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HIGH-LAURIC AND HIGH-MYRISTIC FATTY ACID CHEESE: EFFECTS ON SERUM LIPOPROTEIN PROFILES

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ABSTRACT

Cardiovascular disease (CVD) has been the leading cause of death in the United States for over fifty years. Diet is one of the most widely studied modifiable CVD risk factors. Dietary fatty acids play a key role in the development of CVD. Traditional guidelines for CVD prevention have included recommendations for decreased saturated fat intake. Recent research has suggested, however, that individual saturated fats differentially affect the serum lipoprotein profile. In this study, a treatment cheese high in two saturated fatty acids, lauric and myristic, was compared to a control cheese with a typical fatty acid composition. The control and treatment cheeses were assessed for their effects on total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TC:HDL-C ratio, and triglycerides (TG). In this randomized, cross-over trial, nine individuals with elevated LDL-C levels ($\geq$130 mg/dL) consumed three ounces daily of each cheese type independently during two, three-week feeding periods separated by a two-week washout period. For the overall population, TC:HDL-C significantly increased (6.558%, p=0.0267) from baseline to the end of the treatment cheese period. Treatment cheese consumption did not change any other serum lipids in comparison with control cheese consumption. However, each individual did respond to treatment cheese consumption, and the population could be separated into four individuals who generally experienced beneficial effects versus five participants who experienced more potential adverse effects from treatment cheese consumption. Individuals show variable lipid changes in response to the consumption of different fatty acids. Further research is warranted to determine the differential effects of individual saturated fats on the lipid profile in order to establish optimal dietary recommendations for CVD prevention.

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High-Lauric Fatty Acid and High-Myristic Fatty Acid Cheese: Effects on Serum Lipoprotein Profiles

I. BACKGROUND AND SIGNIFICANCE

A. CARDIOVASCULAR DISEASE

For over fifty years, cardiovascular disease (CVD) has been the leading cause of death in the United States (Cooper et al., 2000; Jemal et al., 2005). In 2006 alone, more than one in four Americans died from CVD (Heron et al., 2009). CVD includes coronary heart disease (CHD), the number one cause of death, as well as stroke, the third leading cause of death. The category of CHD encompasses ischemic heart disease, angina pectoris, and myocardial infarction (US Department of Health and Human Services, 2004).

CHD begins with a buildup of plaque that causes the narrowing and hardening of the vessels supplying blood to the heart in a process known as atherosclerosis. The plaque, which is composed of cholesterol and other lipids, can grow to reduce or fully block blood flow and oxygen supply to the heart. If plaque ruptures, it also can produce blood clots that block the arteries (National Center for Chronic Disease Prevention and Health Promotion, 2009).

The most common symptom of CHD is angina, which is chest pain or discomfort resulting from decreased blood flow to the heart. Over time, CHD can weaken the heart muscle and lead to the abnormal pumping of blood known as heart failure. Arrhythmias, or irregular heartbeats, can develop as well. Apart from these symptoms, a myocardial infarction could be the first sign of CHD. Also called heart attacks, myocardial infarctions occur when heart muscle cells begin to die due to a lack of blood and oxygen flowing through the coronary arteries to the myocardium. Heart attacks frequently produce acute injury, scarring, and permanent damage (US Department of Health and Human Services, 2004). If the myocardial infarction continues without the restoration of blood flow, more damage occurs. The result can be arrhythmia or even the complete termination of heart beats, known as sudden cardiac arrest. CHD is the most common underlying cause of myocardial infarction, which is just one way in which CVD can cause death (National Center for Chronic Disease Prevention and Health Promotion, 2009).

B. NON-MODIFIABLE RISK FACTORS FOR CVD

As CHD consistently is responsible for the greatest number of deaths in America each year, much research has focused on its pathology and prevention. Multiple risk factors have

been associated with America's number one killer. Many of these, such as heredity, gender, and increasing age, are non-modifiable. Nevertheless, they are important to understand in order to promote awareness and prevention among at-risk groups. Individuals with a family history of CHD, for instance, are at a greater risk for developing the disease. While some of this correlation has been linked to common lifestyle characteristics among families (Pohjola-Sintonen et al., 1998), genetic components also have been identified. A single amino acid mutation in apolipoprotein B, for example, has been shown to decrease the ability of low-density lipoproteins (LDL) to bind to their receptors. The resulting decrease in LDL-binding has been correlated with familial hypercholesterolemia and increased CHD risk (Innerarity et al., 1990).

Genetic polymorphisms in angiotension converting enzyme (ACE) likewise have been suggested to increase CHD risk. As part of the renin-angiotension-aldosterone system, ACE is involved in blood pressure regulation. ACE catalyzes the production of angiotensin II, one of the body's most powerful vasoconstrictors (Ganong, 2005). A 2008 meta-analysis suggested a modest positive association between the ACE insertion/deletion polymorphic variant and CAD (Zintzaras et al., 2008). In 2000, Agerholm-Larsen et al. conducted a meta-analysis that also identified converting enzyme (ACE) gene polymorphisms as risk factors for ischemic heart disease and myocardial infarction in small studies only (Agerholm-Larsen et al., 2000). In a 2001 study of 304 individuals, angiotensinogen (AGT) gene polymorphism, but not ACE gene polymorphism, was associated with increased CHD risk independently of hypertension (Rodríguez-Pérez et al., 2001). Ongoing research aims to more firmly establish the relationship between ACE gene polymorphisms and CHD risk.

In addition to mutations in the ACE gene, multiple other genetic polymorphisms have been suggested as CHD risk factors. Due to the role of inflammation in atherosclerosis, much research has focused on polymorphisms in cytokine-encoding genes (Yudkin et al., 2000). For instance, a 1992 study identified variation at the β fibrinogen locus as a CHD risk factor (Fowkes et al., 1992). In 2001, two polymorphisms encoding the interleukin-6 (IL-6) promoter were associated with increased CHD risk among healthy male subjects (Humphries et al., 2001).

Genetic polymorphisms comprise only one class of non-modifiable risk factors. Gender has similarly been identified as an unchangeable indicator of CVD susceptibility. Males are more likely than females to develop atherosclerosis. Among both genders, increasing age is

correlated with higher total cholesterol levels and greater prevalence of atherosclerosis, resulting in an elevated risk for the development of CVD (Castelli, 1984).

Genetics, gender, and age exemplify risk factors for CVD that cannot be willfully changed. Although understanding non-modifiable risk factors is imperative in identifying at-risk populations, current research is aiming to evaluate factors that can be changed in order to prevent CVD. Individuals identified as high-risk based on non-modifiable factors, as well as the general population, should be aware of the risk factors they can control.

C. MODIFIABLE RISK FACTORS FOR CVD

In order to develop guidelines for CVD prevention, many modifiable risk factors are being studied. Data from the National Health and Nutrition Examination Survey (NHANES) 2007-2008 showed that 68.0% of US adults were overweight or obese, with 33.8% in the obese category (Flegal et al., 2010). With the prevalence of obesity greater than ever and continuing to increase, improving weight status has been identified as one of the most important means of CVD prevention. Obesity has been suggested to have several adverse effects on cardiovascular health. Obese individuals are more likely than those with a healthy weight to have hypertension and increased arterial pressure. Independent of arterial pressure, obesity also increases the risk left ventricular hypertrophy (LVH), especially of the eccentric type (Lavie and Messerli, 1986).

Plasma lipid levels also are adversely affected by obesity; higher levels of harmful triglycerides and low-density lipoprotein (LDL) cholesterol as well as lower levels of cardioprotective high-density lipoprotein (HDL) cholesterol are associated with obesity (Pi-Sunyer, 1993). Other diseases related to increased inflammation and cardiovascular mortality, such as insulin resistance, diabetes mellitus, and metabolic syndrome, are more common in the obese population as well. Beyond its contributions to many CVD risk factors, multiple large studies, including the Framingham Heart Study and the Nurses' Health Study, have shown obesity to be an independent risk factor for CHD (Lavie and Messerli, 1986; Pi-Sunyer, 1993).

Physical activity plays an important role in modifying weight status and CHD risk. A literature review through the year 2000 found a causal inverse, dose-response relationship between level of physical activity and CVD incidence and mortality, especially ischemic heart disease (Kohl, 2001). Studies from the past fifty years have consistently demonstrated a reduced incidence of CHD events among more physically active and physically fit people. Exercise has been shown to both prevent and treat atherosclerotic risk factors such as obesity, hypertension,

insulin resistance, glucose intolerance, elevated triglycerides (TG), high LDL-cholesterol (LDL-C), and reduced HDL-cholesterol (HDL-C). A 2003 analysis of fifty-one studies suggested the success of comprehensive, exercise-based cardiac rehabilitation programs in decreasing mortality among patients diagnosed with CHD. For those with heart failure, exercise also was demonstrated as a way to improve cardiovascular health and quality of life. Finally, in patients with peripheral arterial disease and claudication, exercise therapy was shown to improve walking distance (Thompson et al., 2003).

In 2007 the American College of Sports Medicine (ACSM) and the American Heart Association (AHA) published exercise recommendations for optimal cardiovascular health. The report suggested at least thirty minutes of moderate-intensity aerobic exercise five days per week or at least twenty minutes of vigorous-activity aerobic exercise three days per week. In addition, the ACSM and the AHA recommended at least two days per week of activity that increases muscular strength and endurance (Haskell et al., 2007).

Another behavior that can be changed to decrease CVD risk is smoking. Cigarette smoking has been indicated as "the most preventable cause of cardiovascular morbidity and mortality" (Lakier, 1992). Smoking is associated with a two- to four-fold increase in CHD risk, and a greater than 70% increased risk of CHD-related death (Lakier, 1992). The 1994 Report of the Surgeon General first presented data to firmly establish smoking as a major cause of CHD. Ten years later, the Report of the Surgeon General provided further evidence to support this association. Even when controlling for possible confounding lifestyle-related risk factors, smoking has been indicated consistently as an independent risk factor for CHD (US Department of Health and Human Services, 2004).

This study targeted nutritionally modifiable risk factors, which include hypertension and serum levels of cholesterol, lipoproteins, and triglycerides. Hypertension is defined as systolic blood pressure above 140 mmHg or diastolic blood pressure above 90 mmHg (Zieve, 2009). In the Honolulu Heart Program, incidence of CHD was determined during a 10-year follow-up of 7,705 Japanese men. Results indicated systolic blood pressure as the most powerful predictor of all CHD manifestations except angina pectoris, which included fatal CHD, nonfatal myocardial infarction, and acute coronary insufficiency (Yano et al., 1984). Results published from the Framingham Study in 1984 showed a curvilinear relationship between systolic or diastolic blood pressure and CHD risk (Castelli, 1984). In 1996, Kannel published a follow-up from the

Framingham Study that similarly indicated hypertension as a powerful contributor to CVD. On average, hypertension increased risk by two- to three-fold of all major atherosclerotic cardiovascular diseases. Because there was no evidence to suggest a decline in the prevalence of hypertension during the follow-up period, hypertension should be targeted as a means of CVD prevention. Weight control, exercise, and reduced sodium and alcohol intake have been suggested as ways to decrease blood pressure and improve cardiovascular health (Kannel, 1996).

