THE RELATIVE EFFECTS OF DIETS CONTAINING STRUCTURED TRIGLYCERIDE OR PALM OIL/PALM KERNEL OIL ON SERUM LIPIDS AND APOLIPOPROTEINS IN MEN

A Thesis

Presented in Partial Fulfillment of the Requirement for

the degree Master of Science in the

Graduate School of The Ohio State University

by

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The Ohio State University
1991

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To my parents

ACKNOWLEDGEMENTS

I would like to express sincere gratitude to Dr. Gordon Wardlaw for sharing his academic expertise, and for providing continued guidance and support throughout the research study. Also, special thanks to the members of my Master's committee, Dr. Jean Snook and Dr. Betty Bartlett for providing insight and helpful suggestions.

Support from Medical Dietetics graduate students Marian Straub and Kelley Gretz was greatly appreciated as was assistance from Medical Dietetics and Human Nutrition students during the project. This study would not have been possible without them. I would also like to thank Human Nutrition graduate students Myoungsook Lee, Li-Wen Chen, and Sunmin Park for their commitment to all laboratory analyses and continued enthusiasm.

To my family and Deanna I would like to express sincere thanks for encouragement and continued support. To Anil, I am forever grateful for your understanding, refreshing humor, and for just being there. Thank You.

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CHAPTER I

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the United States(22). Diet is one of the major controllable risk factors involved in this degenerative disease. Specifically, saturated fatty acid intake is the major dietary determinant of the serum total cholesterol and low density lipoprotein cholesterol (LDL-C) levels in populations, and thus of CHD risk(90). It has been suggested, therefore, that Americans with elevated serum total and LDL-C levels who lower their intake of saturated fats could reduce CHD risk.

Saturated fatty acids (SFA) of the 12, 14, and 16 carbon atom varieties (lauric, myristic, and palmitic acids respectively) are the fatty acids that are most highly implicated in contributing to the elevated blood cholesterol levels exhibited by men and women in the United States(66,90). Palm oil/palm kernel oil consists primarily of these 12-16 carbon atom saturated fatty acids (see Appendix 1), and is a popular ingredient in the food industry due to its high melting point and semi-solid composition at room

temperature(18). Food products that benefit from the saturated nature of this fat include cakes, cookies, pastries, frostings and deep-fried foods. These are a major part of the American diet.

The health-conscious individuals of today have encouraged food companies to improve and maximize the "healthiness" of their products. An example of this attempt is a unique fat recently developed by The Procter and Gamble Company.

This fat is termed a structured triglyceride. It is solid at room temperature but still may not contribute to an increase in serum cholesterol levels because it contains negligible amounts of C12:0, C14:0, or C16:0. This fat is synthetic, in that chemists have decided which fatty acids should be attached to the glycerol molecule to form the triglycerides. The fatty acids chosen are not themselves synthetic, but are found naturally in nature. Specifically, the structured fat is solid due to approximately one-half of its composition consisting of a particular fatty acid, behenic acid. This is a very long chain saturated fatty acid (22 carbons) found in peanut oil. Very long chain fatty acids are thought to be too long to influence body metabolism of serum cholesterol (due to the length being greater than 18 carbons)(108), and yet have the benefit of being solid at room temperature.

The remaining half of fatty acids utilized in this structured triglyceride are medium chain saturated fatty acids

(8-10 carbon atoms). See Appendix 2 for a complete listing of fatty acid composition of the behenic/medium chain triglyceride structured triglyceride (BMCT). Medium chain fatty acids are commonly found in coconut oil and are used in the clinical setting for fat malabsorption conditions. The metabolism of these medium-chain fats is different than long-chain varieties. These fats are rapidly absorbed by route of the portal vein rather than the lymphatic system, and are metabolized by the liver(47,56,104). It is thus presumed that they do not influence serum cholesterol or serum triglyceride levels(8,48,66,77). Studies that have implied these results in the 1960s were preliminary and not very well designed to show strong evidence of this hypothesis (short duration, not controlling for SFA, using liquid formula diets, etc). More controlled studies are needed to validate the effects of medium chain saturated fatty acids.

Only one previous study involving human subjects has been conducted in relation to behenic acid as part of a structured triglyceride. This study found that only 30 percent of the behenate fed was absorbed. The part absorbed was probably in the 2-monoglyceride form after digestion. The remaining 70 percent was excreted due to its very solid nature at body temperature (communication from The Procter and Gamble Company).

Because this structured triglyceride contains negligible amounts of C12:0-C16:0, it may be possible that this fat will

be beneficial in lowering serum cholesterol levels, and thus the risk of CHD.

If the results of this study show that this structured triglceride can lower serum cholesterol and yet be solid at room temperature, many individuals with an elevated serum cholesterol level will benefit. This product could then be incorporated into various food items that vegetable oils are unsuitable for as a source of fat.

This study is thus designed to compare the effects of this structured triglyceride (BMCT) to palm oil/palm kernel oil with respect to the abilities of each to alter serum total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, apolipoprotein B-100, and apolipoprotein A-1 levels in men.

Objective

The objective of this study is to determine the effects of a diet high in the behenic/MCT structured triglyceride (BMCT) on total serum cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and subfractions HDL-2 and HDL-3, calculated low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), apolipoprotein A-1 (Apo A-1), and apolipoprotein B-100 (Apo B-100) levels as compared to a diet high in palm oil/palm kernel oil.

Null Hypothesis

The BMCT diet fed for 6 weeks will have no significant effect (positive or negative) upon the various lipoproteins and apolipoproteins (TC, LDL-C, HDL-C, HDL-2, HDL-3, TG, Apo B-100, and Apo A-1) when compared to a baseline palm oil/palm kernel oil diet fed for 3 weeks.

Definition of Terms

1. Apolipoprotein - protein constituent of a lipoprotein that serves both structural and functional roles. Some apolipoproteins are ligands for specific cell surface receptors and others are cofactors for enzymes.

2. Apolipoprotein A-1 - one of the subclasses of Apolipoprotein A, and is a major constituent of high density lipoprotein cholesterol. Functions as a mediator to transform free cholesterol into cholesterol esters and lecithin to lysolecithin. (Apo A-1)

3. Apolipoprotein B-100 - one of the subclasses of Apolipoprotein B, and is a major constituent of both low density lipoprotein cholesterol and very low density lipoprotein cholesterol (VLDL-C). Functions as controller of the metabolism of these lipoproteins and is the apolipoprotein recognized by hepatic low density lipoprotein receptors. (Apo B-100)

4. Body Mass Index - mathematical index of obesity status. To calculate: weight (kg) / height^2 (m). (BMI)

5. BMCT - a structured triglyceride consisting of behenic acid and medium chain triglycerides; the experimental fat in this thesis report.

6. Endothelium - squamous epithelium that is flat in structure and lines the blood and lymphatic vessels, heart and other body cavities.

7. Foam Cells - an accumulation of lipid-laden monocytes/macrophages. Their localization subendothelially constitutes the first stage of fatty streak formation in atherosclerosis.

8. Hydrogenation - the addition of hydrogen atoms to an unsaturated fatty acid to produce a more stable, saturated fatty acid.

9. Hypercholesterolemia - excessive cholesterol in the blood; moderate: 200-240 mg/dl; severe: > 240 mg/dl.

10. Hypertriglyceridemia - fasting plasma triglyceride level exceeding 250 mg/dl.

11. High density lipoprotein cholesterol - this lipoprotein is comprised of 45-55 percent protein, 30 percent phospholipid, and 15-20 percent cholesterol esters (3-5 % free cholesterol). High density lipoprotein cholesterol serves as an acceptor of free cholesterol from various tissues and lipoproteins. Serum HDL-C levels have been shown to have an inverse relationship to coronary heart disease risk. Includes subfractions HDL-2 and HDL-3. (HDL-C)

12. Intima - inner coat of a vessel.

13. Lingual Lipase - one of the first lipases that is present to hydrolyze triglycerides; derived from lingual serous glands (von Ebner) located on the back of the tongue in the region of the circumvallate papillae.

14. Lipoprotein - protein/lipid complexes by which lipids are transported through the bloodstream to respective tissues.

15. Low density lipoprotein cholesterol - this lipoprotein is comprised of 45-50 percent cholesterol esters (6-8 % free cholesterol), and approximately 20 percent each of phospholipid and protein. This lipoprotein is the major cholesterol-carrying lipoprotein in normal human plasma and is correlated positively with coronary heart disease risk. (LDL-C)

16. Lymphatic System - the lymphatic vessels, specifically capillaries, collecting vessels, and trunks that collect lymph from the tissues and carry it to the bloodstream; system that transports long chain fatty acids.

17. Mann-Whitney test - A nonparametric statistical testing procedure using rank sum testing; also referred to as the Wilcoxon rank sum test.

18. Macrophages - cells of the reticuloendothelium system that have the ability to phagocytose particles and store colloidal substances.

19. Monocyte - large mononuclear leukocytes having more protoplasm than a lymphocyte.

20. Subintimal - beneath the intima.

21. Total Cholesterol - total amount of serum cholesterol in the blood measured in mg/dl. Derived mostly from VLDL-C, LDL-C, and HDL-C.(TC)

22. Triglycerides - total amount of triglycerides in the blood measured in mg/dl. Derived mostly from chylomicrons and VLDL-C. (TG)

CHAPTER II

REVIEW OF LITERATURE

Coronary heart disease (CHD) continues to be the leading cause of death in the United States(22). Elevated serum cholesterol levels (particularly LDL-C) have been implicated as being a major risk factor associated with the incidence of CHD(15,28,111).

Keys reported that the percentage of calories derived from saturated fatty acids in the food supply is strongly and positively associated with mean population cholesterol levels and CHD rates(62). Predictive formulas for changes in serum cholesterol as a result of changing amounts of dietary saturated, polyunsaturated and cholesterol levels were developed approximately 3 decades ago(49,66). These formulas postulate that saturated fatty acids raise serum cholesterol concentrations about twice as much as polyunsaturated fatty acids lower them. Also, it must be noted that these formulas were designed to be used to predict changes in serum cholesterol levels of large groups versus for individuals, due to large individual variability in responses to dietary lipids and cholesterol(90).

Since then, much research has been conducted in the area of feeding studies of various fatty acid combinations on humans in clinical trials. There has been debate over whether all saturated fatty acids can be grouped together in regards to atherogenicity. This literature review focuses on what is currently known in these various areas of lipid research as they relate to the diet and CHD link. Also, a brief review of the pathogenesis of atherosclerosis will be presented.

SATURATED FATTY ACIDS

Short Chain Fatty Acids

Saturated fatty acids of the short chain variety are those which are classified as having less than six carbons(108). They include acetic (C2:0), propionic (C3:0), butyric (C4:0), and valeric (C5:0) acids.

In the literature, short chain fatty acids (SCFA) have been grouped together with medium chain fatty acids in relation to their effects on serum cholesterol levels. By 1965, there was good reason to believe that fatty acids with fewer than 12 carbons had much less of an effect on serum cholesterol than the longer chain saturated fatty acids(48,49,63,66).

In 1958, Keys(61) tested butyric acid in the diet and reported no effect on serum cholesterol levels. Hashim et al.(48) found similar results while testing humans using a mixture of fatty acids with fewer than 12 carbons. Beveridge et al.(8) provided confirmation to the results of Hashim et al.

Medium Chain Fatty Acids

The classification of medium chain fatty acids (MCFA) in reference to carbon chain length has been variable in the literature. Some authors classify MCFA as including saturated fatty acids of 6 to 12 carbon atoms(108) whereas others classify these fatty acids as including only C8:0 and

C10:0(42). Also, it seems that earlier literature simply referred to fatty acids as either being "long" or "short", categorizing the currently recognized MCFA in the "short" category(49,63,66). Refer to Appendix 3 for a complete listing of names and codes of fatty acids.

Regardless of the classification in terms of carbon length, MCFA (and short chain also) possess several characteristics that distinguishes them from the long chain fatty acids (LCFA). Medium chain fatty acids are more water-soluble than LCFA(108). They thus require minimal amounts of lipase and bile salts for effective solubilization (lingual lipase aids in the initial hydrolysis of MCFA and SCFA)(108). Medium chain fatty acids do not become re-esterified within the enterocyte as do LCFA in order to become absorbed from the small intestine. Due to their dual nature of being highly water-soluble and yet still possessing a lipid-component, these MCFA can easily pass through cell membranes. The MCFA are then transported to the systemic circulation via the portal vein and are quickly metabolized for energy in the liver; MCFA are not stored in fat depots. Many researchers have validated that MCFA are rapidly and preferentially oxidized and are poorly stored into adipose tissue(57,76,77,78). This is in contrast to the transport of LCFA which are carried into the circulation via the lymphatic system, which has a much slower blood flow rate in comparison to the portal circulation(47,56,104). Thus, MCFA have become

a preferred treatment in many fat malabsorption conditions(75,102).

Preliminary studies involving MCFA and its effects on serum cholesterol suggested that these fats were hypercholesterolemic. Ahrens et al.(1) reported rises in serum cholesterol after feeding liquid diets high in butter fat and also diets high in coconut oil (see Appendix 1 for fatty acid composition of these fats). Since both of these fat sources are relatively rich sources of saturated MCFA and short chain fatty acids (SCFA) including C6:0-C12:0, Ahrens et al. postulated that the MCFA and SCFA were responsible for the increased serum cholesterol levels. It was also mentioned by Ahrens et al. that these fats raised the serum cholesterol levels due to the low content of unsaturates. They did not however realize that butter fat contained significant amounts of long chain saturated fatty acids, specifically myristic and palmitic acids, which are currently generally accepted to be hypercholesterolemic agents.

Coconut oil has large amounts of C12:0 (lauric acid) and moderate amounts of myristic acid, both of which are also believed to be hypercholesterolemic as stated by Keys et al.(66). This study by Ahrens et al.(1) seemed to have various experimental flaws. For example, only three subjects were fed the coconut diet. Serum cholesterol levels after the coconut diet for the subjects were 367, 213, and 186 mg/dl. The subject with a cholesterol level of 367 mg/dl had an initial

baseline serum cholesterol of 450 mg/dl. The other baseline values were not reported for the other subjects. For further discussion on lauric, myristic and palmitic acids, see the corresponding respective sections.

