot stir plate until FULLY dissolved (use low heat); be sure to cover with parafilm to avoid evaporation. This step should also be done in a fume hood as Diluent 1 contains acetic acid.
    b. Add 20 ml TBARS Diluent 2, mix.
    c. This solution will be sufficient for one 96-well plate (5 standards and 43 samples).

2. Prepare two sets of standard tubes (1.5mL microcentrifuge) and poke a hole in the lid of one set of the standard tubes with an 18 gauge needle. Prepare sample tubes and poke a hole in the lid of each sample tube as well.

3. Prepare stock solutions of the standards in the tubes without holes (be sure to vortex well):

MDA Std #	[MDA] (nM/mol)	Add MDA Std (ul):	Add MDA Diluent (ul)
0	0	0	100
1	12.5	12.5	87.5
2	25	25	75
3	50	50	50
4	100	100	0

4. Add 30uL of stock standard solution or sample to the respective tube with the hole poked in the lid (for duplicates).

5. Add 30uL SDS to each standard and sample tube.

6. Add 750uL prepared TBA/Buffer Reagent to each tube. Vortex well.

7. Put tubes into 95°C heat block for 60 minutes.

8. Put tubes on ice for 10 minutes.

9. Centrifuge at 3000rpm for 15 minutes at room temperature.

10. Pull of supernatant and re-centrifuge supernatant at 3000rpm for 15 minutes at room temperature to help purify the supernatant.

11. Add 200ul of re-spun supernatant to each well of a 96-well plate.

12. Read absorbance at 532-540nm.

NOTES:
Bring all reagents to room temp.
SDS will be solid in fridge. Leave at RT x 1hr minimum or place in 37° incubator for 30 seconds to liquefy.

Use water bath at 95° - bright colored multi tube racks work best for this assay.

APPENDIX C

CRP ASSAY

APPENDIX C

Laboratory Name
Test Name: CRPHS

Order information

REF	CONTENT		Analyzer(s) on which kit(s) can be used
05401607 190	Cardiac C-Reactive Protein (Latex) High Sensitive (2 x 50 tests)	CRPHS: ACN 217	Roche/Hitachi **cobas c** 111
11355279 216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 656	
11355279 160	Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Code 656	
20766321 322	CRP T Control N (5 x 0.5 mL)	Code 235	
10557897 122	Precinorm Protein (3 x 1 mL)	Code 302	
10557897 160	Precinorm Protein (3 x 1 mL, for USA)	Code 302	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04774230 190	NaCl Diluent 9 % (4 x 12 mL)	Code 951	

Effective date

Effective date for this procedure: _____

Author

Source documentation compiled by Roche Diagnostics
Revised by: _____

Schedule for review

Last date revised: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____
Date Reviewed: _____ Approved: _____

System information

CRPHS: ACN 217

Intended use

In vitro test for the quantitative determination of C-reactive protein (CRP) in human serum and plasma on the **cobas c** 111 system. Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. Highly sensitive measurement of CRP may also be used as an aid in the assessment of the risk of future coronary heart disease. When used as an adjunct to other laboratory evaluation methods of acute coronary syndromes, it may also be an additional independent indicator of recurrent event prognosis in patients with stable coronary disease or acute coronary syndrome.

Summary[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21]

C-reactive protein is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five-member ring having a molecular weight of 105000 Daltons. CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the complement system beginning with C1q. CRP then initiates opsonization and phagocytosis of invading cells, but its main function is to bind and detoxify endogenous toxic substances produced as a result of tissue damage.
CRP assays are used to detect systemic inflammatory processes (apart from certain types of inflammation such as SLE and Colitis ulcerosa); to assess treatment of bacterial infections with antibiotics; to detect intrauterine infections with concomitant premature amniorrhexis; to differentiate between active and inactive forms of disease with concurrent infection, e.g. in patients suffering from SLE or Colitis ulcerosa; to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy; to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to distinguish between infection and bone marrow transplant rejection.
Sensitive CRP measurements have been used and discussed for early detection of infection in pediatrics and risk assessment of coronary heart disease. Several studies came to the conclusion that the highly sensitive measurement of CRP could be used as a marker to predict the risk of coronary heart disease in apparently healthy persons and as an indicator of recurrent event