In addition to hypertension, serum lipid levels are CVD risk factors that also may be modified by diet. Because of their key roles in both the development and prevention of CVD, lipid biomarkers were the primary endpoints for this study. Cholesterol is a soft, waxy steroid metabolite found in the bloodstream and all cells of the body. Due to its hydrophobic nature, cholesterol must be carried in the circulation on lipoproteins. Total cholesterol (TC) is the sum of cholesterol as a component of chylomicrons, very low-density lipoproteins (VLDL-C), low-density lipoproteins (LDL-C), and high-density lipoproteins (HDL-C). Cholesterol provides fluidity to cell membranes and is required for the synthesis of bile acids, steroid hormones, and several fat-soluble vitamins. However, excess LDL-C in circulation can build up in the arteries supplying the heart and the brain to become a component of thick, hard plaque deposits. Blockage of blood flow to the heart leads to myocardial infarction while clots in arteries supplying the brain lead to stroke (AHA, 2010).

Traditionally, the effects of dietary fatty acids on CVD have been estimated from their effects on serum TC and LDL-C. In the Framingham Study, TC and LDL-C were each independently related to CHD risk, and the association increased with age (Castelli, 1984). Among the 325,348 white males in the 1986 MRFIT Study, elevated TC levels were associated with a greater rate of mortality from CHD. The increased risk for CHD attributable to high TC was greater for the older age group (Kannel et al., 1986). In the 2001 Atherosclerosis Risks in the Community (ARIC) Study, males and females in the lowest quintile for LDL-C levels had the lowest risk for CHD, and increased levels were associated with increased risk (Sharrett et al., 2001). The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) has therefore classified LDL-C levels < 100 mg/dL as optimal, 130-159 mg/dL as borderline high, and > 160 mg/dL as an indicator of high CVD risk. TC levels < 200 mg/dL are considered desirable, and TC levels $\geq$ 240 mg/dL indicate high CVD risk (NCEP, 2002).

While past studies have mainly used serum TC and LDL-C as predictors of CHD risk,

HDL-C beneficially impacts cardiovascular health in a number of ways. HDL-C is primarily responsible for moving excess cholesterol from the blood vessel walls to the liver for excretion in a cardioprotective process known as reverse cholesterol transport (Toth, 2003). HDL-C also increases blood flow by promoting vasodilation and reduces blood vessel damage via anti-inflammatory and antioxidant processes (Toth, 2005).

Low HDL-C levels often indicate high levels of atherogenic lipoproteins, and decreased HDL-C frequently accompanies other CVD risk factors such as diabetes and high blood pressure (Vega and Grundy, 1996). Thus, many recent studies have shown an inverse relationship between HDL-C levels and CHD risk. In the ARIC Study, HDL-C was shown to be an independent predictor of CHD risk (Sharrett et al., 2001). Likewise, in a population of 6,408 Japanese men, the incidence of CHD and myocardial infarction was three to four times higher in the lowest quintile than in the highest quintile (Kitamura et al., 1994). The NCEP has classified HDL-C $\geq$ 60 mg/dL as the optimal level for CVD prevention (NCEP, 2002).

Despite the importance of TC and LDL-C levels in past studies, current research now recognizes the ratio of TC:HDL-C as the most specific indicator of CHD risk. That is, a decreased TC:HDL-C ratio is the lipid biomarker response most strongly associated with lower CVD risk (Castelli, 1984). In 2001, Real et al. studied sixty-six heterozygous familial hypercholesterolemic subjects, half of which had CHD. Significantly lower HDL-C and TC:HDL-C levels were present among those with CHD, but associations between CHD and TC and LDL-C were not statistically significant. Therefore, Real and his colleagues suggested treating familial hypercholesterolemia by targeting not only LDL-C but also HDL-C (Real et al., 2001).

In another study of a population of fifty-six women, the TC:HDL-C ratio was the variable most predictive of CAD, and it was the only characteristic associated with CAD severity after adjusting for other risk factors (Hong et al., 1991). The results of the Framingham Study indicated the TC:HDL-C ratio as a predictor of CHD and suggested that the ratio should not exceed 4.5 (Castelli, 1984). In 1994, Kinosian et al. analyzed the prevalence of CHD among the Framingham and Coronary Primary Prevention Trial (CPPT) population and death from CHD in the Prevalence Study population. In all three cohorts, the TC:HDL-C ratio was more indicative of CHD risk than TC or LDL-C alone (Kinosian et al., 1994). Finally, a 2001 epidemiological review indicated the TC:HDL-C ratio as the most predictive indicator of both CHD outcome and

treatment benefit (Criqui and Golomb, 1998). Despite recent evidence, however, the National Cholesterol Education Program (NCEP) does not support the TC:HDL-C ratio as a specified target of lipid therapy. Rather, the NCEP asserts that LDL-C should be maintained as the main target of dietary interventions for hyperlipidemia (NCEP, 2002).

In addition to TC, LDL-C, and HDL-C, triglycerides (TG) also have been correlated with CVD risk and were thus another endpoint in this study. Plasma TG are derived from dietary fats or made endogenously from other energy sources. Ingested calories that are not used immediately for energy are converted into TG and transported to fat cells for storage. Between meals, hormones regulate the release of TG to be used as a valuable energy source (AHA, 2010). However, excess plasma TG, known as hypertriglyceridemia, can increase CHD risk through a variety of proposed mechanisms. High TG levels lead to an increase in the production of atherogenic chylomicron and VLDL remnants; meanwhile, serum TG and HDL-C have been shown to be inversely related. Additionally, LDL-C levels increase with elevated TG levels due to the reduction of hepatic LDL receptors (LDLr) along with the formation of more dense, and thus more atherogenic, LDL-C. Finally, hypertriglyceridemia adversely affects circulation by promoting excess blood coagulation (Geurian et al., 1992).

Much evidence has supported the proposed harmful effects of TG on cardiovascular health. In the fully adjusted models from the ARIC Study, high TG levels were independently associated with increased CHD risk (Sharrett et al., 2001). A 1998 meta-analysis of seventeen population-based studies indicated increased plasma TG as a risk factor for CVD among both men and women, independent of HDL-C status (Austin et al., 1998). Consequently, the NCEP classifies a TG level above 200 mg/dL as an indicator of high CVD risk (NCEP, 2002).

D. EFFECTS OF DIETARY FATS ON LIPID RISK FACTORS

This study focused on decreasing CVD risk by targeting lipid risk factors. Nutrition research has shown diet therapy to be an effective modifier of TC, HDL-C, LDL-C, and TG levels. The 2001 Report of the National Cholesterol Education Program (NCEP) synthesized current scientific research into therapeutic lifestyle changes (TLC) to promote optimal lipid levels for cardiovascular health. The evidence-based report encourages physical activity and healthy weight status while identifying specific nutrient components that effectively modify CVD risk.

The NCEP emphasizes recommendations that modify the intake of dietary fats in order to

decrease LDL-C and promote cardiovascular health. During recent years, numerous studies have identified the differential impacts of the various classes of fatty acids on the serum lipid and lipoprotein profile. A 1992 meta-analysis of twenty-seven studies suggested that, although dietary fats are not the sole or even the main determinant of serum lipids, dietary fatty acids have consistently been shown to modify serum lipid levels regardless of intrinsic starting levels (Mensink and Katan, 1992). At the core of the NCEP's TLC diet is the reduction of saturated fat and cholesterol intakes. The NCEP also recommends minimal intakes of *trans* fatty acids along with the replacement of saturated fats with monounsaturated fats (up to 20% of total energy) or polyunsaturated fats (up to 10% of total energy) (NCEP, 2002).

High-monounsaturated fatty acid intake has been associated with decreased CVD risk. *Cis*-monounsaturated fatty acids decrease LDL-C by replacing saturated fatty acids. Unlike the substitution of saturated fats with carbohydrates, the exchange of monounsaturated fats for saturated fats is associated with neither increased TG nor decreased HDL-C (NCEP, 2002). A 1997 study by Kris-Etherton et al. showed that a diet high in peanut-derived monounsaturated fatty acids was associated with decreased TC, LDL-C, and TG (Kris-Etherton et al., 1999). Similarly, when Mensink et al. compared a carbohydrate-rich diet with a diet rich in olive oil-derived monounsaturated fats, the olive oil diet produced a more favorable lipoprotein profile (Mensink et al., 1989).

A 1995 study of high-carbohydrate intake among diabetes mellitus patients suggested that the TG-lowering effect of monounsaturated fats was due not to increased lipolysis or increased postprandial clearance of TG-rich lipoproteins, but rather a result of reduced hepatic secretion of VLDL-C triglycerides (Blades and Garg, 1995). In a 1998 meta-analysis of diabetes mellitus patients, diets rich in monounsaturated fats versus carbohydrates were associated with a significant lowering of fasting plasma TG, TC, and VLDL-C while HDL-C increased and LDL-C did not change (Garg, 1998).

Like monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) are associated with lower CVD risk. A 1997 review by Kris-Etherton and Yu indicated PUFAs as the most potent hypocholesterolemic fatty acids (Kris-Etherton and Yu, 1997). The long-chain, n-3 PUFAs, namely eicosapentaenoic acid (EPA C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), have been shown to elicit cardioprotective effects via several mechanisms. By replacing arachidonic acid (AA) in phospholipid pools, EPA and DHA act to inhibit the actions

of lipoxygenase and cycloxygenase and to decrease the synthesis of eicosanoids. While reducing the formation of proaggregatory, vasoconstrictive thromboxane A_2 and leukotriene B_4, the n-3 PUFAs promote the synthesis of antiaggregatory prostacyclin I_2 and prostaglandin I_3. As a result, EPA and DHA slow the progression of atherosclerosis, reduce blood pressure and viscosity, inhibit the synthesis of hepatic TG and apoproteins, and control membrane fluidity along with associated enzyme and receptor actions (Kinsella et al., 1990).

A 1995 trial showed that, when substituted for monounsaturated fatty acids (MUFAs), n-3 PUFAs decreased TC and LDL-C and produced a lesser increase in TG. As percent energy from MUFAs decreased and percent energy from PUFAs increased, there was a progressively larger decline in TC and LDL-C (Howard et al., 1995). High intakes of EPA and DHA have been associated with TG reduction, which may be secondary to decreased VLDL production (Nestel 1990, Roche 2000). In general, the marine n-3 fatty acids have not been shown to impact LDL-C, but one review found that large doses reciprocally increase LDL-C in persons with hypertriglyceridemia (Harris 1997).

Because ALA cannot be synthesized endogenously, adequate intakes are especially important for high-risk populations. Both the American Heart Association and the US Department of Agriculture Dietary Guidelines Advisory Committee recommend eating fatty fish at least twice per week to lower CVD risk (Lichtenstein, 2006; USDA, 2005). The NCEP considers the evidence supporting n-3 polyunsaturated fatty intake to be moderate. The TLC dietary recommendations thus include increased marine polyunsaturated fatty acid intake as an option for modifying lipid risk factors, but no specific amount is prescribed (NCEP, 2002).

Limited evidence supports the intake of linoleic acid-derived n-6 PUFAs for CVD prevention. Mensink and Katan's 1992 meta-analysis showed that n-6 PUFA intake was related to decreased concentrations of TC, LDL-C, and HDL-C (Mensink and Katan, 1992). Although some meta-analyses indicate a reduction in CHD risk upon replacing saturated fats with polyunsaturated fats, no large cohorts have consumed high intakes of polyunsaturated fats for an extended period of time. Thus, the NCEP does not suggest large intakes of polyunsaturated fats; the recommended TLC diet includes polyunsaturated fats as replacements for saturated fats in amounts up to 10% of total calories (NCEP, 2002).