Subsequent investigations have failed to verify the preliminary results of Ahrens et al.(1). Beveridge et al.(8) and Hashim et al.(48) tested MCFA (C6:0-C12:0) against diets high in corn oil and reported that the MCFA produced little or no elevation in serum cholesterol concentrations in comparison to the corn oil diets. Hashim et al.(48) also reported that MCFA acted more like carbohydrates than saturates in that they did not raise serum cholesterol levels.

In 1965, Keys et al.(66) stated that saturated fatty acids with fewer than 12 carbon atoms did not affect serum cholesterol levels due to their portal absorption, and thus did not include these fatty acids in their equations to predict serum cholesterol levels based on changes in the diet of various fatty acids. They did however, include lauric acid (C12:0) in their equation (see later section entitled "Lauric Acid"). On the contrary, Hegsted et al.(49) found that both C10:0 and C12:0 did not contribute significantly in their predictive equations, which otherwise were similar to those described by Keys et al.(66).

A recent study by Hill et al.(51) tested formula diets high in medium chain triglycerides (MCT) in comparison to formula diets high in long chain triglycerides (LCT). The

authors reported that the major differences between diets in regards to blood lipids were: 1) a reduction in fasting serum total cholesterol levels with the LCT but not the MCT diet; and 2) a threefold increase in fasting serum triglyceride (TG) levels with MCT but not from the LCT diet. Several comments are worthy to note about this study. This study was quite short (6 days) and perhaps the fats were not given enough time to complete their "course". Also, the authors seemed to contradict themselves, in that the increase in the very low density lipoprotein fraction (from the increase in TG) confounds their interpretation of a stated decrease in LDL-C levels (one would expect to see a rise in LDL-C). The authors stated this decrease in LDL-C as one of the results of the study, but did not directly test this. Since their results contradict past studies, in that the MCT diet significantly affected serum blood lipids, it seems that the results of this study are questionable. No further studies have examined the metabolic effects of MCFA.

Lauric Acid (C12:0)

Lauric acid is a saturated fatty acid with twelve carbon atoms. According to Keys et al.(66), lauric acid is categorized as being hypercholesterolemic along with myristic and palmitic acids, and is thus included in their equations for predicting changes in serum cholesterol levels due to

changes in intakes of dietary fatty acids and cholesterol. However, this conclusion was not confirmed by Hegsted et al.(49), who concluded that lauric acid had very little effect on serum cholesterol levels. Hegsted et al.(49) discussed that lauric acid should hypothetically influence serum cholesterol because this fatty acid is primarily absorbed via the lymph. So, if serum cholesterol levels were truely affected by certain fatty acids in regards to the absorption route, then the distinction between effective and noneffective fatty acids should be made between C10:0 and C12:0, according to Hegsted et al. Nevertheless, they concluded that lauric acid still did not perform as a significant variable in regards to being hypercholesterolemic. In 1985, Reiser et al.(97) reported that a coconut oil based diet (35% of calories from fat with 60% of the fat as coconut oil) raised serum LDL-C levels more than beef fat. This response may have been due to the high myristic acid content (18 percent of total fatty acids) in coconut oil, instead of its lauric acid content. Also, initial serum total cholesterol values of the subjects were < 160 mg/dl which could have affected the lipid response to the various dietary fats. There have been no further investigations done with lauric acid, and its role remains quite uncertain.

It would be interesting to confirm the role of lauric acid in regard to its atherogenicity due to the fact that two tropical oils, namely coconut oil and palm kernel oil which

are used in many food products, contain large proportions of this fatty acid.

Myristic Acid (C14:0)

Myristic Acid is a saturated fatty acid with fourteen carbon atoms and is usually classified as a long chain fatty acid. As was described in the "Lauric Acid" section, Keys et al.(66) have included myristic acid in their predictive formulas due to its hypercholesterolemic properties. Myristic acid was ranked equally with palmitic acid (C16:0) in terms of cholesterol-raising ability. Hegsted et al.(49) also have tested the effects of myristic acid and have postulated that this fatty acid contributes to the accuracy of their predictive formula. However, unlike Keys et al.(66), Hegsted et al.(49) proposed that myristic acid raises serum cholesterol even more than palmitic acid. This claim was based mainly on the fact that butter fat, which is relatively rich in myristic acid (17.7%), increases serum cholesterol levels more than would be predicted than if myristic acid had an equal effect with palmitic acid. A cross-sectional study by Trevisan et al.(115) has confirmed that diets high in butter do have the effect of raising serum cholesterol levels in comparison to diets high in olive oil and diets high in polyunsaturates (soybean, corn, or sunflower oils). Wardlaw

and Snook(119) utilized butter fat as a baseline fat in a recent study. This diet raised serum cholesterol levels in their subjects.

No studies have directly tested the effects of myristic acid on serum cholesterol levels. Nevertheless, since butter fat is the major source of myristic acid, and its consumption remains low, it can be concluded that the diets of most people contain small amounts of this fatty acid.

Palmitic Acid (C16:0)

Palmitic acid is a saturated fatty acid with sixteen carbon atoms and is classified as a long chain fatty acid. This fatty acid is the principal saturated fatty acid in most diets, as it is the major saturate in animal fats and vegetable oils(42). See Appendix 1 for percentage of palmitic acid in these lipids.

Due to its large presence in the diet, it has been tested in various diets by Keys et al.(66) and Hegsted et al.(49), and is included in their formulas for predicting serum cholesterol changes as just described. In these early studies, LDL-C levels were not measured due to the unavailability of advanced analytical methods. But more recent studies have demonstrated that palmitic acid increases LDL-C levels in parallel with total cholesterol concentrations

when it is substituted for carbohydrates or monounsaturated fats in the diet(9,45,79).

Thus, it is well accepted that palmitic acid does possess cholesterol-raising effects. In comparison to lauric and myristic acids, palmitic acid is more abundant in most diets, and thus is most likely the primary contributor of saturated fat in regards to increasing serum cholesterol levels in man.

Stearic Acid (C18:0)

Stearic acid is classified as a long chain saturated fatty acid with eighteen carbons. Early studies done by Ahrens et al.(1) in 1957 revealed that stearic acid may not act as a cholesterol-raising fatty acid. These authors tested diets high in cocoa butter (35% stearic acid) versus diets high in butter (12.5% stearic acid) to test the importance of chain length in regards to raising cholesterol (the saturated fats of cocoa butter are predominantly C16:0 and C18:0 whereas butter has approximately 40% of its saturated fats from C4:0 to C14:0; see Appendix 1). The authors were aware that both of these fats had very similar fat composition percentages (saturated, oleic and linoleic). Initially, it could be argued that cholesterol content of both fats could act as a possible confounding factor. However, as mentioned earlier, saturated fatty acid composition is significantly different in both fats, with cocoa butter possessing almost three times the amount of stearic acid as butter. This thus opened up the

possibility that this saturated fatty acid does not raise serum cholesterol levels. Only two subjects were tested on this diet study, and both subjects experienced a decrease in serum cholesterol levels while on the cocoa butter phase. A second study by Erickson et al.(27) also showed that cocoa butter did not elevate serum cholesterol in comparison to other foods rich in saturated fatty acids. Horlick et al.(54) fed ethyl stearate to human subjects and also showed that stearic acid did not raise the serum cholesterol concentrations.

Subsequent studies validated these initial results. Keys et al.(66) and Hegsted et al.(49) both fed foods high in stearic acid and suggested that stearic acid did not influence serum total cholesterol levels in contrast to other saturated fatty acids. Keys et al.(66) felt that eliminating stearic acid from the dietary mix of saturated fatty acids when calculating the serum cholesterol response would give more accurate predictions, and thus adjusted their results accordingly.

Many researchers were still skeptical of the conclusion that stearic acid was "excused" from being categorized as hypercholesterolemic. They felt that the results of these studies casted doubt since stearic acid was saturated and also a long chain fatty acid(36).

Interest in this topic has recently been rekindled. In 1988, Bonanome and Grundy(9) tested the effects of a liquid

formula diet high in stearic acid (40% of calories) in comparison to isocaloric liquid formula diets high in oleic acid and palmitic acid. Their results showed that relative to the "palmitic" acid diet, the "stearic" acid diet lowered total cholesterol levels as much as the high oleic diet. Low density lipoprotein cholesterol (LDL-C) levels responded similarly. Therefore, the authors concluded that stearic acid is considered neutral as is oleic acid in raising serum total cholesterol or LDL-C levels.

It has been argued that stearic acid may not raise serum cholesterol levels due to its poor absorption from the gastrointestinal tract(42,66). It has been documented that tristearin is unabsorbable in the human gastrointestinal tract(66), but that triglycerides containing one or two stearic acid molecules are digestible and absorbed(66). Bonanome and Grundy(10) tested the absorption of stearic acid in humans recently and suggested that intestinal absorbability of stearic acid is similar to that of palmitic acid, and both of these fatty acids appear to be absorbed almost as well as oleic acid.

Therefore, why does stearic acid seem to be a saturated fat that has no effect on serum cholesterol levels? One possible hypothesis is that stearic acid is rapidly converted to oleic acid in the body, and is done so more readily than are other saturates. One animal study has verified this(26), and it seems that in humans, serum triglycerides contain more

palmitic acid versus stearic acid(9), thus emphasizing the possible rapid conversion of stearic acid to oleic acid. Another interpretation of this phenomenon is that humans consume more palmitic acid in comparison to stearic acid.

Both beef fat and cocoa butter contain substantial amounts of stearic acid.(see Appendix 1). Since stearic acid has been established as not raising serum LDL-C levels, many have advocated the consumption of beef and other animal meats.

In defense of this claim, several studies have tested beef intake to verify whether or not it is "cholesterol-raising". Reiser et al.(97) studied the effects of beef fat (20% stearic acid) in comparison to coconut oil (the fat chosen to represent saturated fat) and safflower oil (the fat chosen to represent polyunsaturated fat) on plasma lipids. These authors found that the beef fat diet produced significantly lower total cholesterol, LDL-C, and high density lipoprotein cholesterol (HDL-C) in comparison to the coconut oil diet (initial serum TC levels of the subjects were < 160 mg/dl which could affect the lack of response seen with coconut oil). As compared to the safflower oil diet, beef fat produced equivalent levels of HDL-C, LDL-C, and marginally higher total cholesterol levels. Thus, they concluded that beef fat, although being highly saturated, should not be grouped with coconut oil and other cholesterol-raising tropical oils.

Sacks et al.(99) studied the ingestion of lean beef in

comparison to a diet devoid of any animal products. They found that when the subjects were switched to the beef diet from the vegetarian diet, serum cholesterol levels rose significantly, and then fell significantly once returning to the vegetarian diet. The authors concluded by saying that even though stearic acid may not promote hypercholesterolemia, beef should not be labeled as a "health" food. Beef consists of various other saturated fatty acids, such as palmitic acid, and also significant levels of cholesterol, which also can be considered cholesterol-raising.

Bonanome and Grundy(9) suggest that complete hydrogenation of vegetable oils may not necessarily transform the oils into cholesterol-raising fats because stearic acid is formed as the end product. They thus propose that food companies could enrich margarines with stearic acid, and this could thus replace butter, lard, or beef tallow in many products. This may not be the case for partially hydrogenated oils as the trans fatty acids that are formed during hydrogenation may be hypercholesterolemic.

Very Long Chain Fatty Acids

Very long chain fatty acids of the saturated variety are classified as having greater than 18 carbon atoms(108). They include arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) acids. These fatty acid have had a minimal role in the research area of hypercholesterolemia related to dietary

intake in humans. Fatty acids of this length have been documented as having very poor absorption due to the length of the carbon chain(108), and thus are believed not to influence body metabolism of serum cholesterol.

One possible exception to this theory could be peanut oil. Peanut oil contains approximately five to ten percent of its saturated fatty acid from very long chain fatty acids(69) (see Appendix 1). It has been noted in animal studies that peanut oil may display atherogenic effects due to these fatty acids(37,103,122), or may be due to a lectin-like substance inherent in peanut oil(68,101). At present, peanut oil research has been confined to animals, and is too preliminary and incomplete to be confirmed at this time. Besides peanut oil, no food products contain substantial amounts of very long chain saturated fats. Only one study involving human subjects has been conducted in relation to behenic acid as part of a structured triglyceride. This study found that only 30 percent of the behenate fed was absorbed. The part absorbed was probably in the 2-monoglyceride form after digestion. The remaining 70 percent was excreted due to its very solid nature at body temperature (communication from The Procter and Gamble Company).

Summary

Saturated fatty acids, in general, have been categorized as being hypercholesterolemic agents in the diet. Research

shows evidence that all saturated fats are not "created equal". Carbon chain length seems to be an important factor in determining atherogenicity. At present, the majority of research shows that myristic (C14:0) and palmitic (C16:0) acids are most effective in elevating serum cholesterol concentrations. Saturated fatty acids of carbon length 10 or less do not seem to influence serum cholesterol levels. Stearic acid (C18:0) also seems to have no effect. The effects of lauric acid appear to be "cholesterol-raising" in some experiments, but the research is not as quantitatively conclusive as that for myristic acid and palmitic acid, and thus requires further investigation.

The mechanism(s) involved with the "cholesterol-raising" ability of SFA is not completely understood. It has been postulated that SFA interfere with LDL-receptor-mediated clearance of LDL-C(42). Saturated fatty acids generally do not raise TG concentrations. An increase could be expected since VLDL-C are a precursor of LDL-C, and LDL-C increases during SFA feeding. Thus, it seems from isotope-kinetic studies (tracing Apo B-100) done on humans that the LDL-C-raising action of SFA is due mainly to impaired removal of LDL-C from the circulation(107).

MONOUNSATURATED FATTY ACIDS

Oleic Acid (C18:1)

Oleic acid is a monounsaturated fatty acid with eighteen carbon atoms in its carbon chain. It contains one double bond in the cis configuration (the two hydrogen atoms attached to the double bond lie on the same side) between the ninth and tenth carbon atoms from the methyl end of the fatty acid.