prognosis. Increases in CRP values are non-specific and should not be interpreted without a complete clinical history. The American Heart Association and the Centers for Disease Control and Prevention have made several recommendations concerning the use of high sensitivity C-Reactive Protein (hsCRP) in cardiovascular risk assessment. Testing for any risk assessment should not be performed while there is an indication of infection, systemic inflammation or trauma. Patients with persistently unexplained hsCRP levels above 10 mg/L (95.2 nmol/L) should be evaluated for non-cardiovascular etiologies. When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values. Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Screening the entire adult population for hsCRP is not recommended, and hsCRP is not a substitute for traditional cardiovascular risk factors. Acute coronary syndrome management should not depend solely on hsCRP measurements. Similarly, application of secondary prevention measures should be based on global risk assessment and not solely on hsCRP measurements. Serial measurements of hsCRP should not be used to monitor treatment. Various assay methods are available for CRP determination, such as nephelometry and turbidimetry. The Roche CRP assay is based on the principle of particle-enhanced immunological agglutination.

Test principle[22,23]

Particle enhanced immuno-turbidimetric assay.
Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

Reagents - working solutions

R1 TRIS buffer with bovine serum albumin and immunoglobulins (mouse); preservative; stabilizers

SR Latex particles coated with anti-CRP (mouse) in glycine buffer; preservative; stabilizers

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

R1 Ready for use.

SR Ready for use. Before use, invert several times, avoiding the formation of foam.

Storage and stability

CRPHS

Shelf life at 2-8 °C:	See expiration date on reagent

On-board in use and refrigerated on the analyzer:	4 weeks

NaCl Diluent 9 %

Shelf life at 2-8 °C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum
Plasma: Li-heparin, K_2-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[24]	11 days at 15-25 °C
	2 months at 2-8 °C
	3 years at (-15)-(-25) °C

Materials provided

See “Reagents - working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section.
General laboratory equipment
Other suitable control material can be used in addition.

_____
_____
_____
_____

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 111 **test definition**

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction direction	Increase
Wavelength A	552 nm
Calc. first/last	17/34
Unit	mg/L (nmol/L, mg/dL)
Reaction mode	R1-S-SR

Pipetting parameters

		Diluent (H_2O)
R1	82 µL	
Sample	6 µL	48 µL
SR	28 µL	14 µL
Total volume	178 µL	

Calibration

Calibrator	Calibrator f.a.s. Proteins
Calibration dilution ratio	1:5, 1:10, 1:20, 1:40, 1:80, performed automatically by the instrument, and Standard 6 = 0 mg/L.
Calibration mode	Linear interpolation
Calibration interval	Each lot and as required following quality control procedures

Enter the assigned lot-specific CRPHS value of the undiluted calibrator (mg/L), indicated in the package insert of C.f.a.s. Proteins.
Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[25]

Quality control

For quality control, use control materials as listed in the "Order information" section.
In addition, other suitable control material can be used.
The control intervals and limits should be adapted to each laboratory's individual requirements.
Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.
If controls do not recover within the specified limits, take the following corrective action:

_____
_____
_____
_____

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors:	mg/L x 9.52 = nmol/L mg/L x 0.1 = mg/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at CRP levels of 3.0 mg/L.

Icterus:[26] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[26] No significant interference up to an H index of 700 (approximate hemoglobin concentration: 435 µmol/L or 700 mg/dL).

Lipemia (Intralipid):[26] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 1200 IU/mL do not interfere.

High-dose hook effect: does not occur at CRP concentrations below 40 mg/L or 380 nmol/L. Samples with concentrations > 40 mg/L are flagged either >TEST RNG or "HIGH ACT".