In contrast to MUFAs and n-3 PUFAs, high *trans* and saturated fatty acid intakes increase CVD risk. *Trans* fatty acids are largely produced by the hydrogenation of vegetable

oils, during which the naturally occurring *cis* double bond formation is changed to the *trans* arrangement (Brown, 2008). The products of linoleic acid hydrogenation, which include *trans* compounds and saturated stearic acid, increase LDL-C and decrease HDL-C compared to the effects of linoleic acid itself (Zock and Katan, 1992).

Extensive evidence indicates the unfavorable effects of *trans* fatty acid intake on the lipid profile. Mensink et al.'s meta-analysis of sixty controlled diet therapy trials found that the most significant reduction in the TC:HDL-C ratio occurred when *trans* and saturated fats were replaced with *cis* unsaturated fats. Replacing *trans* fats had almost double the effect of replacing saturated fats (Mensink et al., 2003). In agreement with the effects of *trans* fats on serum lipids and lipoproteins, large epidemiological studies have associated *trans* fatty acid intake with increased risk for CHD in both women (Willett et al., 1993) and men (Pietinen et al., 1997).

Based on the strong evidence demonstrating the adverse effects of *trans* fat intake on cardiovascular health, the Institute of Medicine (IOM) of the National Academy of Sciences has concluded that there is no safe level of *trans* fatty acid intake (IOM, 2002). In response, the United States Food and Drug Administration (FDA) mandated the inclusion of *trans* fat content on all nutrition labels in 2003 (US FDA, 2003). Similarly, the NCEP has recommended low intakes of *trans* fats and the replacement of *trans* fat-rich foods such as butter, stick margarine, and shortening with liquid vegetable oil, soft margarine, and *trans* fat-free margarine (NCEP, 2002).

Like *trans* fats, saturated fats are traditionally associated with increased CHD risk due to their adverse impacts on lipid biomarkers. Saturated fats are composed of hydrocarbon chains with only single bonds. Typically solid at room temperature, saturated fats are widely consumed in many animal products such as fatty beef, lamb, poultry with skin, lard, cream, butter, cheese, and other dairy products produced from whole or reduced-fat milk. This trial focused on modifying the fatty acid composition of cheese, a dairy product consumed widely in the American diet and a large source of saturated fat.

Saturated fatty acids have been recognized as the single dietary factor with the greatest role in increasing LDL-C concentrations (Fernandez and West, 2005; Hu and Manson, 2001). Analyses have shown that for every one percent increase in calories from saturated fats as a percent of total energy, LDL-C increases by approximately two percent. A one percent decrease in saturated fat consumption results in the reduction of TC by about two percent (Mensink and

Katan, 1992; Kris-Etherton and Yu, 1997; NCEP, 2002). Published in 1998, results of the Dietary Effects on Lipoproteins and Thrombogenic Activity (DELTA) study showed that step-wise reductions in saturated fat intake resulted in parallel reductions of both TC and LDL-C levels. Specifically, when compared to an average American diet, a low-saturated fat diet decreased TC by 9% and LDL-C by 11%. Results were similar across subgroups of male, female, and black participants (Ginsberg 1998). A decrease in saturated fat intake has been suggested to be twice as potent in lowering TC as an equivalent increase in polyunsaturated fat intake (Dietschy 1998 et al., Keys et al., 1957).

The cholesterol-raising effect of saturated fats is primarily related to decreases in the activity, protein, and mRNA abundance of the LDL-C receptor (Mustad et al., 1996). Saturated fatty acids generally do not increase TG levels, which would be expected if they synthesized VLDL-C, the precursor to LDL-C (NCEP, 2002). Some studies have suggested that saturated fats increase the proportion of cholesterol in the regulatory pool by suppressing ACAT, the rate-limiting enzyme of cholesterol esterification (Spady et al., 1993; Fernandez and West, 2005).

The adverse effect of saturated fat consumption on LDL-C levels is reflected by epidemiological evidence relating increased saturated fat intake to increased CHD risk. During 14 years of follow-up of 80,082 women in the Nurses' Health Study, each 5% increase in energy from saturated fat, as compared with equal intake from carbohydrates, was associated with a 17% increase in CHD risk (Hu et al., 1997). The large Seven Countries Study began with 11,579 adult males and included 10 and 15-year follow-ups to assess the relationship between diet and mortality. Data from both time points showed strong correlations between the average percentage of daily energy from saturated fats and both CHD risk and CHD-related mortality (Keys et al., 1986).

Based on clinical and epidemiological research, the NCEP recommends consuming less than 7% of energy as saturated fat in order to minimize LDL-C levels (NCEP, 2002). However, recent findings have suggested the differential impacts of individual saturated fats on the lipoprotein profile. Contrary to traditional guidelines, some saturated fats have been suggested to have hypocholesterolemic effects. The saturated fats evaluated in this study were medium-chain lauric (12:0) and myristic (14:0) acids. Other dietary saturated fats of recent interest include palmitic (16:0) and stearic (18:0) fatty acids.

In a 1997 review, Kris-Etherton and Yu assessed the results of human studies evaluating

the effects of individual fats on serum lipoprotein profiles. Collectively, studies of palmitic acid suggested its cholesterol-raising effect to be less than that of myristic acid, but greater than the individual effects of lauric, stearic, and oleic (18:1) acids. The hypercholesterolemic effect of palmitic acid was also found to be less than that of the lauric-myristic acid combination that was evaluated in this study (Kris-Etherton and Yu, 1997).

Compared with the effects of palmitic acid and other long-chain saturated fatty acids, stearic acid has been shown to produce significant decreases in TC, LDL-C, and HDL-C concentrations (Kris-Etherton and Yu, 1997). In fact, evidence suggests that the effects of saturated stearic acid on the lipid profile are similar to those produced by monounsaturated oleic acid. In a 1988 trial, a high-stearic acid diet and a high-oleic acid diet each significantly decreased TC and LDL-C while exerting no effect on HDL-C in comparison to a high-palmitic acid diet. Furthermore, during the high-stearic acid diet, the oleic acid content of plasma triglycerides and cholesterol esters significantly increased. It has been suggested that stearic acid (18:0) converts rapidly to oleic acid (18:1) and thus induces comparable lipid responses (Bonanome and Grundy, 1988).

Relative to the abundant research confirming the effects of the long-chain saturated fats, evidence regarding the cholesterolemic effects of lauric and myristic acids remains unclear. A 1997 review of studies in animal models suggested that lauric, myristic, and stearic acid all equivalently raised LDL-C by down-regulating hepatic LDLr and increasing LDL-C production via sterol *O*-acyltransferase activity (Nicolosi, 1997). The well-known Keys equation and Hegsted equation were developed in the 1960s to summarize the quantitative effects of dietary fats on serum TC. While Keys considered lauric, myristic, and palmitic acids to have similar hypercholesterolemic effects (Keys, 1966), Hegsted et al. suggested lauric acid's effect on TC to be one-third that of palmitic acid and one-quarter that of myristic acid (Hegsted et al., 1965).

Hegsted et al.'s assertions were supported by a 1992 clinical trial in which lauric acid increased TC and LDL-C relative to oleic acid, but to a lesser extent than palmitic acid (Denke and Grundy, 1992). Although this study found no change in HDL-C, multiple other trials have suggested that lauric acid causes significant increases in HDL-C (Temme et al., 1996; Tsai et al., 1999; Mensink et al., 2003). In Mensink et al.'s meta-analysis of sixty trials, lauric acid was the most potent saturated fat in raising LDL-C. The HDL-C-raising effects of saturated fats increased with decreasing chain length, ranging from no change due to stearic acid to the greatest

change due to lauric acid. Mensink et al. thus showed that lauric acid had a more favorable effect in decreasing the TC:HDL-C ratio than any other fatty acid, either saturated or unsaturated. This ratio was relatively unaffected by the other three saturated fats, although stearic acid had a greater lowering effect than either palmitic or myristic (Mensink et al., 2003). Lauric acid was targeted for analysis in this study because of its suggested beneficial effects on HDL-C and the TC:HDL-C ratio.

Along with lauric acid, the treatment diet in this study also was high in myristic acid. The impact of myristic acid on serum lipoproteins similarly lacks consistent evidence. Based on early human research, Hegsted et al. calculated myristic acid to be the most potent saturated fat in raising TC (Hegsted et al., 1965). A 1992 review of feeding trials with both monkeys and humans found myristic acid to play the largest role in increasing TC (Hayes and Kohsla, 1992). A more recent clinical study by Zock et al. showed that myristic acid could have potential adverse effects when compared to palmitic acid by causing greater increases in LDL-C and TC (Zock et al., 1994).

Zock et al. also showed, however, that myristic acid had a potentially beneficial impact by increasing HDL-C to a greater extent than palmitic acid (Zock et al., 1994). A trial by Tholstrup et al. likewise indicated that postprandial HDL-C concentrations were greater after high-myristic acid intake than after high-stearic acid intake (Tholstrup et al., 2003). In hamsters, a 2002 study indicated that high-myristic acid intake increased HDL-C while other lipoproteins remained unaffected. This animal model research associated myristic acid with the decreased expression of liver scavenger receptor B1, which normally promotes the hepatic uptake of HDL-C (Loison et al., 2002).

It is important to note that in most studies asserting myristic acid's potentially adverse hypercholesterolemic effects, myristic acid has represented a very high percentage of the total energy intake (16-20%). More recently, a study by Dabadie et al. correlated myristic acid intake with potential beneficial decreases in TC and LDL-C along with an increase in HDL-C using a moderate intake of myristic acid (0.6% or 1.2% energy intake) as part of a mixed-fat diet in hamsters. This moderate intake also decreased plasma TG and increased n-3 and n-6 phospholipid levels (Dabadie et al., 2005). These results warrant further study of the effects of myristic acid on serum lipids as part of a more realistic mixed-fat diet.

E. CHEESE WITH A CARDIOPROTECTIVE FAT PROFILE

The specific treatment in this study was a modified bovine cheese with high concentrations of lauric and myristic fatty acids. Both fats are commonly found in coconut oil, palm-kernel oil, and milk fat. When consumed together, lauric and myristic acids have been suggested to decrease CVD risk. Countries in which coconut oil is a primary source of dietary fat, such as Sri Lanka and regions of the South Pacific, have the lowest prevalence of CVD. The high concentrations of lauric and myristic acids in coconut oil may be important contributors to the decreased CVD risk in these countries (Mann, 1998; Kaunitz, 1986).

In a clinical study by Sunmin et al., consumption of a lauric-myristic acid combination caused a two-fold to four-fold greater increase in HDL-C than palmitic-myristic or stearic-myristic treatments. Sunmin et al. attributed the HDL-C increase primarily to lauric acid. Although precise mechanisms have not been determined, the apo A-1 mechanism was suggested because the lauric-myristic acid diet produced the greatest increase in this apolipoprotein. It is unlikely that the lauric-myristic combination works through the reverse cholesterol transport system because all diets similarly impacted cholesterol esterification. Interestingly, the lauric-myristic combination was also shown to modify CHD risk by increasing the lag time for platelet aggregation (Sunmin et al., 1996).