This fatty acid is the major monounsaturate in the diet and thus, is the primary fatty acid that has been studied to determine the effects of monounsaturated fats in the diet as they relate to serum lipids. Monounsaturated fatty acids (MUFA) have been the neglected fat, with more attention focused on saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) in the research world for the past three decades. The SFA have generally been recognized to increase serum cholesterol, with PUFA generally noted to decrease it. Meanwhile, the MUFA have been referred to as being a neutral fat in the "lipid-lowering" theatre of research. However, very early reports involving olive oil, (which is rich in oleic acid) showed that olive oil, when substituted for SFA, decreases serum cholesterol levels, as did other fats rich in PUFA(1,12,16,60,64). Keys et al.(64) reported that oleic acid, when substituted for carbohydrates in the diet (isocalorically), did not affect serum cholesterol levels. Later, in 1965, it was postulated by Keys et al.(65) that the inclusion of MUFA did not add to the accuracy of the

predictive formulas for determining serum cholesterol changes, as did SFA and PUFA. Hegsted et al.(49) found similar results in their equations, and thus, these two studies were probably the ones most responsible for the "neutral" label given to these fats.

Since these earlier studies, a number of more recent controlled clinical trials re-examined the effects of MUFA, specifically, oleic acid on serum lipids and apolipoproteins. In 1983, Becker et al.(7) reported that feeding liquid diets high in MUFA (source: high oleic safflower oil) and free of cholesterol lowered serum TC and LDL-C levels significantly, as did diets high in PUFA, when replacing the subject's usual intake (baseline). Plasma TG and HDL-C levels did not change significantly from baseline. In a metabolic ward study Mattson and Grundy(79) fed liquid diets high in MUFA from the same source of fat used by Becker et al.(7) and also reported that oleic acid lowered LDL-C levels as effectively as linoleic acid (a polyunsaturated fat). They also reported that oleic acid reduced HDL-C levels less frequently than did linoleic acid. Sirtori et al.(109) studied a population at high-risk for atherosclerosis. In comparison to a low-fat diet (30 percent of calories from fat) of corn oil, Sirtori et al. found that a low-fat diet (isocaloric) of olive oil did not lower HDL-C levels as did the corn oil diet. Also, plasma Apo A-1 levels were increased with the olive oil diet. Both diets produced an equal reduction in Apo B levels, and thus,

the Apo A-1:B ratio rose only with the olive oil diet. In 1986, Grundy(39) showed that a diet high in MUFA (high oleic safflower oil) was comparable to a diet high in carbohydrates (20 percent of calories from fat with equal amounts of SFA, PUFA and MUFA; and 63 percent of calories from carbohydrate provided by dextrose). Both diets lowered plasma total cholesterol levels and LDL-C levels in obese subjects when compared to a diet high in SFA (palm oil and coconut oil). However, the MUFA diet did not have an effect upon serum HDL-C or TG levels, whereas the high carbohydrate diet raised TG levels and significantly reduced HDL-C, both in comparison to the high SFA diet. The author concluded that a diet rich in MUFA appears to be at least as effective in lowering plasma cholesterol as a diet low in fat and high in carbohydrate. Mensink and Katan(85) also studied the effect of diets high in MUFA (olive oil) and diets high in complex carbohydrates. This study was different from the Grundy study in that the diets consisted of conventional mixed foods and the subjects were healthy males and females. All subjects were initially placed on a "western" diet (38 percent of calories from fat; 13 percent of calories from C12:0-C16:0 fatty acids). Their results showed that the olive oil rich diet (MUFA diet) caused an increase in HDL-C levels and an increase in TG levels on the complex carbohydrate diet. Both diets reduced serum TC levels. The results for the olive oil diet were more pronounced in men than in women. Grundy et al.(43) confirmed

the results of Mensink and Katan(85), in that a solid-food diet rich in MUFA is equivalent to a low-fat, high carbohydrate diet for reducing serum cholesterol but does not reduce HDL-C levels.

In 1988, Bonanome and Grundy(9) studied liquid formula diets high in stearic acid, palmitic acid (SFA diet) and oleic acid (high MUFA diet). Their results revealed that in comparison to the palmitic acid diet, plasma TC and LDL-C levels decreased significantly on both the oleic and stearic acid diets. No significant differences were observed for plasma TG or HDL-C among all three of the diets. Baggio et al.(5) tested the effects of mixed solid-food diets high in olive oil (38 percent of calories from fat; 25 percent of calories from MUFA) or low-fat diets (28 percent of calories from fat) on serum lipid levels in men. The results showed that a significant decrease of TC, Apo B, LDL-C, and TG was observed for the olive oil enriched diet in comparison to the low-fat diet. Total HDL-C, HDL subfractions, and Apo A-1 levels remained unchanged from the low-fat diet period. The authors conclude that "olive oil may be a natural fat that can be used for the control of TC and LDL-C levels as an alternative to PUFA".

Mensink and Katan(86) fed mixed solid-food diets either rich in olive and sunflower oil (MUFA diet) or rich in sunflower oil alone (PUFA diet) after an initial SFA diet in healthy men and women. Their results showed that both diets

significantly lowered LDL-C levels on both the MUFA and PUFA diets. In men, HDL-C levels decreased, but not significantly, on both diets. In women, no change in HDL-C levels was observed on either diet. Therefore, the authors concluded that a mixed diet rich in MUFA was as effective as a diet rich in PUFA for lowering LDL-C. McDonald et al.(81) reported that both PUFA and MUFA diets produced similar decreases in plasma TC and LDL-C in comparison to a diet high in SFA in normolipidemic men. Plasma HDL-C and TG levels were not altered by either diet.

Mensink and Katan(84) tested diets either high in carbohydrate and fiber (22 percent of calories from fat) or a high fat diet enriched with olive oil (24 percent of calories from MUFA; 41 percent of calories from total fat) after administration of a high saturated fat diet (20 percent of calories from SFA; 38 percent of calories from total fat). Serum TC, LDL-C, and HDL-C levels decreased in both diets. VLDL-C levels increased and HDL-3 levels decreased on the carbohydrate diet whereas VLDL-C levels decreased and HDL-3 levels increased on the olive oil diet. Mensink and Katan(87) studied the effects of diets high in olive oil or diets high in carbohydrates and fiber on healthy Dutch adults. Replacement of energy from SFA by either MUFA or complex carbohydrates caused equal decreases in serum TC. HDL-C levels decreased on the carbohydrate but remained unchanged on the olive oil diet.

Wardlaw and Snook(119) fed diets high in either corn oil (PUFA) or high oleic sunflower oil (MUFA) in comparison to a diet high in butter and reported that both vegetable oil diets reduced LDL-C, TG and Apo B-100. The corn oil diet produced a greater decrease in these profiles in comparison to the MUFA diet. They reported no significant changes in serum HDL-C or Apo A-1, and thus suggested that when men on diets high in SFA reduce their SFA intake but not their total fat intake, many can still experience a significant lowering of serum TC.

Ginsberg et al.(33) conducted a study in which they evaluated the effects of both an American Heart Association Step 1 diet (30 percent of calories from fat; 10 percent SFA; 10 percent PUFA; 10 percent MUFA; 250 mg of cholesterol/day) and a MUFA-enriched Step 1 diet (38 percent of calories from fat; 10 percent SFA; 18 percent MUFA; 10 percent PUFA; 250 mg cholesterol/day) in comparison to a diet that is typical of the American diet (38 percent of calories from fat; 18 percent SFA; 10 percent MUFA; 10 percent PUFA; 500 mg of cholesterol/day). Both the Step 1 diet and the MUFA-enriched Step 1 diet lowered TC and LDL-C levels in comparison to the American diet. Plasma TG and HDL-C were not changed significantly on either diet in comparison to the baseline diet (American diet).

Last, a study by Dreon et al.(23) tested two solid-food diets (30 percent of calories from fat), either high in PUFA or MUFA in healthy males and females in a cross-over design.

The results indicated that plasma TC, LDL-C and HDL-C concentrations did not change significantly on exchanging fat type. However, HDL-2 levels were 50 percent higher and HDL-3 levels were 7 percent lower for the PUFA diet when compared to the MUFA diet. They also reported that Apo B was lowered significantly after subjects transferred to the PUFA diet. Thus the authors concluded that they find no advantage with respect to plasma HDL-C levels in using predominantly MUFA rather than PUFA in subjects who consume reduced-fat, solid-food diets.

Several recent cross-sectional studies have investigated the effects of olive oil in relation to serum lipid/apolipoprotein concentrations. Aravanis et al.(4) studied the diet of Cretan boys (aged 7-9 years), due to their high intake of olive oil, and the effect that their diets have on serum lipid patterns. A two-day dietary recall was utilized to assess the diets. Fat accounted for 45 percent of calories with oleic acid providing 27 percent of calories. Saturated fat intake was low in comparison to oleic acid consumption (10 percent of total calories). The authors stated that this information correctly reflected a high consumption of olive oil. Results indicated contrary to the authors expectations, that the Cretan boys did not show a more favorable serum lipoprotein pattern than boys from more westernized countries. Thus, they concluded that these results did not support their hypothesis that a typical, olive

oil rich Cretan diet causes a relatively high HDL-C to TC ratio. These findings may have been different if an adult population was studied. The second study was recently published by Trevisan et al.(115) and involved a quite large sample of Italian men and women (4903 total subjects). The intake level of fats commonly consumed by these subjects was ascertained via an interviewer-administered questionnaire. Results indicated that an increased consumption of butter (and margarines) was associated with significantly higher serum cholesterol levels for men. In both sexes, consumption of olive oil and vegetable oil (oils rich in PUFA such as corn, soybean, and sunflower) were inversely associated with serum cholesterol concentrations (other confounding factors for CHD were controlled). Thus, the authors concluded by reporting that these cross-sectional findings from a large population sample suggest that consumption of butter may detrimentally affect coronary risk factors, while PUFA and MUFA fat may be associated with a lower coronary risk profile.

Summary

Thus, there is a growing body of research that has provided evidence that people on diets high in oleic acid may show lower levels of serum TC and LDL-C in comparison to SFA, and that MUFA can protect against HDL-C lowering as is seen by PUFA and high carbohydrate diets in some studies.

It is yet to be proven whether diets high in MUFA are

more effective than low-fat diets, but evidence of this could have various dietary implications for the high-risk CHD individual and the general health-conscious public.

The possible mechanism(s) responsible for the "cholesterol-lowering" effect seen with MUFA is unclear. As discussed in the "Saturated Fatty Acids" section, if LDL-receptor activity is the key reason in determining serum LDL-C levels, then the replacement of SFA by MUFA may simply be allowing for the natural expression of LDL-receptor activity. Whatever the mechanism may be, substituting MUFA for SFA (C12:0-C:16:0) in the diet seems to be a prudent dietary replacement as an attempt to lower the risk for coronary heart disease.

TRANS FATTY ACIDS

Elaidic Acid (C18:1)

Elaidic acid is a monounsaturated fatty acid with eighteen carbons and one double bond. It is similar to oleic acid except that the double bond in elaidic acid is in the 'trans' configuration (the hydrogen atoms lie on opposite sides of the double bond) between carbons nine and ten. The trans nature of this fatty acid allows it to possess a 'solid' texture versus a liquid form as seen with oleic acid, which possesses a cis configuration. Elaidic acid is found in nature only in very small amounts(40). Hydrogenation of vegetable oils (the predominant fatty acid in vegetable oils that are hydrogenated is linoleic acid) results in various fatty acids, one of which is elaidic acid. The process of hydrogenation is used to solidify many liquid oils in order to create more stable and textured fats, such as shortenings and margarines.

Elaidic acid and its isomers are the most abundant trans fatty acids in the diet(105). Concern regarding the hypercholesterolemic effects of this fatty acid was initially documented in 1956 as was discussed in the recent paper by Booyens et al(11). This early work postulated that hydrogenation of plant oils could have contributed to coronary artery disease. Unfortunately, since then, few controlled clinical trials in humans have been conducted and results are inconsistent, and thus unable to verify these

initial concerns. One study in 1972 reported that trans fatty acids raised cholesterol concentrations(118), whereas other studies have shown that these fatty acids are neutral and do not influence serum cholesterol levels(27,35,74,80,83). Of these studies, the report by Mattson et al.(80) was the most thorough and best controlled. They tested the effects of varying amounts of trans fatty acids (up to 60 percent of MUFA and 50 percent of PUFA in the trans configuration) in 33 men while keeping constant the proportions of MUFA, SFA, and PUFA. After feeding these diets for 4 weeks, there were no differences in serum TC or TG levels.

A recent study by Mensink and Katan(88) reported that trans fatty acids are at least as unfavorable as cholesterol-raising saturates because they not only raise LDL-C levels but also lower HDL-C levels. This study has been referred to as the "standard" by which other clinical trials in this area should be conducted, as it was a very well designed and executed investigation(40). The authors note that the trans isomers produced in this study may be different than the isomers found in the American diet.

At present, it is a great controversy as to whether trans fatty acids play an important role in CHD due to their limited intake in the diet. Trans fatty acid intake has been reported to supply only 3 to 4 percent of total energy in the American diet(40,55). Nevertheless, intake of the "atherogenic" SFA in the diet come from a variety of foods which are consumed in

small amounts also. It may be that in the future, food labeling will need to be extended to report not only grams of SFA, PUFA and MUFA, but to specifically identify certain fatty acids that have been determined to possess certain atherogenic qualities.

POLYUNSATURATED FATTY ACIDS

Linoleic Acid (C18:2)

Linoleic acid is the predominant dietary polyunsaturated fatty acid in the w-6 family. It is found mainly in plant oils. Linoleic acid consists of eighteen carbon atoms with two double bonds. The first double bond is positioned between the sixth and seventh carbons from the methyl end of the fatty acid (thus in the w-6 family). Linoleic acid content ranges from one to two percent of total fatty acids in coconut and palm kernel oils, and up to fifty to sixty percent in corn, cottonseed and soybean oils. Safflower oil, in its natural form, can consist of up to seventy-five percent linoleic acid(90).

Linoleic acid has been recognized as a "cholesterol-lowering" fatty acid since the 1950's. Initial feeding studies (liquid formula and solid food diets) by Kinsell et al.(67) and Ahrens et al.(1) have demonstrated that various vegetable oils that are high in this fatty acid possessed hypocholesterolemic qualities when substituted for SFA. Coconut oil and dairy fats were used as the sources of saturates in most of these initial studies, with corn and soybean oils as the source of the PUFA. It is interesting to note that these very early studies were concerned with the iodine number (total degree of saturation) of the various oils. Ahrens et al.(1) had postulated a rough inverse relationship between the serum cholesterol level and the

iodine number of various oils. This implied that monounsaturates were half as effective as fats with two double bonds such as linoleic acid. The literature at that time was just beginning to recognize the importance of the presence of a double-bond in fats as a factor in reducing serum cholesterol, which was the motivator for studies that followed these initial ones.