Drugs: No interference was found at therapeutic concentrations using common drug panels.[27,28] Exception: Significantly degreased CRP values may be obtained from samples taken from patients who have been treated with carboxypenicillins.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results. [29]

Although measures were taken to minimize interference caused by human anti-mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Special wash requirements
No interfering assays are known which require special wash steps.

Limits and ranges

Measuring range
0.15-20.0 mg/L (1.43-190 nmol/L, 0.015-2.0 mg/dL)

Lower limits of measurement
Lower detection limit of the test
0.15 mg/L (1.43 nmol/L, 0.015 mg/dL)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Functional sensitivity (Limit of Quantitation)

0.3 mg/L (2.86 nmol/L)
The functional sensitivity (Limit of Quantitation) is the lowest CRP concentration that can be reproducibly measured with an inter-assay coefficient of variation < 10 %.

Expected values

Consensus reference interval for adults:[30]

IFCC/CRM 470

mg/dL	mg/L	nmol/L
< 0.5	< 5.0	< 47.6

The CDC/AHA recommended the following hsCRP cut-off points (tertiles) for CVD risk assessment:[21,31]

hsCRP level (mg/L)	hsCRP level (nmol/L)	Relative risk
< 1.0	< 9.52	low
1.0-3.0	9.52-28.6	average
> 3.0	> 28.6	high

Patients with higher hsCRP concentrations are more likely to develop myocardial infarction and severe peripheral vascular disease.
5-95 % reference intervals of neonates and children:[32]
Neonates (0-3 weeks): 0.1-4.1 mg/L (0.95-39.0 nmol/L)
Children (2 months-15 years): 0.1-2.8 mg/L (0.95-26.7 nmol/L)
Roche has not evaluated reference values in pediatric population.

It is important to monitor the CRP concentration during the acute phase of the illness.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.
Increases in CRP values are non-specific and should not be interpreted without a complete clinical history.
When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values. Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Measurements should be compared to previous values. When the results are being used for risk assessment, patients with persistently unexplained hsCRP levels of above 10 mg/L (95.2 nmol/L) should be evaluated for non-cardiovascular origins. Testing for any risk assessment should not be performed while there is indication of infection, systemic inflammation or trauma.[21]

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability n = 21, intermediate precision (3 aliquots per run, 1 run per day, 10 days).
The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	mg/L (nmol/L, mg/dL)	*mg/L (nmol/L)*	*%*
Precinorm Protein	11.4 (109, 1.14)	0.0 (0, 0.0)	0.4
CRP T Control N	4.06 (38.7, 0.406)	0.01 (0.1, 0.01)	0.3
Human serum 1	0.49 (4.66, 0.049)	0.01 (0.07, 0.001)	1.5
Human serum 2	4.02 (38.3, 0.402)	0.02 (0.2, 0.002)	0.6
Human serum 3	16.9 (161, 1.69)	0.1 (1, 0.01)	0.3

Intermediate precision	*Mean*	*SD*	*CV*
	mg/L (nmol/L, mg/dL)	*mg/L (nmol/L)*	*%*
Precinorm Protein	11.3 (108, 1.13)	0.1 (1, 0.01)	0.5
CRP T Control N	3.90 (37.1, 0.390)	0.04 (0.4, 0.004)	1.0
Human serum 4	0.48 (4.57, 0.048)	0.01 (0.10, 0.001)	2.0
Human serum 5	3.91 (37.2, 0.391)	0.05 (0.5, 0.005)	1.4
Human serum 6	16.8 (160, 1.68)	0.1 (1, 0.01)	0.7

Method comparison

CRP values for human serum and plasma samples obtained on the **cobas c** 111 analyzer (y) were compared to those determined with the same reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 79

Passing/Bablok[33]	Linear regression
y = 1.035x – 0.111 mg/L	y = 1.051x – 0.202 mg/L
τ = 0.962	r = 0.999

The sample concentrations of the reference system (x) were between 0.21 and 18.6 mg/L (2.0 and 177 nmol/L, 0.021 and 1.86 mg/dL).