A 1994 dietary intervention in normocholesterolemic men showed that a high intake of the lauric-myristic combination increased TC and LDL-C to a greater extent than did palmitic acid intake. Importantly, the lauric-myristic combination also increased HDL-C to a greater extent (Sundram et al., 1994). However, a 1995 study found conflicting results in a hypercholesterolemic population of males and females. Palmitic acid produced higher TC and LDL-C than did the lauric-myristic combination, and neither diet affected HDL-C (Cox et al., 1995).

The lauric-myristic fatty acid combination may beneficially affect cholesterol, and research has especially highlighted the favorable effects of these fats as components of dairy products. This study used a dairy food, cheese, as the vehicle for lauric and myristic acid consumption. The relationship between cheese consumption and CVD risk is controversial. The NHANES III Survey found that cheese consumption was associated with unfavorable body composition, hypertension, high LDL-C, and low HDL-C in men; however, in women, cheese consumption favorably reduced LDL-C and increased HDL-C (Houston et al., 2008). A 2009

report published by the New England Journal of Medicine indicated that, despite the contribution of dairy products to the saturated fatty acid component of the diet, there is no consistent evidence to associate high-dairy consumption with increased CVD risk. Thus, the review encourages the re-evaluation of dietary guidelines for heart health with consideration of the diverse nature of saturated fats in different milk products. Multiple studies have suggested that cheese has a lesser adverse impact on lipid profiles than do other milk products (German et al., 2009).

High-dairy fat intake has been associated with increased HDL-C (Sjogren et al., 2004; Mensink et al., 2003; German et al., 2009). In contrast to fats in milk and butter, the fat in cheese is encapsulated by the casein structure and thus may be absorbed and metabolized via unique mechanisms (Tholstrup et al., 2004). Dairy fat from cheese has been shown to produce less of an LDL-C-raising effect than dairy fat from butter (Nestel et al., 2005; Tholstrup et al., 2004). Fat from cheese also may beneficially impact the LDL-C particle size distribution by decreasing the proportion of small, dense LDL-C molecules (Sjogren et al., 2004).

Apart from inducing changes in serum lipids, cheese may exert additional cardioprotective effects. The National Heart, Lung, and Blood Institute has compiled scientific evidence to establish the Dietary Approaches to Stop Hypertension (DASH) eating plan. In addition to recommendations for increased fruit and vegetable intake with decreased fat and saturated fat intake, the DASH diet suggests two to three daily servings of low-fat or non-fat dairy. In comparison to the average American diet, which includes minimal low-fat or non-fat dairy products, the DASH diet has been shown to effectively reduce high blood pressure (Appel et al., 1997), while decreasing TC, LDL-C, and HDL-C concentrations (Obarzanek et al., 2001).

In a review of dairy products related to CHD mortality risk, consumption of ice cream, cream, whole milk, butter, or margarine was correlated with increased risk while intake of fermented dairy products, such as yogurt and cheese, resulted in decreased risk (Moss and Freed, 2003). The hydrolysis of milk fats and proteins during fermentation may account for the differential impacts of milk products. Furthermore, potentially protective compounds, such as phospholipids or calcium, may be removed during the production of butter (German et al., 2009).

The overall relationship between dairy intake and CVD risk has not been firmly established. It is evident, however, that cheese is a widely consumed food in the American diet. Although cheese may not increase CVD risk to the same magnitude as whole milk or butter, it is nevertheless a significant contributor to total energy, fat, and saturated fat intakes among

Americans. NHANES III Data 1999-2000 data indicated cheese or cheese spreads as the eighth greatest contributor to total energy intake in the US, accounting for 2.6% of total caloric consumption. Moreover, many cheese-containing foods were ranked even higher; pizza, for instance, was the fourth greatest contributor to energy intake (Block, 2004). NHANES II data, collected from 1976-1980, indicated cheese as the eleventh greatest contributor to energy intake, the eighth greatest contributed to total fat intake, and the third greatest contributor to saturated fat intake. The top sources of saturated fat were hamburgers, cheeseburgers, and meatloaf, which were followed by whole milk and whole milk products (Block et al., 1985). With CVD as a prevalent concern, a modified cheese with a cardioprotective impact could have great effects on the health of the American population.

Cheese provides a wide array of nutrients with beneficial impacts beyond cardiovascular health. A three-ounce serving of cheddar cheese contains 21 grams of complete protein, which provides all of the essential amino acids necessary for growth and function. Bone health is a prevalent concern in the United States, where osteoporosis affects one in four women and one in eight males. Cheese consumption promotes the growth and maintenance of bones by providing calcium, phosphorous, and vitamin D.

The mineral content of bones and teeth is largely composed of calcium and phosphorus. A three-ounce serving of cheese provides 60% of the Recommended Dietary Allowance (RDA) for calcium, a mineral that is consistently under-consumed by the American population. In addition to facilitating bone mineralization, adequate calcium consumption is required for dental health, muscle function, nerve activity, and blood clotting. Phosphorus is a key component of nucleic acids, phospholipids, adenosine triphosphate (ATP), and a number of enzymes and coenzymes. The metabolism of calcium and phosphorous requires the action of vitamin D, another beneficial component of cheese.

In addition to calcium and phosphorous, important minerals found in cheese include zinc, sodium, potassium, and iodine. Zinc activates protein metabolism and serves as a component of insulin and several enzymes. Sodium and potassium are important electrolytes that regulate the balance of acids, bases, and water in the body while contributing to nerve activation. Iodine is involved in the regulation of energy production and growth as a functional part of thyroid hormones.

Cheese is also a valuable source of B vitamins, which are involved in energy metabolism

and are required for normal growth and neuromuscular function. Two other vitamins found in cheese are folate, an important methyl donor, and vitamin A, which contributes to visual function and mucous membrane production. For its abundant nutrient composition with widespread health benefits, the current Dietary Guidelines for Americans suggest at least three servings of low-fat milk, cheese, or yogurt daily (Brown, 2008).

Evaluating the effects of a high-lauric and –myristic acid cheese on lipids in hypercholesterolemic humans was prompted by a study of hypercholesterolemic hamsters that was conducted by Dr. Thomas Wilson's laboratory at the University of Massachussetts in Lowell, MA. In this experiment, after consuming a high-cholesterol diet for two weeks, the hamsters were fed either control cheese or high-lauric and –myristic acid cheese for four weeks. Data showed that the cheese high in lauric and myristic acids caused significant reductions in TC and TC:HDL-C compared to the control cheese. The treatment cheese did not have a significantly different impact on HDL-C or non-HDL-C concentrations (Hristov, 2010).

Increasing the concentrations of lauric and myristic acids in cheese is an efficient and economical process. In 2009, the research group led by Dr. Alexander Hristov successfully produced high-lauric and –myristic acid cheese by manipulating the diet of the dairy cow. When the diets of lactating cows were supplemented with coconut oil, the milk produced contained a 2.2 to 2.6-fold increase in lauric acid and a 1.23-fold increase in myristic acid concentrations compared to cheese produced by cows fed a conventional diet.

Many modifiable risk factors are being studied in order to develop recommendations for CVD prevention. The lipoprotein profile is an important indicator of CVD risk that can be modified by dietary intake. In particular, the increased consumption of lauric and myrisitic fatty acids may favorably affect lipid biomarkers. The concentrations of these fatty acids can be altered in cheese, a popular component of the American diet. This study sought to evaluate the potential cardioprotective effects of a diet therapy that replaces typical cheese with cheese high in lauric and myristic fatty acids.

II. OBJECTIVE

To determine whether a three-week diet that includes 3 oz/day of treatment cheese containing high levels of lauric and myristic fatty acids improves the lipoprotein profile compared to consumption of control cheese.

III. **HYPOTHESES**

A. Daily intake of treatment cheese will decrease TC:HDL-C when compared to daily intake of control cheese.

B. Daily intake of treatment cheese will decrease TC:HDL-C cholesterol ratio when compared to baseline.

IV. METHODS

A. SUBJECTS AND ELIGIBILITY

All procedures were approved by the Institutional Review Board of the Pennsylvania State University (IRB #30582). Before enrolling in the study, all participants reviewed and signed an Informed Consent document. Ten generally healthy men and women aged 21-65 years with body mass index (BMI) 20-35, LDL-C 130-175 mg/dL, TG <350 mg/dL, and blood pressure ≤140/90 mmHg were recruited. To determine eligibility, each participant completed a phone interview followed by a clinical screening appointment. All subjects were "cheese-eaters" who typically consumed cheese daily. Participants replaced all cheese and cheese products they would normally eat with the daily servings of cheese provided by the study. A registered dietitian provided subjects with nutrition education principles to aim for maintenance of their baseline body weight (± 2 kg).

Additional exclusion criteria for all subjects included:

- Smokers
- A history of myocardial infarction, stroke, diabetes mellitus, liver disease, kidney disease, and thyroid disease (unless controlled on medication)
- Lactation, pregnancy, or desire to become pregnant during the study
- Cholesterol-lowering medications
- Intake of putative cholesterol-lowering supplements (psyllium, fish oil capsules, soy lecithin, niacin, fiber, flax, and phytoestrogens, stanol/sterol supplemented foods)
- Veganism
- Lactose intolerance

Blood pressure-lowering medications were acceptable if blood pressure was controlled (≤140/90 mmHg).

B. CHEESE PRODUCTION

Two cheddar-type cheeses were produced by Dr. Alexander Hristov's laboratory in the Department of Dairy Nutrition at the Pennsylvania State University in University Park, PA. Cows were fed a conventional diet to produce the control cheese with a typical fatty acid composition. A coconut oil diet was fed to other cows to produce the treatment cheese that was high in lauric and myristic fatty acids (Table 1).

Table 1. Fatty Acid Composition (g/100g Total Fatty Acids) of Cheeses

	Control	Treatment[1]
4:0	3.22	3.59
6:0	2.07	1.98
8:0	1.26	1.09
10:0	3.09	2.51
cis-9 10:1	0.29	0.25
11-cyclo 11:0	0.01	0.01
12:0	**3.80**	**8.52**
cis-9 12:1	0.10	0.17
trans-9 12:1	0.09	0.18
13:0 iso	0.03	0.02
13:0 anteiso	0.01	0.01
14:0	**11.91**	**14.67**
14:0 iso	0.12	0.07
cis-9 14:1	0.97	1.20
trans-9 14:1	0.02	0.03
15:0	1.43	0.90
15:0 iso	0.20	0.19
15:0 anteiso	0.42	0.28
cis-9 15:1	0.02	0.01
trans-5 15:1	0.09	0.06
trans-6 15:1	0.01	0.01
16:0	32.75	32.35
16:0 iso	0.28	0.23
cis-9 16:1	1.91	1.32
cis-11 16:1	0.02	0.06
cis-13 16:1	0.14	0.11
trans-6,-7,-8 16:1	0.04	0.03
trans-9 16:1	0.03	0.03
trans-12 16:1	0.16	0.27
trans-13 16:1	0.01	0.03
Σ *cis* 16:1	2.07	1.50
Σ trans 16:1	0.24	0.36
Σ 16:1	2.31	1.86
cis-9, *cis*-12 16:2	0.01	0.01
17:0	0.70	0.47
18:0	8.41	8.50
18:0 iso	0.05	0.03
Σ *cis* 18:1	17.21	13.16
Σ *trans* 18:1	2.95	3.28
Σ 18:1	20.16	19.08
Σ 18:2[2]	2.87	2.67
Σ CLA	0.38	0.55
18:3 n-3	0.39	0.31
18:3 n-6	0.03	0.05