Keys et al.(66) and Hegsted et al.(49) were the main researchers who refined the idea that linoleic acid may be a hypocholesterolemic agent. They suggested that linoleic acid lowered TC levels more than oleic acid and carbohydrate (isocaloric exchanges) through linear regression analysis. Equations for predicting serum cholesterol changes were thus developed with these initial data by these authors. It was determined later that the cholesterol lowering action of linoleic acid occurs mainly in the LDL fraction(90).

A large number of clinical studies have been performed since the initial discovery of the cholesterol-lowering nature of linoleic acid, and it would be beyond the scope of this thesis to discuss them all. At present however, a controversy remains as to whether linoleic acid is a stronger hypocholesterolemic agent than oleic acid or low-fat (high carbohydrate) diets.

Many studies have shown that substituting PUFA for carbohydrates in the diet does not lower TC or LDL-C levels in humans(13,14,44,52,53,73,121), but these results can be viewed

as uncertain due to various experimental design weaknesses seen in some of the studies (such as small samples, studies involving only females, short-duration studies, not controlling for other dietary constituents such as cholesterol and SFA, etc.).

Several studies have shown that linoleic acid may not have a greater LDL-C lowering action than oleic acid. In 1985, Mattson and Grundy(79) fed liquid formula diets either high in SFA, MUFA or PUFA (40 percent of total calories from fat). They reported that, in comparison to the SFA diet, oleic acid was as effective as linoleic acid in lowering LDL-C levels in normolipidemic subjects, and oleic acid reduced HDL-C levels less frequently than linoleic acid (initial mean serum TC levels of the subjects were 263 $\pm$ 50 [SD] mg/dl). Sirtori et al.(109) studied the effects of low fat diets (30 percent of calories from fat) containing either corn oil (PUFA) or olive oil (MUFA). They reported that both the diets reduced serum TC levels, but olive oil did not reduce HDL-C levels as did the corn oil diet (initial mean serum TC levels of the subjects was 246 $\pm$ 13 [SEM] mg/dl). Similarly, McDonald et al.(81) and Mensink and Katan(86) reported that diets rich in either oleic acid or linoleic acid equally reduced serum cholesterol levels when compared to a SFA-rich diet. Wardlaw and Snook(119) showed that both oleic acid-enriched and linoleic acid-enriched diets reduced serum TC, LDL-C, TG, and Apo B-100 levels, but the linoleic acid-

enriched diet (corn oil) produced a greater drop in these values.

Thus, these studies have raised the question of whether linoleic acid possesses a specific LDL-C lowering effect or not, as opposed to just replacing SFAs. Several possible mechanisms have been postulated to explain the hypocholesterolemic "power" of linoleic acid.

One hypothesis suggests that when PUFA are substituted for SFA in the diet, there should be an increased excretion of excess cholesterol from the bloodstream because linoleic acid lowers serum LDL-C(42). However, it is unclear if the excess cholesterol is excreted or just redistributed into body tissues. These questions stimulated the development of cholesterol-balance studies. To date, the results of these studies have been inconclusive with varying results. The question of whether exchange of PUFA for SFA consistently promotes excretion of cholesterol products has not been resolved(17,38).

A second mechanism involves the hypothesis that when serum lipids are enriched with polyunsaturates, they occupy more space within lipoprotein particles than when the lipids are poor in polyunsaturates(112). Thus, in the presence of polyunsaturates, fewer cholesterol ester molecules can occupy the core of LDL-C particles. So, if this is so, the number of LDL-C particles (in the plasma) should not be reduced by polyunsaturates, but the cholesterol content of each particle

would be decreased. However, studies have shown this not to be the case, in that the dietary exchanges of polyunsaturates for saturates does reduce the number of LDL particles (as measured by a decrease in LDL Apo B levels)(72,117).

It also has been postulated that an increased ingestion of PUFA may lower serum LDL-C levels due to the inhibition of hepatic synthesis of Apo B-containing lipoproteins(42). This is seen in some individuals, but not all. Therefore, this LDL-lowering mechanism probably cannot primarily be due to a decreased hepatic secretion of Apo B particles.

A final hypothesis suggests that linoleic acid may up-regulate LDL-receptor activity, thus increasing the uptake of LDL-C from the plasma(42). Saturated fats have been recognized as suppressing LDL receptor activity(73). Thus, it may be that substituting PUFA for SFA in the diet may simply be allowing for the natural expression of LDL receptor activity, making the effects of linoleic acid strictly passive. If this is so, linoleic acid would technically have no greater effect on LDL receptor function than oleic acid.

Nevertheless, there has been concern recently about advocating overconsumption of linoleic acid rich products(2,90). Many studies, but not all, have documented lowering of the protective HDL-C lipoproteins as a result of greatly increased PUFA consumption(79,106,109). It should be noted that most of the studies performed to evaluate the effects of linoleic acid on serum lipids provided 15-30

percent of total calories from PUFA in the diet. The typical intake of PUFA in the American diet is approximately only 7 percent. Other potential adverse effects of excess PUFA consumption include potential for carcinogenic properties, immunosuppressive activity and increased TG levels (for some hypertriglyceridemic individuals)(79,90).

W-3 Fatty Acids

The second category of PUFA to be discussed are the w-3 fatty acids. These fatty acids are labelled as 'w-3' because the first double bond on the carbon chain begins at the third carbon atom from the methyl end of the fatty acid. Alpha-linolenic acid (C18:3) is the predominant dietary w-3 fatty acid. It is found mostly in vegetable oils; soybean, rapeseed (canola), and linseed oils are the richest sources of alpha-linolenic acid. Eicosapentaenoic acid (EPA) (C20:5) and docosahexaenoic acid (DHA) (C22:6) are two PUFA that are also in the w-3 category of fatty acids. Both of these very long chain fatty acids are found predominantly in fish oils. Eicosapentaenoic acid and DHA constitute approximately 26 percent of fatty acids in fish oils(42). Cold-water fatty fish such as salmon, mackerel and eel contain large amounts of EPA and DHA, but other fatty fish (albacore tuna, trout, menhaden) also have substantial amounts of these w-3 fats(50).

Alpha-linolenic acid has received little attention in the literature regarding its hypocholesterolemic effects. This

could be due to the fact that linoleic acid (from the w-6 family) is more widespread in vegetable oils in comparison to linolenic acid; thus more emphasis has been placed on the w-6 PUFA.

The first documented hypocholesterolemic effects of fish oil were reported soon after the cholesterol-lowering effects of PUFA were discovered in 1952(90). This finding remained dormant and unexplored until the mid seventies when reports of low rates of CHD were noted among fish-eating Greenland Eskimos(6,24). Dyerberg et al.(24) found that levels of TC and LDL-C were significantly lower and that levels of HDL-C were higher among Greenland Eskimos than among Danes in all age groups and both sexes. The cultural differences in these diets did not show variations in total fat consumed, but in the composition and source of the fat consumed. The Eskimos were found to consume large amounts of fat from seals, walruses, and whales. These sources of fat contain large amounts of EPA and DHA. The Danes on the other hand consumed twice as much saturated fat and had substantially more w-6 PUFA, with negligible amounts of fatty fish (w-3 fatty acids).

Since then, many studies have evaluated the effects of fish oils on serum lipoproteins and apolipoproteins in man(20,21,91,94,100,116). The results of these studies are inconclusive (in regards to serum TC, LDL-C, and HDL-C), in that high doses show different results in comparison to low doses in different clinical populations. Most studies failed

to control for saturated fat as a variable. Nestel et al.(91) showed a decrease in serum LDL-C levels in subjects with high initial serum LDL-C levels, after administration of very high doses of fish oil. However, fish oils do seem to show consistency in reducing plasma VLDL and TG levels, especially in hypertriglyceridemic patients(46,91,94). This may be due to the inhibition of the synthesis of VLDL-triglycerides(91). Typically, when a hypertriglyceridemic patient is treated with fish oils as a therapeutic agent, the lowering of plasma TG is often associated with an increase in LDL-C levels(90). This effect has been seen as a result of feeding moderate doses of 10-15 grams of fish oil per day(46) as is also seen with quite large doses of 75-100 grams per day in initial studies(46).

Nevertheless, regular consumption of fish has been postulated in several epidemiological studies as being a preventative factor in relation to CHD(58,70,71). It is likely that the anti-atherogenic effects of fish and its oils may be due to its eicosanoid-related properties in altering platelet activity(46), and not due to any lipid-lowering effect.

At present, it seems that the data is too inconclusive to recommend the supplementation of fish oils as a mode of diet therapy for CHD. As I have just reviewed, LDL-C levels often increases with fish oil use in hypertriglyceridemic patients. However, it seems prudent to recommend a regular intake of fish, as it is lower in fat (especially saturated fats) in

comparison to other meats in the diet, and does seem to possess anti-coagulatory qualities as evidenced by the epidemiological studies mentioned.

DIETARY CHOLESTEROL

There is much controversy surrounding the atherogenic effects of dietary cholesterol in humans. Evidence of dietary cholesterol being a risk factor in CHD in humans is variable and conflicting(90). At present, it is well-accepted that there is considerable variability in plasma lipid responses to dietary cholesterol among individuals(25,59,82). McNamara et al.(82) studied the effects of dietary fat quality (SFA versus PUFA) on the metabolic response to a low versus a high cholesterol diet in a cross-over design study in males. The results indicated that 69 percent of the subjects compensated for the increased cholesterol intake by decreasing cholesterol fractional absorption and/or endogenous cholesterol synthesis. When an increased intake of cholesterol was reported in the other subjects, there was a failure to suppress endogenous cholesterol synthesis. Also, the authors found that the quality of fat had more of an effect on serum cholesterol levels when compared to the quantity of cholesterol intake.

Many cross-sectional studies of individuals within populations have revealed that there is little or no association between dietary cholesterol intake and concentration of serum TC levels(90). However, due to the great inter/intra individual differences noted from the previous studies just mentioned, cross-sectional studies are inappropriate for studying the effects of dietary cholesterol

on serum TC in humans(90). Several long-term cohort studies have reliably shown that dietary cholesterol was positively associated with risk of CHD after adjustment for age, blood pressure, cigarette smoking, and serum cholesterol level(90). It should be noted when elevations in serum cholesterol result from dietary cholesterol intake, these elevations are due primarily to an elevation of LDL-C and apolipoprotein B concentrations(3).

Many researchers have studied the effects of dietary cholesterol on serum lipid and apolipoprotein levels. Studies performed in the outpatient setting have suggested that dietary cholesterol has little or no effect on serum cholesterol levels(29,96,110). Eggs were used as the source of cholesterol and were either added or removed to the usual diets of the subjects. These studies lack strict dietary control of other nutritional factors, but nevertheless are useful to interpret for practicality in terms of dealing with a free-living population.

On the other hand, controlled studies in metabolic ward settings consistently show that increasing the intake of cholesterol produces a substantial rise in serum TC levels(16,49,59,66). However, a recent study by Wardlaw and Snook(119) demonstrated that the addition of approximately 300 mg of dietary cholesterol per day (in the form of eggs) did not affect mean TC, LDL-C, HDL-C, TG, Apo A-1 or Apo B-100 concentrations in normocholesterolemic males following a diet

low in saturated fat.

The mechanism for dietary cholesterol to raise LDL-C concentrations in humans has been presumed to be due to suppression of LDL receptor activity. Packard et al.(92) have reported that a decrease in fractional catabolic rate and an increase in production rates for LDL are seen with increased cholesterol intake.

At present, diminished importance has been assigned to dietary cholesterol as an atherogenic agent, with more attention being focused on various fatty acids, as discussed throughout this review. However, it has been noted recently that dietary cholesterol, especially oxidized forms, may enhance atherogenicity of postprandial lipoproteins(41). Therefore the importance of dietary cholesterol as an atherogenic factor cannot be ruled out. The current recommendation from the Committee on Diet and Health(90) (which is directed towards healthy, North American adults and children) includes limiting the intake of cholesterol to less than 300 mg daily.

PATHOGENESIS OF ATHEROSCLEROSIS

The pathogenesis of atherogenesis in man has been studied for many years(98). Atherosclerosis is defined as a form of arteriosclerosis (hardening of the arteries) in which plaques of fatty deposits form in the inner layer, or intima, of the arteries(89). Current concepts regarding the pathogenesis of

atherosclerosis include three distinct phases. The first is "initiation" which involves some form of arterial injury which could be caused by lipid oxidation products (to be discussed below), smoking and/or hypertension. "Progression" is the next phase in this disease process which involves formation of the atherosclerotic plaque. Last, "termination" involves the final phase in which a myocardial infarction, thrombosis or spasm occurs as a result of a combination of the first two phases.

Injury to the intima by the denudation of endothelial cells has been documented as being an initiating event in atherogenesis(98). This then creates arterial lesions due to subsequent platelet aggregation. The platelets are attracted to the arterial surface due to the release of a growth factor named platelet-derived growth factor (PDGF). This PDGF has been speculated to promote the smooth muscle cell proliferation that characterizes early fibrous plaque lesions(98). This theory characterizes the "endothelial injury hypothesis", whereas others claim that the fatty streak precedes the formation of the fibrous plaques and is developed under a structurally intact endothelial surface(19,95). The "lipid-infiltration" theory defined the origin of fat-laden cells coming from smooth muscle cells. However, it is now believed that the origin of fatty streaks could be due mainly to monocyte/macrophage origin(30,32). It is currently widely accepted that the natural progression of lesions in

experimental atherosclerosis is first the development of fatty streaks and later the development of fibrous plaques and more complicated lesions(114).

Currently, there is interest in the origin of the fatty streak lesion. As was mentioned, monocytes have been elucidated as being the cells to create the lipid-laden cells within the endothelial surface. These cells enter tissues, become macrophages, and are then termed foam cells as they take up fat. Studies have shown that circulating monocytes that penetrate the cell endothelium do not initially contain lipid droplets(31,95). They become filled with lipid droplets in the subendothelial space. Since LDL is the major atherogenic lipoprotein generating foam cell-rich lesions, it would be expected that incubation of monocytes with LDL would lead to foam cell development. However, Goldstein et al.(34) have reported that this is not the situation that is seen. Current research shows that, in order for LDL to be taken up by monocytes/macrophages, certain chemical modifications are necessary.

Oxidative modification of LDL is believed to show the strongest biological evidence of this phenomena of altered LDL being the form taken up(114). This new theory suggests that LDL can be oxidized by endothelial cells, smooth-muscle cells, and macrophages, all of which are involved with the formation of the arterial lesion. It has been documented that oxidized LDL is taken up by monocytes significantly faster than is

native (unoxidized) LDL. This thus would enhance foam cell production. Also, oxidized LDL may directly alter the vasomotor properties of coronary arteries. It is beyond the scope of this paper to thoroughly review the specific mechanisms involved. In summary, however, it seems that oxidized LDL plays an important role in atherosclerosis, and may do so in other ways besides macrophage lipid accumulation. Currently, research is underway to determine ways to decrease the oxidative modification of LDL(114). Various antioxidant-type therapies such as the use of probucol, Vitamin E, Vitamin C and selenium are under investigation(114). Of interest to the focus of this paper is the fact that diets rich in MUFA make LDL less prone to oxidation than diets rich in PUFA(93). This could provide further impetus for promoting MUFA as the fat source of choice for the American diet.

CHAPTER III

METHODS

The protocol and procedure for informed consent utilized in this study were approved by The Ohio State University Biomedical Review Committee. A randomized two-phase experimental design was employed. The first phase consisted of a three-week baseline diet (main fat source used was a combination of palm oil/palm kernel oil, and will be referred to as PALM) which was representative of a typical American diet. In this diet, 38 $\pm$ 1 (mean $\pm$ SEM) percent of the energy (en%) was derived from fat, of which 17 $\pm$ 1 en% was saturated, 12 $\pm$ 1 en% was monounsaturated and 4 $\pm$ 1 en% was polyunsaturated fat (see Table 1). After completion of the baseline phase, half of the subjects were randomized (as later described) to the experimental fat diet (EXP) described as BMCT (structured triglyceride made from behenic acid and medium chain fatty acids) for six weeks. The other half of the subjects remained on the original baseline diet (PALM) for the same six weeks. This last six-week phase will be referred to as the "experimental phase". Both fats were supplied by The Procter and Gamble Company, Cincinnati, Ohio. Please see

Appendix 2 for fatty acid composition of both fats.

During the experimental phase, the EXP diet provided 34 ± 1 en% from fat, whereas the PALM diet provided 38 ± 1 en% from fat. This difference in fat intake will be further discussed in this section under the "Diets" heading. Saturated fat (C12:0, C14:0, and C16:0) provided 5 ± 1 en% and 17 ± 1 en% on the EXP and PALM diets respectively. The EXP diet consisted of 6 ± 1 en% of medium chain fatty acids (MCFA) with the PALM diet containing minimal amounts of MCFA (1 ± 1 en%). The EXP diet consisted of 6 ± 1 en% of behenic acid, whereas the PALM diet was essentially free of behenate. See Table 2 for a complete listing of nutrient consumption for both groups of subjects during the experimental phase.

The baseline diet was utilized as a means to create a uniform fat intake among the subjects that represented a typical American diet. Also, the baseline diet allowed the subjects to adapt to a regular and standardized three meal per day plan. In addition, the baseline diet allowed for the observance of the effect of a constant dietary intake over a substantial time period of nine weeks in half of the subjects.

The study was originally designed to be subject and investigator-blind, but due to the necessity to change cake size after the baseline phase for the EXP subjects, the subjects were able to distinguish who was assigned to the "new" (EXP) diet (see "Diets" in this section to see why cake size changed). However, the subjects were not informed of

their response to the diets until the study was completed. Both principal investigator (GMW) and student investigator (PKP) supervised the feeding of the subjects and remained blinded to the changes in serum chemistries. The other principal investigator (JTS) supervised all laboratory analyses and was blinded, as were her laboratory staff, to the dietary assignments.

Subjects

Potential subjects were recruited from advertisements in the student paper and from notices posted on campus. Specific subject qualifications included: male, age range of 20-60 years, fasting serum total cholesterol level between 4.65-6.72 mmoles/l (180-260 mg/dl), fasting serum triglyceride level of less than 2.26 mmoles/l (200 mg/dl), not currently under treatment for a disease that affects serum cholesterol levels, avoidance of use of prescription medications that affect serum cholesterol levels, and in good health as determined by a physical exam. After an initial cholesterol screening, completion of a detailed health questionnaire (see Appendix 4), and signing of the consent form (see Appendix 5) by 79 subjects, 38 subjects qualified for the study. Some subjects were solicited from a previous diet study whose total cholesterol levels met the guidelines of this study, and who also met all previous criteria just described. Further screening of possible subjects was then performed by physical

examination and a detailed blood chemistry analysis (Diagnostic Multi-Chem, Roche Biomedical Laboratories, Columbus, OH). A total of 34 subjects then remained after fulfilling all the subject qualifications. All 34 subjects completed the study.

Each subject's entry dietary habits (self-selected diet) was determined utilizing a computerized food frequency questionnaire (Right Byte) developed by N-squared Computing, Salem, Oregon. Subjects were matched for age, body mass index, and serum cholesterol levels in pairs and randomly assigned to either diet before the experimental phase of the study. See Table 3 for clinical profiles of subjects after randomization.

Diets

Table 4 describes the meal plan utilized in this study. Both diets were precisely calculated and then formulated to provide approximately the same percentage of energy from carbohydrate, protein and fat. Dietary cholesterol levels were calculated to provide 400 mg/day (400 $\pm$ 50 mg). See Tables 1 and 2 for the actual nutrient composition of both diet phases. Seventy percent of the fat was provided by either the EXP or PALM fats; the remaining 30 percent of fats was provided by meats and milk. This was in agreement with the original goal of test fat intake for this study.

On weekdays, the subjects were required to consume

breakfast and evening meals under the supervision of a registered dietitian (PKP) in the metabolic kitchen of the Medical Dietetics Division and were given sack lunches. On weekends and national holidays, the subjects were provided with sack lunches and dinners that were prepared for them in the metabolic kitchen. These meals were picked up in the morning after consuming breakfast in the metabolic kitchen. All meals were prepared from standardized recipes; a one-week cycle menu was utilized. A local bakery prepared the bread (utilizing either the EXP or PALM fats as specified by the investigators) that was utilized for french toast and hamburger buns during the study. All food intake for each subject during the study period was recorded and analyzed using an IBM PC and Food Processor II software (ESHA Research, Salem, Oregon). This data base was updated using Handbook 8 (United States Department of Agriculture, 1979) in order to account separately for C8:0-C10:0, C12:0-C16:0, C18:0 and C22:0 intake from total saturated fat values.

Each subject selected an appropriate energy level (ranging from 9.2 - 16.3 mjoules) after being instructed to choose the amount of food they felt would not induce weight change (the goal was to maintain weight within $\pm$ 2 percent of entry weight). Body weight was then monitored daily by the investigators. Energy intake was increased for any subject who required additional energy by providing extra cookies and sandwiches, and vice versa for those subjects requiring a

reduction in energy intake.

Both diets were color-coded during the experimental phase. No color-coding was utilized during the initial three weeks on the baseline phase. On the experimental phase, subjects were assigned to either the "blue" or "red" diet (after randomization as discussed). The EXP and PALM diets were color-coded blue and red respectively, but the subjects were not informed of the diet-type, just color. This color-coding allowed for the investigators and employees in the kitchen to maintain correct diet assignments when serving subjects their meals. Plates were marked with either red or blue stickers to assure that the correct diet was served to the correct subject. The subjects on the "blue" diet soon were able to distinguish that they were receiving the "new" fat (EXP) because their cake servings increased in comparison to the "red" cake on the PALM diet (which was the original cake size for all subjects while on the baseline diet). This topic of increased cake size on the EXP diet will be discussed later in this section.

The experimental fats were incorporated into food items such as cakes, cookies, salad dressings, rolls, muffins, biscuits and mayonnaise. Fat as a condiment was limited to one tablespoon per day (corn oil margarine). The range of fat intake as percent of total calories varied slightly due to a certain amount of latitude given in terms of allowing the subjects to substitute certain food items. For example,

cookies would be requested instead of cake for the dinner dessert occasionally. Subjects were permitted up to 15 grams of alcohol per day, and were cautioned not to overindulge on an occasional basis. Most subjects adhered to this recommendation. Mean intake averaged 5 grams per day. The highest intake was 19 grams per day.

Overall, items fed were identical except for the type of fat incorporated into the various foods. The exception to this was the cake on the EXP diet. According to communication from Procter and Gamble, fat-balance studies performed on the BMCT (EXP) showed that of the 37.5 kjoules (9 kcalories) provided per gram of the experimental fat, only 20.8 kjoules (5 kcalories) were utilized by the body. The remaining kjoules were reported to be unabsorbable and thus excreted in the stool. Due to the net utilizable energy value of the BMCT of 20.8 kjoules per gram of fat, we had to compensate for this energy deficit in comparison to the PALM diet. To do this, the serving size of the cake on the EXP diet was increased by approximately 80 percent and canola oil was used to replace 45 percent of the fat in the recipe.

Because of the increased amount of cake consumed on the EXP diet, the subjects consumed more carbohydrate than when on the baseline diet. These changes resulted in increasing the total percentage of energy from carbohydrate from 50 en% on the baseline diet to 55 en% on the EXP diet (see Table 2). Fat intake then fell from 38 en% on baseline to 34 en% on the

EXP diet. This procedure also "broke" the subject-blind in the study, due to an increase in cake size.

Total mean energy intake reported in Table 2 represents utilizable energy. We assigned a value of 20.8 kjoules per gram to all energy from BMCT on the EXP diet. If this calculation had not been adjusted to account for the unutilizable "fat energy", then the EXP subjects would have consumed 1.5 more mjoules (mj) per day than when on the baseline diet. This would have yielded about a 2.5 kilogram (kg) weight gain from the end of baseline to end of study (42 days). The calculation for the 2.5 kg weight gain was performed as shown:

1.5 mj x 42 days = 63 mj

63 mj / 24.9 mj/kg of body fat = 2.5 kg wt.gain

Weight gain on average was less than 0.15 kg (0.2%) for subjects on both diets throughout the study period, thus giving creedance to our adjustment (see Table 3).

It should also be noted that the physiological fuel value of MCFA is 34.6 kjoules per gram (Mead Johnson Product Handbook, 1990), which is slightly less than 37.5 kjoules per gram for long chain fats. This may have affected the energy deficit to some extent, but it cannot account for the majority of the theoretical energy deficit seen on the EXP diet.

Serum Lipid and Apolipoprotein Analysis

Chemical analyses were conducted in the laboratories of

the Department of Human Nutrition and Food Management under the direction of JTS. Blood samples for serum lipid analyses were obtained by venipuncture before breakfast, generally after a greater than 12 hour fast at various times throughout the study. Depending on the requirements of the procedure, serum samples obtained by centrifugation were analyzed fresh, or were frozen at -70 degrees Celsius for later analysis.

All serum lipids were analyzed from previously-frozen serum samples. Serum total cholesterol (TC) was analyzed using an enzymatic assay (procedure #352, Sigma Chemical Company, St. Louis, MO). Total serum HDL-C was obtained by precipitating non-HDL lipoprotein components (such as VLDL and LDL) with dextran sulfate and magnesium (Solomon Park Research, Kirkland, Washington). Total HDL-C was then determined using an enzymatic assay as just described for TC. The original supernatant (containing total HDL-C) was then further precipitated with a higher concentration of the dextran sulfate/magnesium solution to obtain HDL-3 in the "new" supernatant. An enzymatic assay was performed as before to obtain HDL-3 values. Serum HDL-2 cholesterol was calculated by subtracting serum HDL-3 cholesterol from the total serum HDL-C. Serum TG were measured enzymatically (Solomon Park Research, Kirkland, Washington). Serum LDL-C was calculated using the following formula in mg/dl units:

LDL-C = TC - Total HDL-C - (TG/5).

Conversion factor to convert to SI units were then calculated

by dividing by 38.67.

Aliquots of a previously frozen serum sample were analyzed along with the standard in all runs. The run-to-run precision (coefficient of variation) for the various serum lipid analyses were as follows: total cholesterol, 2.9 percent; Total HDL-C, 1.6 percent; HDL-3, 4.1 percent; HDL-2, 13.6 percent; and TG, 1.5 percent.

Fresh serum samples were analyzed for apolipoproteins A-1 and B-100 by radial immunodiffusion (Tago. Inc., Burlingame, CA). Three levels of reference sera were run with each determination. The serum apolipoprotein concentrations were calculated from a standard curve using the square of precipitin ring diameter and antigen concentration. Standards run with each set of samples showed a coefficient of variation throughout the study of 1.9 percent for Apo A-1 and 1.2 percent for Apo B-100.

Blood Chemistry Analyses

All of the blood chemistry analyses performed during the study (almost weekly for each subject) were performed by Roche Biomedical Laboratories (Diagnostic Multi-Chem, Columbus, OH). See Table 7 and Chapter IV for these results.

Data Analysis

We compared end of baseline values to end of study values for all serum lipid-related parameters utilizing a Mann-

Whitney test (Minitab, State College, PA). Statistical significance was set at $p < 0.05$. We used this nonparametric test because distributions of the lipid parameters studied were not normally distributed (based on dotplot analyses). All means and standard errors of the mean (SEM) were calculated utilizing Minitab (State College, PA).

CHAPTER IV

RESULTS

Clinical Profile

Table 3 lists the mean values for age, body mass index, height and weights for subjects on the EXP and PALM diets. Percent weight change from entry to completion of the study (63 days) was 0.2 $\pm$ 0.5 on the EXP diet and 0.1 $\pm$ 0.4 on the PALM diet. Weights remained within $\pm$ 2 percent of start weight in 29 subjects. Weight gain up to 5 percent of start weight was seen in 3 subjects. Weight loss up to 5 percent of start weight was seen in 2 subjects. Gains and losses were essentially equally distributed between both groups of subjects (EXP and PALM groups).