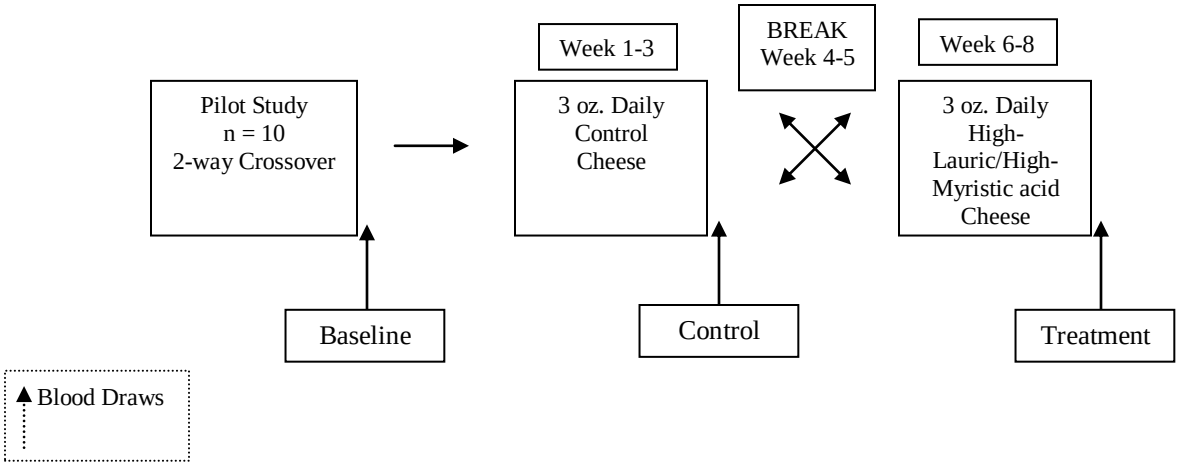
19:0	0.08	0.12
20:0	0.14	0.13
cis-6,-7,-8 20:1	0.08	0.05
cis-9 20:1	0.10	0.08
cis-11 20:1	0.02	0.03
Σ 20:1	0.20	0.15
20:2 n-6	0.04	0.03
20:3 n-3	0.01	0.01
20:3 n-6	0.11	0.10
20:4 n-3	0.02	0.02
20:4 n-6	0.13	0.14
20:5 n-3	0.03	0.04
22:0	0.05	0.05
Σ 22:1	0.01	0.01
22:4 n-6	0.02	0.02
22:5 n-3	0.06	0.06
22:5 n-6	0.02	0.02
23:0	0.03	0.03
24:0	0.03	0.03
26:0	0.01	0.01
Σ unidentified	0.66	0.60
Σ saturated fatty acids	70.75	72.08
Σ trans fatty acids	3.82	5.81
Σ MUFA[3]	24.53	23.45
Σ PUFA[3]	4.14	4.01

[1]Average of 2 cheese samples; no statistical analyses were performed. [2]Sum of 18:2 excluding isomers of CLA.

C. EXPERIMENTAL DESIGN

This clinical trial followed a randomized, two-period, cross-over, free-living design (see diagram below). Participants consumed a daily 3 oz-serving of control cheese or treatment cheese exclusively for three consecutive weeks each. Treatment order was randomly assigned and the diet periods were separated by a two-week compliance break.

Figure 1. Participant Procedures



Participants had two, consecutive days of fasting blood draws at each of three time points: at the beginning of the study (baseline) and at the end of each diet period. Approximately 50 milliliters (mL) of blood were collected at baseline (BL) and at the subsequent two time points (~25 mL on two, consecutive days). Therefore, over the 6-week study, blood was taken on 7 days for a total amount of 165 mL, which included 15 mL at screening and 150 mL for the other time points. Blood pressure was only measured on Day 1 of each time point. All screening and blood draw appointments were conducted by trained study staff and registered nurses at the General Clinical Research Center (GCRC) of the Pennsylvania State University in University Park, PA.

Subjects incorporated the control and treatment cheeses into their daily diets as replacements for all cheeses and cheese products that they would typically consume. Each week during the diet periods, participants picked up seven servings of cheese at the Metabolic Diet Study center. During these weekly visits, body weight was measured and a registered dietitian provided diet monitoring and counseling. Subjects were encouraged to maintain their regular diets with the exception of substituting the treatment and control cheeses for their usual cheeses. Counseling aimed to encourage a diet consistent with the goals of the study so the cheese could be incorporated without producing changes in body weight. On a daily basis during the diet periods, participants recorded the amount and manner in which the cheese was consumed. The registered dietitian reviewed these cheese logs on a weekly basis. For one week during each diet period, participants also completed a daily diet record of all food consumed. These logs were reviewed by the dietitian to monitor compliance and dietary changes.

D. MEASUREMENTS

Height was measured only on Day 1 of the BL appointment at the GCRC. The subject was instructed to stand erect with feet together against a wall-mounted measuring strip. On Day 1 of each time point, trained nursing staff members also measured systolic and diastolic blood pressure with a standard blood pressure cuff. Participants were instructed to sit quietly, with their legs uncrossed, for five minutes before blood pressure was measured. Three consecutive measurements were taken, and the data shown represent the average of the second and third values. Weight data for each time point are the averages of two, consecutive day measures. Weight was measured at the GCRC on Day 1 and Day 2 of each time point using a calibrated, digital scale.

Blood draws were performed by trained nursing staff members on Day 1 and Day 2 of each time point. Blood was drawn after measurements of height, weight, and blood pressure were completed. For forty-eight hours before each blood draw, participants refrained from alcohol consumption. For twelve hours before, subjects abstained from strenuous exercise and excluded any food or drink except water. Blood draws were performed only if participants denied illness, medication use, and deviance from the study protocol.

Approximately 7 mL of blood were collected, centrifuged, and refrigerated before being sent to the certified lipid laboratory at Quest Diagnostics in Pittsburgh, PA. Lipid panel results were obtained within 24 hours of each blood draw. Quest Diagnostics provided the serum lipid and lipoprotein profile data, which included TC, LDL-C, HDL-C, TC:HDL-C, and TG measures. Except where indicated, all biochemical results represent the average of the two, consecutive-day values. Additional samples were frozen for potential analyses in future studies.

E. DATA ANALYSIS

Data were analyzed as a randomized, cross-over design using the univariate mixed procedure or *proc* mixed in the Statistical Analysis Software (SAS) for WINDOWS, release 9.1 (SAS Institute Inc., Cary, NC). There were no significant differences in lipid values between the control and treatment diets when baseline was analyzed as covariate. Therefore, baseline, control diet, and treatment diet were included as the main effects in the final model. Three hypervariable lipid values were removed before analysis.

Table 3 and Table 7 show the results of the *proc* mixed analyses. Table 3 indicates the estimated lipid values at the end of each diet period. The null hypothesis was the lipid profile at

the end of each diet period was the same. Table 7 shows percent changes from baseline. The null hypotheses were that there would be no changes from BL to the end of the control period nor from BL to the end of the treatment period. The effects of age, sex, and diet order were not statistically significant. In Table 6, the lipid responses of Group 1 and Group 2 were compared using the unpaired student t-test. For all tests, significance was declared at $p \leq 0.05$.

V. RESULTS

Ten participants who met study criteria were enrolled. One subject completed only the control cheese feeding period because insufficient treatment cheese was produced. Data analyses included only the nine participants who completed feeding periods for both cheese types. The baseline characteristics of the nine-subject study population are shown in Table 2.

Table 2. Baseline Characteristics of Study Population (N=9)

ID	Age (years)	Sex	Order[a]	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TC: HDL-C	TG (mg/dL)
1	39	M	1	24	115	82	272.5	161	55	4.95	280.5
2	47	F	1	22	105	70	254	161.5	61	4.2	157.5
3	50	F	2	26.1	122	83	243.5	162.5	59.5	4.1	105.5
4	54	F	2	24.5	135	83	221	141	63	3.5	84
5	47	M	2	31.5	122	84	215	126	46	4.65	215
6	54	F	2	23.6	126	75	194	128.5	49.5	3.9	80
7	31	M	1	25.4	110	80	239.5	172.5	50	4.8	84
8	63	F	1	24.4	118	78	201.5	138.5	47.5	4.25	77.5
9	56	F	2	28.7	112	84	242	160.5	57.5	4.2	121
Mean ± SD	**49 + 9.5**			**25.58 ± 2.73**	**118.33 ± 9.10**	**79.89 ± 4.78**	**235.46 ± 6.90**	**152.23 ± 5.28**	**52.53 ± 1.78**	**4.45 ± 0.13**	**158.79 ± 15.18**

[a]Order 1 indicates that control cheese was consumed first followed by treatment cheese, and Order 2 indicates consumption of treatment cheese followed by control cheese.

Table 3. Dietary Effects on Lipid Biomarkers of Study Population

Diet	Baseline	Control	Treatment
TC (mg/dL)	235.46 ± 6.90	238.29 ± 5.98	238.39 ± 7.41
LDL-C (mg/dL)	152.23 ± 5.28	153.33 ± 5.57	155.47 ± 7.72
HDL-C (mg/dL)	52.53 ± 1.78	54.20 ± 1.60	51.43 ± 2.54
TC:HDL-C	4.45 ± 0.13	4.46 ± 0.13	4.60 ± 0.15
TG (mg/dL)	158.79 ± 15.18	152.32 ± 11.29	146.28 ± 13.98

Table 3. Estimated values at each time point based on *proc* mixed analysis.

Overall, lipid values after eating the treatment cheese were not different from lipid values after eating the control cheese (Table 3). The lack of change for the total population may have been related to the differential responses of the individuals to treatment cheese consumption (Table 4). In general, body weight change remained within the desired 2 kg range. Weight, gender, and treatment order had no effects.