Serum lipids and apolipoproteins

Table 5 gives a complete listing of all the lipid parameters studied. All changes from baseline are insignificant unless otherwise noted.

Mean total serum cholesterol (TC) levels from entry to end of baseline showed an overall decrease of 4 percent for all of the subjects (see Table 5). The mean change from

baseline to the completion of the study was a 0.6 percent increase on the EXP diet and a 0.4 percent decrease on the PALM diet.

Mean calculated LDL-C from entry to end of baseline showed an overall decrease of 7 percent in the entire subject sample (see Table 5). The mean calculated LDL-C levels increased 8 percent on the EXP diet from end of baseline to the end of study. The PALM diet showed an increase of 1 percent from end of baseline to the end of study.

Mean serum Apo B-100 levels showed an overall increase of 7 percent from entry to end of baseline in the entire subject sample. The mean serum Apo B-100 levels increased 4.9 percent on the EXP diet from end of baseline to the end of study. The PALM diet also showed an increase of 3.5 percent from end of baseline to the end of study. Figure 1 displays more closely the fluctuations in serum values seen throughout the study.

Mean serum TG levels showed an overall decrease of 13 percent for all subjects from entry to end of baseline (see Table 5). The mean serum TG levels decreased 7 percent on the EXP diet from end of baseline to the end of study. The PALM diet showed a 0.7 percent decrease from end of baseline to the end of study.

Mean serum total HDL-C concentrations increased 9 percent overall for all subjects from entry to end of baseline. The mean serum total HDL-C levels decreased 16 percent on the EXP diet from end of baseline to the end of study ($p<0.01$). The

PALM diet showed a 1.1 percent increase from end of baseline to the end of study. Mean serum HDL-2 cholesterol levels decreased 1 percent overall for all subjects from entry to end of baseline. The mean serum HDL-2 cholesterol levels decreased 28 percent on the EXP diet from end of baseline to end of study ($p<0.05$). The PALM diet showed a 9.4 percent increase from end of baseline to the end of study. Mean serum HDL-3 cholesterol levels increased 12 percent overall for all subjects from entry to end of baseline. The values for mean serum HDL-3 cholesterol levels decreased 16 percent ($p<0.05$) and 0.5 percent on the EXP and PALM diets respectively from end of baseline to end of study.

Mean serum Apo A-1 levels increased overall 15 percent for all subjects from entry to end of baseline. A decrease of 2.4 percent was seen on the EXP diet from end of baseline to end of study. The PALM diet showed an increase of 10.3 percent ($p<0.05$) from end of baseline to end of study.

The TC/HDL ratio decreased 11 percent overall for all subjects from entry to end of baseline. An increase of 20 percent was seen on the EXP diet from end of baseline to end of study for this ratio ($p<0.05$). The PALM diet showed no change from end of baseline to end of study for the TC/HDL ratio.

The LDL/HDL ratio decreased 13 percent overall for all subjects from entry to end of baseline. An increase of 30 percent was seen on the EXP diet from end of baseline to end

of study for this ratio ($p<0.05$). The PALM diet showed a 1 percent increase from end of baseline to the end of study.

Health Reaction to Both Diets

Table 6 reports the symptoms that were most frequently noted by the study subjects from their weekly health assessments. Flatulence and loose stools were reported on average by 3 subjects each week on the EXP diet. Symptoms of stomach ache were reported on average by 1 subject each week on the EXP diet. PALM diet subjects rarely reported any side effects throughout the study (see Table 6).

Heart, Liver and Kidney Function

Table 7 gives a complete listing of changes seen in various serum indices of heart, liver and kidney function in the study subjects. Only two subjects had moderately high liver function tests and they started the study with these high-normal values. Mean values, and most values as well, remained within normal limits for the total group of subjects during the study.

Diet History of Subjects Prior to Study

Table 8 gives the nutrient intake profile of subjects prior to the study (self-selected diet). On the food-frequency questionnaire, the subjects reported consuming diets

that were somewhat lower in fat and cholesterol than the typical American diet. The mean reported values for the group as a whole were 2800 kcalories, of which 50 percent of energy was carbohydrate, 14 percent protein, and 35 percent fat (9% of energy from saturated fat). An average of 370 mg of cholesterol was reported by all of the subjects.

Table 1. Calculated nutrient consumption on the baseline diet

	EXP*	PALM
Energy (mjoules/d)	12.4 ± 0.4** (9.6 - 15.5)	13.3 ± 0.5 (9.8 - 17.5)
Carbohydrate (en%)[a]	50 ± 1 (48 - 52)	50 ± 1 (47 - 51)
Protein (en%)	13 ± 1 (11 - 14)	12 ± 1 (11 - 14)
Fat (en%)	38 ± 1 (35 - 41)	38 ± 1 (37 - 41)
Saturated fatty acids (C12:0-C16:0) (en%)	17 ± 1 (15 - 19)	17 ± 1 (16 - 18)
Monounsaturated fatty acids (en%)	11 ± 1 (10 - 13)	12 ± 1 (11 - 13)
Polyunsaturated fatty acids (en%)	4 ± 1 (3 - 4)	4 ± 1 (4)
C18:0 (en%) (stearic acid)	3 ± 1 (2 - 4)	3 ± 1 (2 - 4)
C8:0 - C10:0 (en%) (MCT)	1 ± 1 (1)	1 ± 1 (1)
C22:0 (en%) (behenic acid)	0	0
Cholesterol (mg/day)	420 ± 10 (360 - 470)	430 ± 10 (380 - 480)
Alcohol (g/day)	5 ± 2 (0 - 19)	4 ± 1 (0 - 13)

* EXP represents subjects eventually randomized to the BMCT-based diet (n = 17) and PALM represents subjects eventually randomized to the palm oil/palm kernel oil-based diet (n = 17).

** Values are means ± SEM with a range of values in parentheses.

[a]en% refers to a percentage of total diet energy.

Table 2. Calculated nutrient consumption on the experimental phase

	EXP*	PALM
Energy (mjoules/day)**	12.3 ± 0.3[a] (10.3 - 15.7)	13.0 ± 0.4 (10.5 - 16.0)
Carbohydrate (en%)[b]	55 ± 1 (53 - 56)	50 ± 1 (47 - 53)
Protein (en%)	13 ± 1 (12 - 15)	13 ± 1 (11 - 14)
Fat (en%)	34 ± 1 (29 - 37)	38 ± 1 (35 - 40)
Saturated fatty acids (C12:0-C16:0) (en%)	5 ± 1 (4 - 6)	17 ± 1 (16 - 18)
Monounsaturated fatty acids (en%)	8 ± 1 (7 - 9)	12 ± 1 (11 - 13)
Polyunsaturated fatty acids (en%)	5 ± 1 (4 - 5)	4 ± 1 (3 - 4)
C18:0 (en%) (stearic acid)	2 ± 1 (1 - 3)	3 ± 1 (2 - 4)
C8:0 - C10:0 (en%) (MCT)	6 ± 1 (6)	1 ± 1 (1)
C22:0 (en%) (behenic acid)	6 ± 1 (5 - 6)	0
Cholesterol (mg/day)	430 ± 10 (400 - 490)	430 ± 10 (390 - 480)
Alcohol (g/day)	4 ± 1 (0 - 14)	5 ± 1 (0 - 17)

* EXP refers to the BMCT-based diet and PALM refers to the palm oil/palm kernel oil-based diet.

** Adjusted energy intake, based on total fat energy calculated as 20.9 kjoules per gram (5 kcals/g) for the fatty acids from the BMCT fat and 37.6 kjoules per gram (9 kcals/g) for all other fatty acids (see METHODS). Unadjusted energy consumption for subjects on the EXP diet was 13.9 ± 3.6 mjoules (11.8 - 17.1). Conversion factor for energy: 239kcals/mjoule; .24kcals/kjoule.

[a]Values are means ± SEM with a range of values in parenthesis.

[b]en% refers to a percentage of total diet energy

Table 3. Clinical Profile

	EXP* (n = 17)	PALM (n = 17)
Age (years)	32 ± 3 ** (22 - 58)	27 ± 2 (20 - 49)
Body Mass Index ($wt[kg]/ht^2[m]$)	26.8 ± 1.2 (20.7 - 40.6)	26.3 ± 1.0 (18.5 - 43.2)
Height (cm)	180 ± 2 (162 - 198)	179 ± 2 (169 - 190)
Start Weight (kg)	79.9 ± 3.0 (49.1 - 101.8)	80.8 ± 3.2 (63.2 - 112.7)
End Weight (kg)	80.0 ± 3.0 (50.5 - 102.7)	80.7 ± 3.2 (63.2 - 110.9)
Weight Change (percent)	0.2 ± 0.5 (-3.5 ± 4.2)	0.1 ± 0.4 (-3.7 ± 3.0)

* EXP refers to the BMCT-based diet and PALM refers to the palm oil/ palm kernel oil-based diet.

** Values are means ± SEM with the range of values in parentheses.

Table 4. Meal plan

Breakfast:	fruit juice; egg; french toast, muffin, biscuit, or pancakes; milk (1% milk fat)
Lunch:	roast beef, turkey or ham sandwich; cookies; fruit; diet soda
Dinner:	salad with dressing; beef, poultry, pizza, turkey, pork or shrimp; starch; vegetable; cake; milk (1% milk fat)

Table 5. Serum lipid values and ratios for entry, end of baseline, and end of experimental diet.

	ENTRY	END OF BASELINE	END OF STUDY	PERCENT CHANGE FROM BASELINE
Total Cholesterol (mmoles/l)				
EXP*	6.0 ± 0.2**	5.9 ± 0.2	5.9 ± 0.2	0.6 ± 1.6
PALM	6.2 ± 0.2	5.8 ± 0.2	5.8 ± 0.2	0.4 ± 1.0
Calculated LDL-C (mmoles/l)				
EXP	4.1 ± 0.2	3.9 ± 0.2	4.2 ± 0.2	8.0 ± 3.0
PALM	4.3 ± 0.1	4.0 ± 0.2	4.0 ± 0.2	1.0 ± 2.0
HDL-C (mmoles/l)				
EXP	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	-16.0 ± 2.4[b]
PALM	1.2 ± 0.1	1.2 ± 0.0	1.3 ± 0.1	1.1 ± 2.4
HDL-2 Cholesterol (mmoles/l)				
EXP	0.25 ± 0.02	0.24 ± 0.02	0.16 ± 0.02	-28.5 ± 6.4[a]
PALM	0.22 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	9.4 ± 9.0
HDL-3 Cholesterol (mmoles/l)				
EXP	0.88 ± 0.06	1.04 ± 0.04	0.87 ± 0.05	-16.1 ± 4.2[a]
PALM	0.94 ± 0.05	1.00 ± 0.03	0.98 ± 0.05	-0.5 ± 4.2
Triglycerides (mmoles/l)				
EXP	1.7 ± 0.2	1.5 ± 0.1	1.3 ± 0.1	-7.2 ± 4.7
PALM	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	-0.7 ± 5.2
Total Cholesterol: HDL Ratio				
EXP	5.4 ± 0.4	4.8 ± 0.3	5.7 ± 0.3	20.0 ± 3.0[a]
PALM	5.4 ± 0.3	4.8 ± 0.2	4.8 ± 0.3	0.0 ± 2.0
LDL-C: HDL-C Ratio				
EXP	3.7 ± 0.3	3.2 ± 0.2	4.1 ± 0.3	30.0 ± 4.0[a]
PALM	3.8 ± 0.2	3.3 ± 0.2	3.3 ± 0.2	1.0 ± 4.0
Apolipoprotein A-1 (mg/l)				
EXP	1542 ± 63	1746 ± 45	1693 ± 52	-2.4 ± 3.3
PALM	1487 ± 57	1727 ± 51	1889 ± 43	10.3 ± 2.8[a]
Apolipoprotein B-100 (mg/l)				
EXP	1153 ± 60	1277 ± 83	1321 ± 73	4.9 ± 3.0
PALM	1118 ± 45	1157 ± 64	1169 ± 50	3.5 ± 4.9

* EXP refers to the BMCT-based diet and PALM refers to the palm oil/palm kernel oil diet. All subjects consumed the PALM diet during the baseline phase of the study.

** Values are means ± SEM for 16-17 subjects.

[a] $p < 0.05$

[b] $p < 0.01$

Table 6. Symptoms reported by study subjects

Symptom	Diet *	Week								
		1	2	3	4	5	6	7	8	9
Flatulence	EXP **	1[a]	-	-	2	4	3	3	5	3
	PALM	1	-	1	-	-	-	-	-	-
Loose stools, diarrhea	EXP	-	-	-	-	4	3	-	3	2
	PALM	-	-	-	-	-	-	-	-	-
Stomach ache	EXP	1	-	-	2	2	1	1	1	1
	PALM	-	1	-	1	-	-	-	-	-

* All 34 subjects consumed the PALM diet during the first 3 weeks of the study. Then 17 subjects each followed the diets listed for 6 weeks.

** EXP refers to the BMCT-based diet and PALM refers to the palm oil/palm kernel oil-based diet.

[a]Number of subjects reporting this symptom in the study period for that week. Other minor complaints appearing only once during the entire study period are not listed.