Table 4. Dietary Effects on Lipid Biomarkers and Body Weight of Subjects

	Participant	Baseline	Control Cheese	Treatment Cheese	BL to Control	BL to Treatment	Control to Treatment[a]
TC (mg/dL)	1	272.5	276	262	3.5	-10.5	-14
	2	254	271.5	190	17.5	-64	-81.5
	3	243.5	235.5	251	-8	7.5	15.5
	4	221	215.5	218.5	-5.5	-2.5	3
	5	215	228.5	243	13.5	28	14.5
	6	194	206[b]	187[b]	12	-7	-19
	7	239.5	228[b]	280	-11.5	40.5	52
	8	201.5	218	218	16.5	16.5	0
	9	242	225	240	-17	-2	15
LDL-C (mg/dL)	1	161	169.5	166	8.5	5	-3.5
	2	161.5	187	126	25.5	-35.5	-61
	3	162.5	151.5	165	-11	2.5	13.5
	4	141	135	140	-6	-1	5
	5	126	139	146	13	20	7
	6	128.5	132[b]	120[b]	3.5	-8.5	-12
	7	172.5	159[b]	210	-13.5	37.5	51
	8	138.5	149	151.5	10.5	13	2.5
	9	160.5	139	150.5	-21.5	-10	11.5
HDL-C (mg/dL)	1	55	54	53	-1	-2	-1
	2	61	60	42	-1	-19	-18
	3	59.5	66	67.5	6.5	8	1.5
	4	63	61	58	-2	-5	-3
	5	46	47	44	1	-2	-3
	6	49.5	61[b]	52[b]	11.5	2.5	-9
	7	50	40[b]	52	-10	2	12
	8	47.5	52.5	49	5	1.5	-3.5
	9	57.5	59	59	1.5	1.5	0
TC:HDL-C	1	4.95	5.1	4.95	0.15	0	-0.15
	2	4.2	4.55	4.55	0.35	0.35	0
	3	4.1	3.6	3.75	-0.5	-0.35	0.15
	4	3.5	3.55	3.75	0.05	0.25	0.2
	5	4.65	4.85	5.55	0.2	0.9	0.7
	6	3.9	3.4[b]	3.6[b]	-0.5	-0.3	0.2
	7	4.8	5.7[b]	5.4	0.9	0.6	-0.3
	8	4.25	4.15	4.45	-0.1	0.2	0.3
	9	4.2	3.85	4.05	-0.35	-0.15	0.2

(Table 4 ctd)	Participant	Baseline	Control Cheese	Treatment Cheese	BL to Control	BL to Treatment	Control to Treatment[a]
TG (mg/dL)	1	280.5	261.5	214.5	-19	-66	-47
	2	157.5	121	109.5	-36.5	-48	-11.5
	3	105.5	89.5	92.5	-16	-13	3
	4	84	97.5	103	13.5	19	5.5
	5	215	212	265.5	-3	50.5	53.5
	6	80	67[b]	74[b]	-13	-6	7
	7	84	145[b]	91	61	7	-54
	8	77.5	82	88	4.5	10.5	6
	9	121	134.5	153	13.5	32	18.5
Weight (kg)	1	85.3	84.95	84.87	-0.35	-0.43	-0.08
	2	60.4	61.85	63.45	1.45	3.05	1.6
	3	80.85	83	81.9	2.15	1.05	-1.1
	4	70.4	70.45	70.5	0.05	0.1	0.05
	5	102.85	105.85	103.35	3	0.5	-2.5
	6	67.25	69.15	68	1.9	0.75	-1.15
	7	80.45	81.9	80.7	1.45	0.25	-1.2
	8	55.25	56.35	56.55	1.1	1.3	0.2
	9	73.15	73.05	73.9	-0.1	0.75	0.85

[a]Change from control to treatment was used to define a potential adverse effect as any decrease in HDL-C or any increase in TC, LDL-C, TC:HDL-C, or TG. A potential beneficial effect was defined as any increase in HDL-C or any decrease in TC, LDL-C, TC:HDL-C, or TG from the control period to the treatment period. [b]Value represents a one-day measurement rather than the mean of measurements from two, consecutive day blood draws.

For each lipid biomarker, approximately half of the population experienced potential adverse changes while half experienced potential beneficial changes from the end of the control period to the end of the treatment period (Table 5). A potential adverse effect was defined as any decrease in HDL-C (-1 mg/dL, -3 mg/dL, -3 mg/dL, -3.5 mg/dL, -9 mg/dL, -18 mg/dL) or any increases in TC (+3 mg/dL, +14.5 mg/dL, +15 mg/dL, +15.5 mg/dL, +52mg/dL,), LDL-C (+2.5 mg/dL, +5 mg/dL, +7 mg/dL, +11.5 mg/dL, +13.5 mg/dL, +51 mg/dL), TC:HDL-C (+0.15, +0.2, +0.2, +0.2, +0.3, +0.7), or TG (+3 mg/dL, +5.5 mg/dL, +6 mg/dL, +7 mg/dL, +18.5 mg/dL, +53.5 mg/dL) from the end of the control cheese period to the end of the treatment cheese period. A potential beneficial effect was defined as any increase in HDL-C (+1.5 mg/dL, +12 mg/dL) or any decreases in TC (-14 mg/dL, -19 mg/dL, -81.5 mg/dL), LDL-C (-3.5 mg/dL, -12 mg/dL, -61 mg/dL), TC:HDL-C (-0.15, -0.3), or TG (-11.5 mg/dL, -47 mg/dL, -54 mg/dL).

Table 5. Adverse and Beneficial Effects of Treatment Cheese on Lipid Biomarkers

	Adversely Affected[a]	Mean Adverse Effect[a]	Beneficially Affected[b]	Mean Beneficial Effect[b]	Unaffected[c]
TC	5	20	3	-38.1667	1
LDL-C	6	15.08333	3	-25.5	0
HDL-C	6	-6.25	2	6.75	1
TC:HDL-C	6	0.291667	2	-0.225	1
TG	6	15.58333	3	-37.5	0

[a]Adverse effect defined as any decrease in HDL-C or any increase in TC, LDL-C, TC:HDL-C, or TG from control period to treatment period. [b]Beneficial effect defined as any increase in HDL-C or any decrease in TC, LDL-C, TC:HDL-C, or TG from control period to treatment period. [c]A biomarker was unaffected by treatment cheese consumption if it had the same value at the end of the control period as at the end of the treatment period.

Those showing adverse effects were not consistent for each biomarker, but two sub-groups could be identified (Table 6). Of the five lipid biomarkers assessed, each of the five participants in Group 1 experienced four or more adverse effects while each of the four participants in Group 2 experienced three or fewer adverse effects. In Group 1, Participants 4 and 5 experienced adverse effects for all five lipid biomarkers. Participants 3 and 9 were adversely affected for all biomarkers except HDL-C; Participant 3 showed an increase in HDL-C while Participant 9 showed no change in HDL-C. Participant 8 was adversely affected for all biomarkers except TC, which did not change. All members of Group 1 except Participant 8 consumed the treatment cheese first and then the control cheese (Table 1).

The five members of Group 2 experienced more beneficial effects from treatment cheese consumption than did the members of Group 1. The only adverse effect experienced by Participants 1 and 2 was a decrease in HDL-C. Participant 6 experienced decreases in TC, LDL-C, and HDL-C along with increases in TC:HDL-C and TG. Participant 7 showed decreases in TC:HDL-C and TG and increases in TC, LDL-C, and HDL-C. All members of Group 2 except Participant 6 consumed the control cheese followed by the treatment cheese (Table 1).

Overall, the two sub-groups differed in their mean changes in TC:HDL-C ($p = 0.0396$) and TG ($p = 0.0342$) from the end of the control period to the end of the treatment period. Group 1 showed a mean increase in TC:HDL-C ($+0.31 \pm 0.22$) while Group 2 showed a mean decrease in TC:HDL-C (-0.0625 ± 0.21). Group 1 also showed a mean increase in TG ($+17.3 \pm 21.12$ mg/dL) while Group 2 showed a mean decrease in TG (-26.375 ± 29.00 mg/dL).

Table 6. Sub-Groups Differentially Affected by Treatment Cheese

	Participant	Biomarkers Adversely Affected[a]	Biomarkers Beneficially Affected[b]	Biomarkers Unaffected[c]
Group 1	3	TC, LDL-C, TC:HDL-C, TG	HDL-C	
	4	TC, LDL-C, HDL-C, TC:HDL-C, TG		
	5	TC, LDL-C, HDL-C, TC:HDL-C, TG		
	8	LDL-C, HDL-C, TC:HDL-C, TG		TC
	9	TC, LDL-C, TC:HDL-C, TG		HDL-C
Group 2	1	HDL-C	TC, LDL-C, TC:HDL-C, TG	
	2	HDL-C	TC, LDL-C, TG	TC:HDL-C
	6	HDL-C, TC:HDLC, TG	TC, LDL-C	
	7	TC, LDL-C	HDL-C, TC:HDL-C, TG	

[a]Adverse effect defined as any decrease in HDL-C or any increase in TC, LDL-C, TC:HDL-C, or TG from control period to treatment period. [b]Beneficial effect defined as any increase in HDL-C or any decrease in TC, LDL-C, TC:HDL-C, or TG from control period to treatment period. [c]A biomarker was unaffected by treatment cheese consumption if it had the same value at the end of the control period as at the end of the treatment period.

Analyses of changes in the overall population indicated a significant increase in TC:HDL-C from baseline to the end of the treatment cheese period (Table 7). The majority of subjects showed increases in percent change of TC:HDL-C from baseline to treatment. TC:HDL-C increased in five participants, decreased in three participants, and did not change in one participant from baseline to treatment (Figure 2).

Table 7. Percent Change in Lipid Biomarkers from Baseline

	Control	p-value	Treatment	p-value
TC	1.83 ± 0.02	0.3288	2.03 ± 0.03	0.5605
LDL-C	3.08 ± 3.02	0.3172	3.72 ± 3.59	0.3095
HDL-C	-0.23 ± 3.06	0.9411	-4.66 ± 4.58	0.3181
TC:HDL-C	3.20 ± 2.23	0.1626	6.56 ± 2.80	**0.0267**
TG	1.17 ± 7.78	0.8322	3.16 ± 7.43	0.6743

Table 7. Estimated percent change from baseline using *proc* mixed analysis. Significance defined as $p \leq 0.05$.

Figure 2. Percent Change in TC:HDL-C from Baseline to Treatment

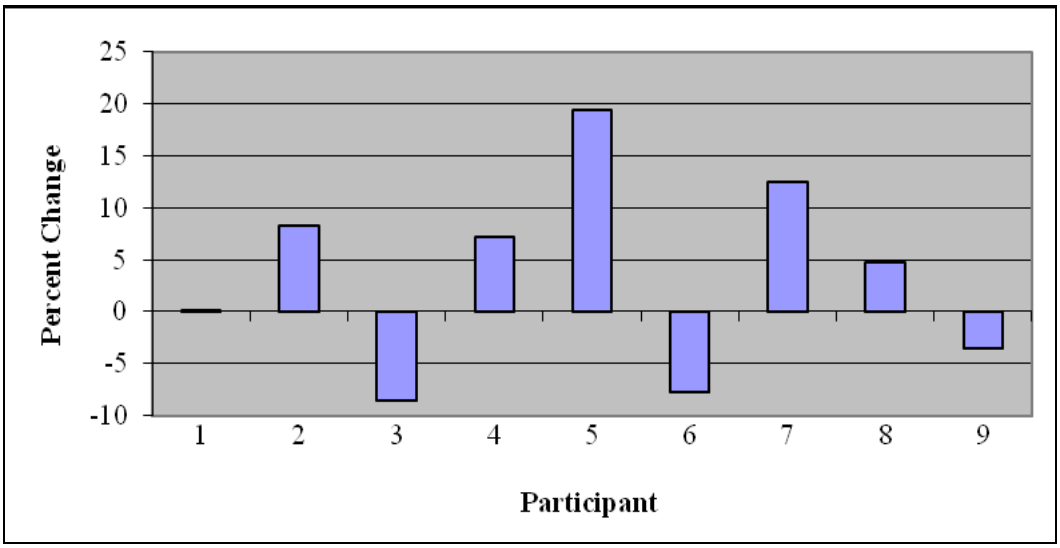


Figure 2. Percent change calculated as (mean TC:HDL-C at treatment - mean TC:HDL-C at baseline)/(mean TC:HDL-C at baseline) for each participant.

V. DISCUSSION

A. OBSERVED INCREASE IN TC:HDL-C

Treatment cheese consumption had variable effects on the lipid and lipoprotein profiles of the nine participants in this study. The only overall effect of the treatment cheese on the total population was to cause a significant percent increase in TC:HDL-C from baseline values. Based on Mensink et al.'s 2003 meta-analysis of sixty human trials and from the results of Wilson's 2009 study of hamsters, it had been expected that the treatment cheese would have the opposite effect on TC:HDL-C (Mensink et al., 2003; Hristov, 2010). These conflicting results may suggest the limitations of evaluating two fats in combination and of translating animal model findings to human subjects. Mensink et al.'s meta-analysis indicated lauric acid to be the most potent of all fatty acids in decreasing TC:HDL-C, while myristic acid had a much lesser effect. Perhaps a treatment cheese high in lauric acid alone would have had the expected beneficial impact on TC:HDL-C.

Due to differences in cholesterol metabolism, hamsters and humans may show different lipid biomarker changes in response to dietary fat consumption. Because hamsters use HDL-C and VLDL-C as major cholesterol carriers whereas humans do not, it has been suggested that TC is the only comparable measure between the species (Hayes et al., 1995). Furthermore, in order to compare animal models to human subjects, the level of dietary cholesterol required to produce similar lipid responses must be determined.

The hypercholesterolemic individuals in this study (baseline LDL-C $\geq$ 130 mg/dL) represented a human population experiencing LDLr down-regulation. To show similar down-regulation, hamsters must be fed a diet with 0.12% of energy intake from cholesterol. This represents an unrealistically large amount of dietary cholesterol for humans (at least 1,000 mg/day based on 2,200 daily caloric intake or 4,500 mg/day based on 70 kg relative weight). It has thus been suggested that humans must have cholesterol levels > 240 mg/dL to produce lipid responses similar to those of hamsters fed hypercholesterolemic diets (Hayes et al., 1995). The hamsters in Wilson's study were fed a diet with 0.06% of energy from cholesterol. Although this is a more realistic proportion of cholesterol for human intake, the hamsters in Wilson's study thus reflected the lipid responses of normocholesterolemic humans. The TC:HDL-C-lowering effects shown by the hamsters, therefore, may not have been applicable to the hypercholesterolemic human population in this study.

TC:HDL-C was the primary targeted outcome in this study because recent research has shown that high TC:HDL-C is more closely associated with increased CVD risk than is any other lipid biomarker (Hong et al., 1991; Real et al., 2001; Castelli, 1984; Kinosian et al., 1994; Criqui and Golomb, 1998). According to a 1984 review of human studies by Castelli, to minimize CVD risk, the TC:HDL-C ratio should not exceed 4.5 (Castelli, 1984). The estimated mean TC:HDL-C at the end of the treatment cheese period (4.60 ± 0.15) exceeded this limit. The treatment cheese elevated the ratio from low-risk levels at baseline (4.45 ± 0.13) and after control cheese consumption (4.46 ± 0.13) to above the recommended limit. High levels were seen in three participants at baseline (total range 3.5 – 4.95), in four participants at the end of the control cheese period (total range 3.4 – 5.7), and in five participants at the end of the treatment cheese period (total range 3.6 – 5.55). These findings could suggest potential adverse effects of treatment cheese consumption on CVD risk.

The percent increase in TC:HDL-C is largely a result of a great decrease in HDL-C (- 4.66%) from baseline to the end of the treatment period (Table 7). Mensink and Katan's 1992 meta-analysis, however, indicated that all fatty acids had the opposite effect when substituted for carbohydrates. The meta-analysis associated greater increased saturation of fats with greater increases in HDL-C (Mensink and Katan, 1992). The only exceptions are stearic acid, which has no effect on HDL-C, and *trans* fats, which decrease HDL-C (Yu et al., 1992; Aro et al., 1997). The control and treatment cheeses were designed to produce equivalent physiological effects from stearic fatty acid and *trans* fatty acids. However, each of these LDL:HDL-increasing fats comprised a greater proportion of total fatty acids in the treatment cheese (8.50% stearic, 5.81% *trans*) than in the control cheese (8.41% stearic, 3.82% *trans*) (Table 1). Low HDL-C levels from treatment cheese intake may have been associated with differences in fatty acids other than lauric and myristic.

Apart from the potential increased effects of stearic acid and *trans* fatty acids in the treatment cheese, the lauric-myristic combination was expected to increase HDL-C. The decrease in HDL-C from baseline to treatment was largely attributable to Participant 2, whose HDL-C changed by -19 mg/dL (Table 4). The changes in HDL-C of the other eight participants were much lower, from -5 mg/dL to +8 mg/dL. The unexpected decrease in TC:HDL-C thus may be explained by the large decrease in HDL-C shown by Participant 2. The HDL-C values for Participant 2 should not be excluded, however, because they were not hypervariable.

Interestingly, Participant 2 had the lowest baseline BMI of all participants (BMI = 22, Table 2). Perhaps Participant 2 was unique from other study participants in genetics, compliance, or other lifestyle factors. Evidently, it is important to consider inter-individual variability in lipid responses to altered fatty acid intakes.

As expected, control cheese consumption did not change TC:HDL-C from baseline because it contained the fat composition of typical cheese. Participant 2's HDL-C changed by only -1 mg/dL from baseline to the end of the control period. Because the nine participants identified themselves as regular cheese consumers, the control cheese period did not alter their baseline proportion of saturated fat intake and thus did not affect their lipid profiles. Treatment cheese consumption did cause a significant percent increase in TC:HDL-C from baseline, which reflected a lipid profile response to the increased intake of lauric and myristic fatty acids. However, treatment cheese consumption did not change lipids from the end of the control period, which was likely due to reciprocal responses of individuals within the study population.

B. MAJORITY OF LIPID BIOMARKERS UNAFFECTED

Apart from TC:HDL-C, the treatment cheese affected no other baseline lipid biomarker values when the population was analyzed as a whole (Table 7). Lipid values also did not change from the end of the control period to the end of the treatment period for the total population (Table 3). Results of previous research suggested that the treatment cheese would be expected to affect lipid biomarkers. Some of these studies were conducted in animal models and thus presented the same limitations as Wilson's research. A 1997 review of lipid studies in animal models showed that lauric and myristic acid each had an equivalent LDL-C-raising effect (Nicolosi, 1997). In hamsters, a 2002 study showed that myristic acid increased HDL-C but did not affect other lipoproteins (Loison et al., 2002). A 1992 review by Hayes and Kohsla showed that both human and monkey studies experienced a strong TC-raising effect of myristic acid (Hayes and Kohsla, 1992). Monkeys may therefore be a more appropriate model for human lipid metabolism than hamsters.

Although much research has used animal models, human evidence also has indicated an effect of lauric and myristic acids on lipoprotein levels. High intake of the lauric-myristic acid combination in coconut oil has been associated with decreased CVD risk in the South Pacific (Kaunitz, 1986; Mann, 1998). However, this epidemiological association cannot establish a casual relationship. The potential effects of other unique lifestyle habits of the South Pacific

culture cannot be disregarded. Decreased CVD risk could also be related to a cardioprotective mechanism of coconut oil that is not reflected by changes in the endpoints assessed in the study. Although lipids were largely unaffected by consumption of the lauric-myristic combination, perhaps measurements of inflammation, for instance, were modified.

Both a 1994 and a 1996 human trial suggested the potential beneficial HDL-C-raising effect of the lauric-myristic combination (Sundram et al., 1994; Sunmin et al., 1996). However, both of these studies evaluated only normocholesterolemic populations while the present study included only hypercholesterolemic individuals. In a past study of hypercholesterolemic humans, the lauric-myristic combination did not affect HDL-C, but produced lower TC and LDL-C than did high palmitic acid intake (Cox et al., 1995). Hypercholesterolemic individuals may be less likely to experience potential beneficial changes in HDL-C related to consumption of the lauric-myristic combination.

In addition to studying the effects of the lauric-myristic combination, human research has also assessed lauric acid and myristic acid independently. Each has been suggested to adversely increase TC and LDL-C (Hegsted et al., 1965; Denke and Grundy, 1992; Hayes and Kohsla, 1992; Zock et al., 1994; Dabadie et al., 2005) and also to beneficially increase HDL-C (Temme et al., 1996; Tsai et al., 1999; Mensink et al., 2003; Zock et al., 1994; Tholstrup et al., 2004; Dabadie et al., 2005) in humans. Perhaps the treatment cheese evaluated in this study showed no effects on these biomarkers because lauric and myristic acid were evaluated in combination. Alternatively, the treatment cheese may have contained an insufficient amount of either fatty acid to produce the expected effect. Future studies could evaluate lauric and myristic acid independently, or in different proportions, to determine the optimal consumption for CVD prevention.

A 2005 study by Dabadie et al. suggested the potential beneficial effects of increased myristic acid consumption on lipid biomarkers in humans. Apart from using a diet high in only myristic acid, Dabadie et al.'s trial exemplifies many ways in which past research differs from the present study. For instance, Dabadie et al. used two diets with 0.6% or 1.2% of daily kilocalories as myristic acid. Both levels of myristic acid intake produced decreases in TC, LDL-C, and TG. The diet with 1.2% myristic acid, although perhaps reflecting an unrealistically high level of intake, decreased TG and increased HDL-C to an even greater extent than the 0.6% myristic acid diet (Dabadie et al., 2005). Based on such research, the

treatment cheese was expected to have potential beneficial effects on lipid biomarkers.

The present study may have produced unexpected results for a variety of reasons. First, Dabadie et al.'s population had normal cholesterol levels and was entirely male. Perhaps baseline cholesterol levels or gender impacted the lipid responses of the nine individuals in this study. In addition, Dabadie et al. incorporated myristic acid as a component of both milk and cheese while this study used only cheese. Fatty acids in cheese are encapsulated by casein and may be absorbed and metabolized differently from those in milk (Tholstrup et al., 2004).

Because Dabadie et al.'s study included only myristic acid, lauric acid may have produced the unexpected effects of the treatment cheese in this study. Although percent of daily kilocalories from each fat type cannot be calculated due to the free-living design of this study, the ratio of lauric to myristic acids in the cheeses may be compared. The proportions of fatty acids in the diet, rather than their percentages of total calories, have been shown to be the strongest predictors of lipid biomarker changes (Hegsted et al., 1965). The trial by Dabadie et al. included half-skimmed milk products with a lauric:myristic ratio (LMR) of 0.39 and whole milk products with an LMR of 0.36. The control cheese in this study had an LMR of 0.32 while the treatment cheese had a much higher LMR of 0.581 (Table 1). The potential beneficial effects shown from Dabadie et al.'s high-myristic acid trial contrasted with the lack of changes from the lauric-myristic combination suggest the importance of analyzing lauric and myristic acids independently.

C. INTER-INDIVIDUAL VARIABILITY IN LIPID RESPONSES

While treatment cheese consumption caused no changes in TC, LDL-C, HDL-C, or TG levels in this population as a whole, each individual did show a response to treatment cheese consumption (Table 4). The lack of overall significant findings, therefore, was likely due to reciprocal changes within the population. The population could be divided into two groups. Group 1 experienced at least four potential adverse effects from treatment cheese consumption whereas Group 2 responded more favorably to treatment cheese consumption with three or fewer potential adverse changes. Only one member of Group 1, Participant 3, showed a potentially beneficial change in any of the five lipid biomarkers. In contrast, one member of Group 2 showed four beneficial responses, two showed three beneficial responses, and one showed two beneficial responses. Group 1 showed potentially adverse increases in TC:HDL-C and TG which were different from Group 2's potentially beneficial decreases in TC:HDL-C and

TG (Table 6).