Table 7. Indices of heart, liver and kidney function for study subjects

	END OF BASELINE	END OF STUDY	PERCENT CHANGE
Lactic acid dehydrogenase* (LDH)			
EXP	149 ± 4 (119-186)	167 ± 7 (136-260)	12 ± 3 (-15 to 44)
PALM	154 ± 6 (117-206)	156 ± 5 (131-189)	2 ± 3 (-22 to 28)
Alanine aminotransferase* (ALT)			
EXP	23 ± 2 (10-36)	33 ± 3 (18-63)	48 ± 10 (-18 to 125)
PALM	24 ± 4 (9-60)	29 ± 5 (10-88)	20 ± 6 (-20 to 80)
Gamma-glutamyl transferase* (GGT)			
EXP	25 ± 4 (7-60)	24 ± 4 (5-65)	-8 ± 4 (-40 to 27)
PALM	18 ± 3 (7-49)	18 ± 3 (7-51)	-1 ± 4 (-26 to 36)
Serum aspartate aminotransferase* (AST)			
EXP	23 ± 1 (17-36)	32 ± 3 (17-75)	37 ± 9 (-8 to 117)
PALM	24 ± 2 (14-35)	24 ± 2 (14-55)	1 ± 6 (-37 to 72)
Alkaline phosphatase* (ALK PHOS)			
EXP	90 ± 8 (49-188)	82 ± 8 (44-189)	-8 ± 2 (-23 to 6)
PALM	85 ± 4 (57-122)	86 ± 5 (61-126)	1 ± 9 (-9 to 33)
Total bilirubin[a] (T Billi)			
EXP	0.66±0.04 (0.4-1.0)	0.65±0.05 (0.4-1.2)	1.3±6.0 (-28.6 to 60)
PALM	0.75±0.06 (0.4-1.3)	0.78±0.08 (0.4-1.5)	3.6±5.4 (-22.2 to 50)
Blood urea nitrogen[a] (BUN)			
EXP	14 ± 1 (11-20)	14 ± 1 (9-19)	2 ± 4 (-19 to 33)
PALM	13 ± 0 (9-16)	13 ± 0 (10-16)	-1 ± 4 (-29 to 33)
Creatinine[a]			
EXP	1.2 ± 0.1 (1.0-1.4)	1.2 ± 0.1 (1.0-1.4)	-1 ± 1 (-8 to 9)
PALM	1.2 ± 0.1 (1.0-1.5)	1.2 ± 0.1 (1.0-1.5)	1 ± 2 (-8 to 15)

* units in IU/L

[a]units in mg/dl

Normal ranges: heart--LDH 100-250;
liver--ALT 0-50; GGT 0-65; AST 0-50; ALK PHOS 40-150;
T Billi 0.1-1.2;
kidney--BUN 7-26; Creatinine 0.5-1.5.

Table 8. Nutrient profile of subjects prior to study (self-selected) compared to baseline diet values.*

	self-selected diet	baseline diet
Energy (mjoules)	10.9 ± 0.8**	12.8 ± 0.5
Carbohydrate (en%)[a]	50 ± 2	50 ± 1
Protein (en%)	15 ± 1	13 ± 1
Total Fat (en%)	32 ± 2	38 ± 1
Saturated fat (en%)	9 ± 1	17 ± 1
Cholesterol (mg/dl)	313 ± 33	425 ± 10

* Diet history questionnaire: Right Byte (see METHODS). Value given is average of all 34 subjects.

** Values are mean ± SEM

[a]en% refers to a percentage of total diet energy.

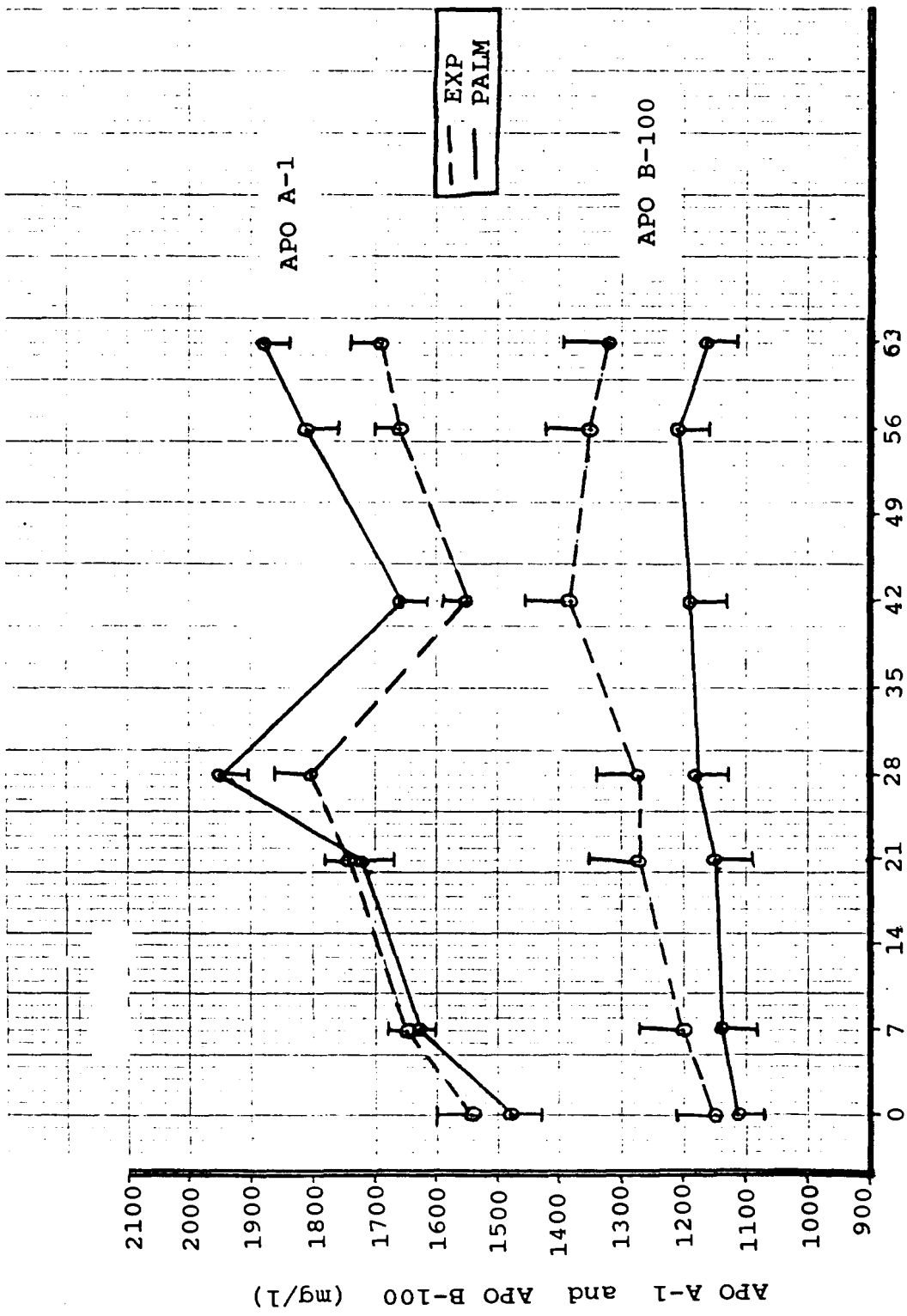

FIGURE 1 : Apolipoprotein A-1 and apolipoprotein B-100 values in the EXP and PALM groups during the baseline and experimental phases.

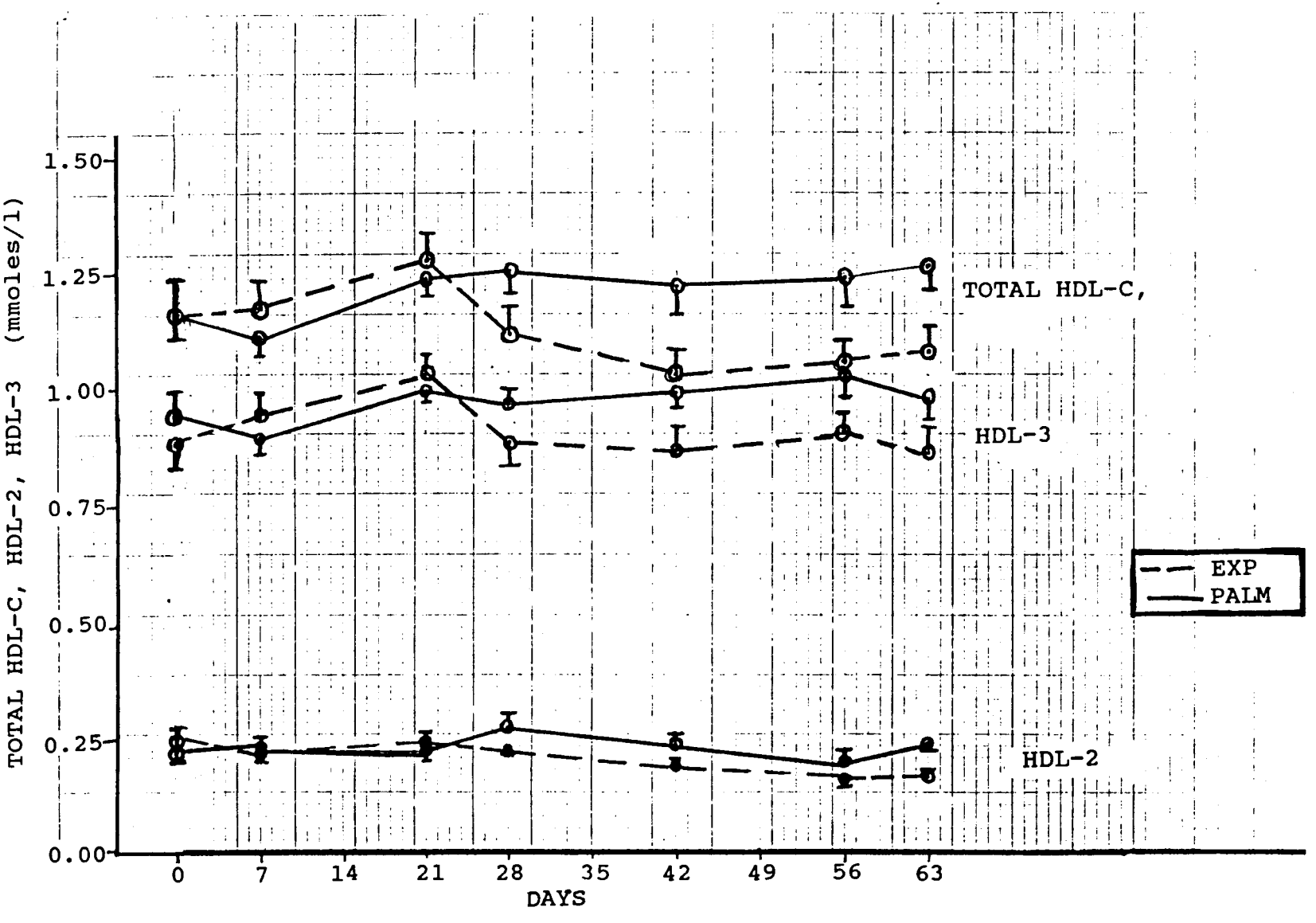

FIGURE 2: Total HDL-C, HDL-2, and HDL-3 values in the EXP and PALM groups during the baseline and experimental phases.

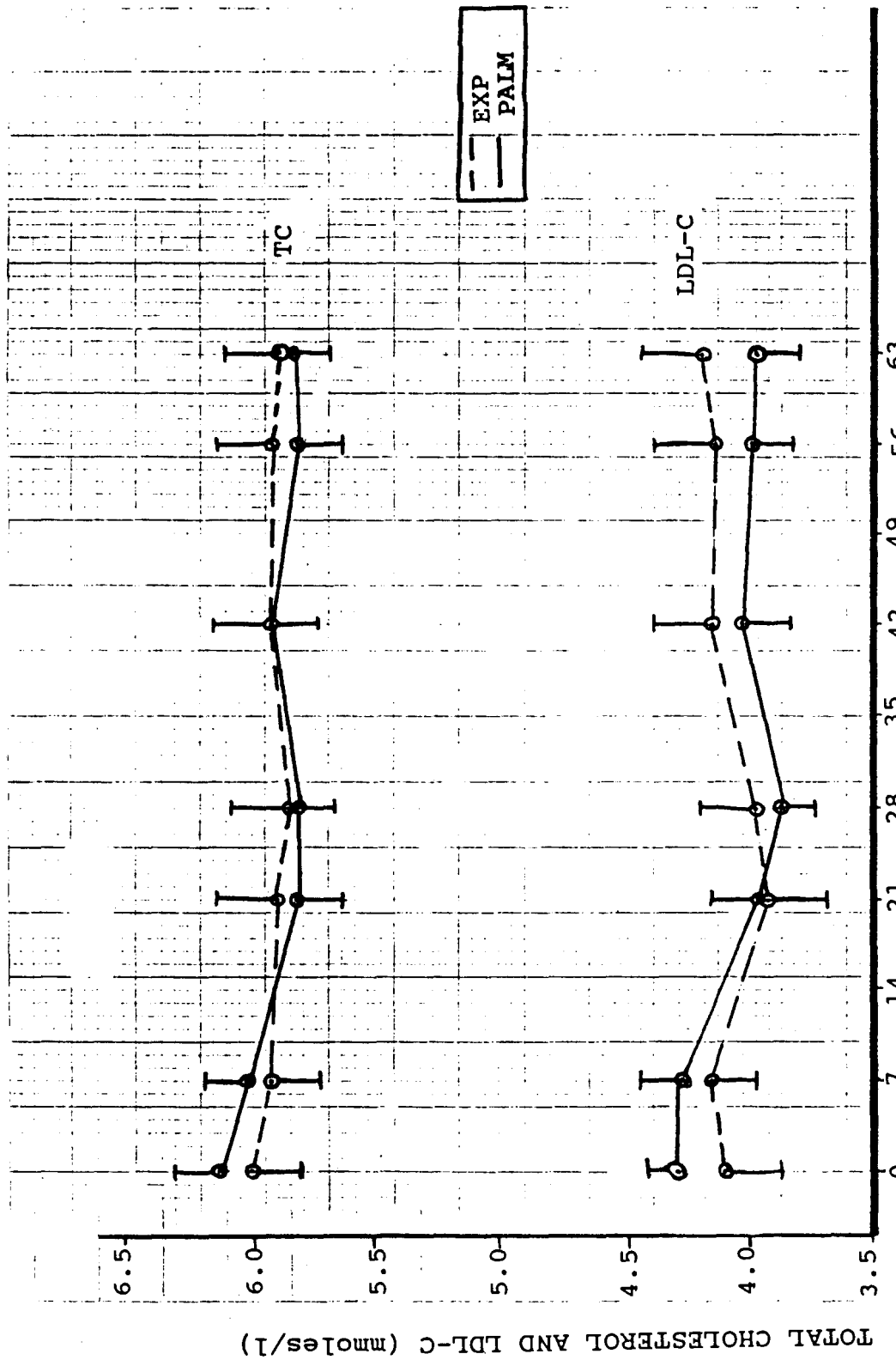

FIGURE 3: Total cholesterol and calculated LDL-C values in the EXP and PALM groups during the baseline and experimental phases.