Although all statistical analyses indicated no effects of diet order, sub-group comparisons suggested a relationship between diet order and response to treatment cheese consumption. All members of Group 1, excepted Participant 8, consumed the treatment cheese first and all subjects in Group 2, except Participant 6, consumed the control cheese first. Therefore, independent of diet, seven of nine participants had a less favorable lipid profile at the end of the first diet period and a more favorable lipid profile at the end of the second diet period. This finding could indicate potential carryover effects or differences in dietary compliance despite the lack of interaction shown by statistical analyses.

Participants may have experienced adverse effects after the first diet period and then showed improved lipid profiles at the end of the second diet period due to the body's homeostatic response to increased cheese consumption. Consuming 3 oz/day of either cheese may have had similar adverse effects on the lipid profile during the first period. The composition of both cheeses, therefore, may have produced detrimental effects on the lipid profile. Although all subjects identified themselves as frequent cheese consumers before beginning the trial, the daily 3 oz cheese consumption may have exceeded their usual intake. During the second diet period, fat metabolism and LDLr activity may have adapted to the increased cheese and saturated fat consumption to restore a more favorable lipid profile. Although diet order may have impacted the body's response to cheese consumption, the two-week washout period was designed to counteract the effect of homeostatic adaptations.

Alterations in lipid profiles based on diet order may have indicated the effects of changes in compliance. Various factors affect dietary adherence in clinical nutrition studies. Important considerations include diet palatability, study duration, motivation, and participant characteristics such as age and gender (Hall and Most, 2005). Many steps were taken to promote study compliance. Before enrollment in the trial, each participant tasted a sample of cheese and agreed that the product was palatable. Weekly cheese intake logs were mandated to monitor consumption. In these logs, all participants indicated that they consumed three ounces of the cheese daily. However, when asked face-to-face about their cheese intake, multiple participants said that the cheese had an unfavorable taste or that the daily amount prescribed was more than preferred. At the conclusion of the diet periods, many expressed relief that they would finally be freed from the monotony of the daily cheese intake.

Participants suggested no difference in taste between the cheeses. Consumption of either cheese may have been higher during the first diet period because participants were not yet tired of the daily cheese consumption. Greater compliance may have produced the greater number of potential adverse effects observed during the first diet period.

Compliance may not fully explain the observed inter-individual variability because, despite their expressed difficulties, all but one participant confirmed daily consumption of the prescribed amount of cheese. Participant 1 was the only individual who admitted to being unable to completely finish the daily cheese requirement on one or two occasions, and none of his values were hypervariable.

In addition to requiring the completion of cheese intake logs, further steps were taken to encourage compliance. All participants were given a booklet of cheese preparation ideas and recipe suggestions. The two-week break after the first diet period was designed not only to wash out lipid profile responses but also to promote compliance during the second diet period.

All participants received professional advice about dietary adherence from a registered dietitian. Further verbal motivation was provided by study staff during visits to the GCRC and the Diet Center as well as via e-mail. The study's potential importance for the promotion of cardiovascular health was emphasized. Participants were asked if they were experiencing difficulties with compliance, and in return were offered new ideas such as incorporating the cheese into different foods or consuming the cheese at different times during the day. Participants were allowed to omit one day of cheese-eating by consuming 6 oz of cheese the day before or after the omitted day; 3 oz of cheese intake was required, however, for all three consecutive days prior to blood draws. Upon study completion, compensation included all blood work results along with a $50 reward.

For future studies, improving the taste of the cheese, producing a variety of flavors, or incorporating the cheese into a variety of foods could further encourage compliance. These modifications would not only strengthen experimental results, but also would enhance the success of the cheese if it were to be marketed as a consumer product in the food industry. To monitor dietary adherence, biochemical assays could be developed to evaluate compliance based on the presence of cheese or fatty acid metabolites in the blood, feces, or urine (Farquhar et al., 1963).

Apart from diet order and compliance, inter-individual variability in response to fatty acid consumption could be related to any factor that modifies LDLr activity. The down-regulation of LDLr activity adversely alters the lipoprotein profile by decreasing LDL-C clearance and increasing the conversion of VLDL-C to LDL-C. Human and animal research have shown a down-regulation of LDL receptor (LDLr) activity in individuals with LDL-C concentrations above 130 mg/dL (Hayes and Kohsla, 1992), which characterizes the population observed in this study. However, high LDL-C concentrations in these nine individuals could have been related to a variety of factors. Common causes of inter-individual variability in lipid responses include differences in diet, genetics, and anthropometrics.

An evident limitation of a free-living study design is the prevalence of inter- and intra-individual variability in diet. It has been suggested that individuals with daily cholesterol intakes >400 mg are more likely to show lipid responses to fat intake that are inconsistent with standardized predictive equations (Hayes and Kohsla, 1992). The dietary cholesterol intakes of study participants were not controlled. Therefore, increased lipid levels in response to treatment cheese consumption among Group 1 participants may have been related to LDLr down-regulation resulting from high dietary cholesterol intake.

It is important to consider not only inter-individual differences in diet but also intra-individual dietary changes. Perhaps more favorable responses to treatment cheese consumption among Group 2 were due to increased physical activity or more heart-healthy eating habits during the treatment cheese period. Although the importance of maintaining constant diet and exercise habits was emphasized, participants may have impacted study results by either inadvertently or consciously changing their lifestyle habits.

Before enrolling in the study, all subjects were provided the results of their screening blood draws which indicated that they had the required elevated LDL-C ($\geq$ 130 mg/dL). They were also aware that the study was aiming to reduce LDL-C in order to decrease CVD risk. Thus, some participants may have changed their diet and physical activity habits during the study to modify their CVD risk. Diet and exercise changes require time to impact lipid profiles, and thus could explain the more favorable lipid profiles observed at the end of the second diet period (Table 6).

Because subjects participated in the study on a rolling basis, from July 2009 through January 2010, seasonal weather changes, vacations, and holiday celebrations may have also

influenced lifestyle habits. Some participants may have exercised more during nicer weather or eaten more high-fat and high-cholesterol foods during the holiday season. Future studies could enroll all participants during the same time period. Physical activity questionnaires or more frequent dietary logs could improve compliance monitoring. Ideally, a controlled-feeding diet design, in which participants consume only foods and beverages provided by the Diet Center throughout the study period, would eliminate the effect of individual dietary differences.

Although all participants had elevated LDL-C at baseline, not all high levels may have been related to modifiable factors such as diet and physical activity. Genetic differences, for instance, may contribute to inter-individual variability in lipid responses to fatty acid consumption. Over 300 mutations in the LDLr gene have been identified which result in familial hypercholesterolemia. A 2010 review showed that existing evidence does not suggest an effect of diet therapy on the lipid profiles of individuals with familial hypercholesterolemia (Shafiq et al., 2010). Therefore, individuals in this study whose high LDL-C levels were related to genetic defects may have produced unique lipid responses.

Familial hypercholesterolemia is just one example of the important relationship between the diet and the genome. The emerging field of nutrigenetics explores the impact of an individual's genetic composition on his/her response to a dietary intervention. Various heritable factors have been shown to influence lipid changes in response to the consumption of different fatty acids. Apolipoprotein E has been suggested to modulate TC and LDL-C levels, cholesteryl ester transfer protein and apoplipoprotein A have been shown to impact HDL-C responses, and lipoprotein lipase and apolipoprotein A-V have been associated with changes in TG concentrations (Ordovas, 2006).

Individuals in this study may have shown different responses to treatment cheese consumption due to genetic variability. Individuals in Group 1, for example, may have shared a genetic composition that made them more susceptible to potential adverse changes in the lipid profile related to treatment cheese intake. Future clinical studies will compare sub-groups based on genetic differences. The study of nutrigenetics will ultimately aim to create individualized dietary recommendations based on genetic profiles.

In addition to genetic mutations, body composition, age, and gender also impact the lipid profile. Group 1 had a higher BMI at baseline. Higher BMI has been associated with greater CVD risk (Lavie and Messerli, 1986; Pi-Sunyer, 1993) and a more unfavorable lipid profile as

characterized by decreased HDL-C and increased TC, LDL-C, TC:HDL-C, and TG (Bertsias et al., 2003). The increased BMI of Group 1 participants may have made them more susceptible to potential adverse changes in the lipid profile from treatment cheese consumption.

Although the prevalence of adverse lipid profiles is greater in older populations (Castelli, 1984), there was no difference in age between Group 1 and Group 2. Similarly, while males are more likely than females to develop atherosclerosis at an earlier age, this study population was too small to draw conclusions based on gender. There were only two males in Group 1 and one male in Group 2. In general, a small sample size was a large limitation in this study. Future studies could provide more powerful results with a greater number of participants. Large sample sizes with sub-groups based on age, gender, and body weight would provide the ability to further assess the potential impacts of these non-modifiable risk factors.

VII. CONCLUSIONS

Treatment cheese consumption was expected to have potential beneficial effects on the lipid profiles of nine individuals with moderately elevated LDL-C. However, the only change produced by the treatment cheese was a potential detrimental increase in TC:HDL-C. No other effects were seen for the overall population, which was likely due to the contradictory responses of individuals to treatment cheese consumption. Inter-individual differences may have been related to variability in diet order, compliance, genetics, anthropometrics, age, gender, race, dietary intake, or exercise habits.

Despite unexpected findings, the strengths of this study should not be discredited. Importantly, this food-based intervention evaluated the effects of treatment cheese consumption in a way that was applicable to the everyday cheese consumer. High-lauric and high-myristic fatty acid intake was administered through cheese, a popular food product. The effect of the treatment was assessed without changing any other dietary or exercise habits. Therefore, the results realistically reflected the manner in which an average consumer would incorporate the cheese product into their normal lifestyle.

Many steps were taken to promote participant adherence to the study protocol. Subjects completed weekly cheese intake logs along with one, three-day dietary recall per diet period. Weight was measured weekly and contact with a registered dietitian and study staff members provided continuous support and monitoring. All participants tasted the cheese before beginning the trial to confirm palatability, and a guide with recipes and cheese preparation ideas was provided. A two-week break was designed to encourage compliance and to eliminate the effect of diet order.

Despite the careful design of this food-based clinical trial, future studies could be modified to provide stronger evidence regarding the effects of treatment cheese consumption on the lipid profile. A controlled-feeding study design along with a monitored physical activity log would decrease the impact of variable diet and exercise habits. Starting and ending all subjects at the same time would help to diminish the effects of lifestyle changes. The assessment of metabolites could be a means of compliance monitoring. Dietary adherence could be improved by producing different varieties of cheeses or by incorporating lauric and myristic acids into other foods. This study evaluated the effect of only one unique fatty acid profile on lipid biomarkers. Future studies could produce foods with different proportions of lauric and myristic

fatty acids in addition to exploring the effects of other saturated fats on CVD risk factors.

In future trials, hereditary effects could be determined by relating the genomic profiles of study participants to their lipid profile responses. A greater number of subjects within different age, race, and gender sub-groups could help to identify the effects of these non-modifiable risk factors. The role of body composition could be evaluated by comparing participants within different weight classifications or by assessing the effect of intra-individual weight changes on lipid profile responses. While all participants in this study had high LDL-C, further research could compare the lipid biomarker changes of normocholesterolemic individuals to those of hypercholesterolemic participants.

Until recently, all saturated fats were considered to adversely affect cardiovascular health. The nine individuals in this study showed variable lipid biomarker changes in response to the consumption of cheese with high levels of lauric and myristic fatty acids. Existing evidence has yet to differentiate the effects of individual saturated fats on the lipid profile. Further research is warranted to establish optimal dietary fat recommendations for the promotion of cardiovascular health.

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