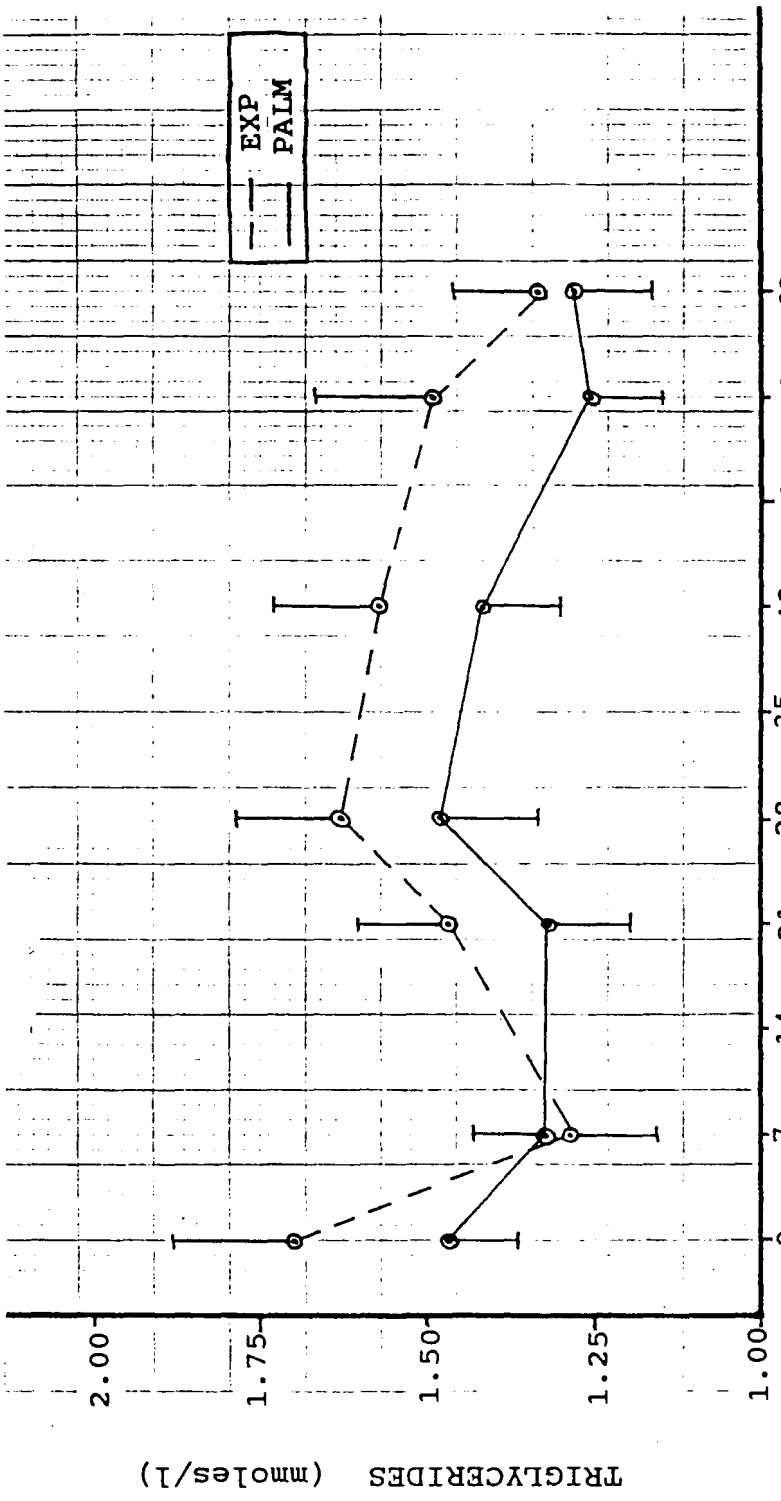

FIGURE 4 : Triglyceride values in the EXP and PALM groups during the baseline and experimental phases.

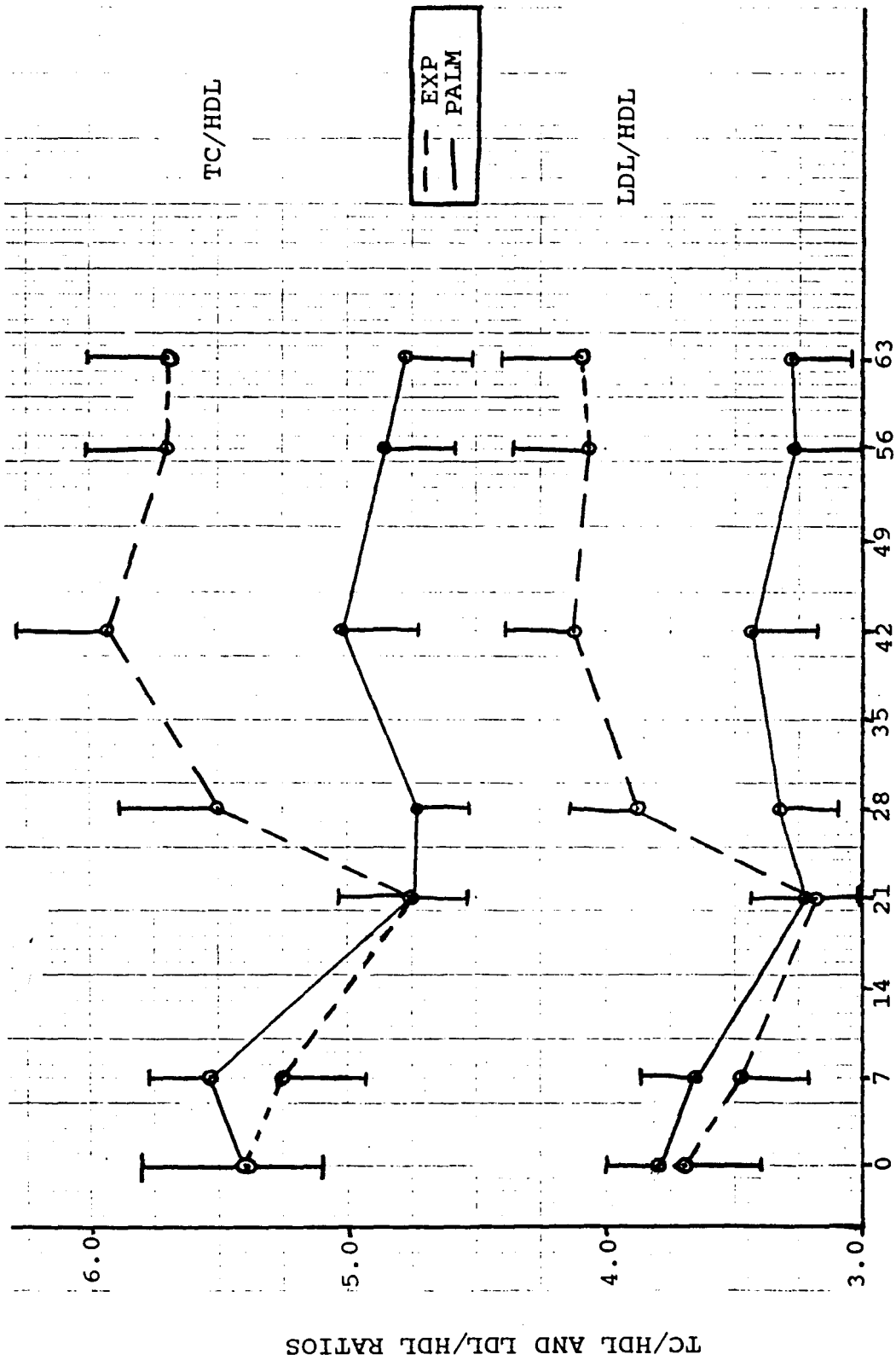

FIGURE 5: Ratios of Total cholesterol:HDL-C and LDL-C:HDL-C in the EXP and PALM groups during the baseline and experimental phases.

CHAPTER V

DISCUSSION

Many observations were noted upon completion of this study. First, mean serum total cholesterol levels (and LDL-C) levels decreased overall from the beginning of the study to the end of baseline. This could be attributed to a controlled and consistent dietary intake that provided less saturated fat in the diet when compared to the subject's diets (self-selected) previous to participation in the study. Although the level of saturated fat provided during the baseline phase was designed to imitate a typical American diet (17 percent of total diet energy), it could be that individual saturated fat intakes among some subjects were higher than the "average" typical saturated fat intake of Americans. Many controlled feeding trials utilizing a free-living population have demonstrated this similar finding of an initial drop in serum lipid levels (9,33,39,79).

The design of this study also allowed us to evaluate the effects of a constant dietary intake (PALM diet subjects only) over a substantial time period (nine weeks). Mean serum LDL-C and Apo B-100 levels did not increase significantly as a result of feeding a high-saturated fat intake in the PALM

subjects. A study by Ginsberg et al.(33) similarly reported stable plasma cholesterol levels after feeding diets high in saturated fat to subjects over a ten week period. Also, serum total HDL-C levels remained relatively stable throughout the entire study period in the PALM subjects (see Figure 2), with the subfraction HDL-2 even showing a slight increase of 9 percent from end of baseline to end of study. In addition, Apo A-1 levels showed a significant increase ($p<0.05$) from end of baseline to end of study on the continuous PALM diet. These results were probably seen due to the fact that the PALM subjects were maintained on a relatively constant and consistent intake for nine weeks. One would perhaps expect that over a period of time of feeding an "atherogenic fat" as we did, would result in a transient increase in serum LDL-C levels. As mentioned earlier however, it could be that the majority of our subjects were consuming a potentially higher content of saturated fat (previous to entering the study), and this thus would explain this observation of no transient increase in serum LDL-C levels. It could be that the type of fat we used, namely palm oil/palm kernel oil, may not be as atherogenic as thought over a substantial period of time. This topic of saturated fat "source" deserves further attention.

The next point of interest deals with the initial null hypothesis (H_o) proposed in Chapter I. The results of our study show that, in fact, the BMCT diet did not have a

significant effect on serum TC or LDL-C levels. It was proposed that since this structured TG was essentially free of the atherogenic SFA (C12:0-C16:0), that it may not affect serum lipids adversely, and may even lower TC and LDL-C levels. Unfortunately, this was not the case. Serum total HDL-C levels were significantly decreased ($p<0.01$) on the EXP diet from end of baseline to end of study, a result that was not expected. Both HDL subfractions also decreased significantly ($p<0.05$). This in turn affected the TC/HDL and LDL/HDL ratios adversely. The increase in LDL-C from end of baseline to end of study also contributed to the significant increase seen in both ratios. Although Apo A-1 levels did not decrease significantly, one has to wonder whether it would have significantly decreased over a longer time period.

These results have perplexed us as well as the developers of this structured TG. As discussed previously, medium chain fatty acids have been documented as being metabolized quickly by the liver, thus not affecting serum lipid levels(57,76,77,78). This was the basis for incorporating these fatty acids in the structured TG (see Appendix 3 for fatty acid composition of BMCT fat). The other main fatty acid utilized in the structured TG was behenic acid, which is a very long chain saturated fatty acid. Very long chain fatty acids have also been assumed to have no effect on serum lipids(108). Our study was the first study conducted in which the BMCT fat was utilized as a major source of lipid energy in

humans.

There are several possible suppositions as to why the BMCT diet did not lower serum TC, LDL-C and Apo B-100 levels significantly. It could be that perhaps medium chain triglycerides (C8:0-C10:0), in actuality do affect serum lipids. More controlled trials are needed on this question. This topic is discussed in detail in Chapter II. The same is also possible about behenic acid; only one clinical pilot study has been performed to date involving human consumption of behenate, and this study only lasted one week (communication from Procter and Gamble). Thirdly, it could be that the combination of the behenate and the MCFA could have had a synergistically negative effect on serum lipids.

Also, lauric acid (C12:0) is considered to be a hypercholesterolemic fatty acid, but may in actuality be neutral or act as a MCFA. This controversy deserves further attention. Very little information is available in the area of lauric acid and its effect on serum lipids.

Thus, one can see that many questions come to mind after observing the perplexing results of this study.

At present, further research should focus on examining the C22:0 and C8:0-C10:0 fatty acids separately to see if one can be shown to be the lipid-raising moiety of the BMCT product. Until then, the results of this study clearly fail to promote the use of this fat (BMCT) as a major dietary component of the American diet.

CHAPTER VI

CONCLUSION

As the results of this study show, the experimental structured triglyceride (BMCT) failed to perform as a "cholesterol-lowering" fat. Even in comparison to the saturated fat source of palm oil/palm kernel oil, this newly developed fat did not show any benefit. The null hypothesis (H_o) states that the BMCT diet fed for 6 weeks will have no significant effect (positive or negative) upon the various lipids and apolipoproteins (TC, LDL-C, HDL-C, HDL-2, HDL-3, TG, Apo A-1, and Apo B-100) when compared to a baseline palm oil/palm kernel oil diet fed for 3 weeks. We accepted the null hypothesis when evaluating the results of TC, LDL-C, TG, Apo A-1, and Apo B-100, in that no significant effect, either positive or negative was noted from end of baseline to end of study (based on Mann-Whitney testing). However, we rejected H_o when evaluating the results of total HDL-C, HDL-2, HDL-3, in that these levels were significantly lower at the end of the study than when compared to end of baseline. See Table 5 for all results and p-values of significant results.

It was unfortunate that this "new" fat did not perform as planned by the lipid scientists at Procter and Gamble. It was

their hope that this unique fat would enable the food industry to maintain the quality of many products that require a "solid" (saturated) fat, but yet not influence the serum cholesterol levels of the consumers. Hopefully, as mentioned in Chapter V, further research in the various areas of saturated fatty acids will encourage the development of a more beneficial semi-solid "structured TG".

At present, the average American consumes almost about 1.5 times the amount of saturated fat as recommended by the Committee of Diet and Health of the National Research Council(90). Its current recommendations are aimed towards healthy, North American adults and children. In the area of fat intake, it recommends that Americans: 1) reduce total fat intake to 30 percent or less of calories; 2) reduce saturated fatty acid intake to less than 10 percent of calories and 3) reduce the intake of cholesterol to less than 300 mg daily. The intake of fat and cholesterol can be reduced by substituting fish, poultry without skin, lean meats, and low- or nonfat dairy products for fatty meats and whole-milk products; by choosing legumes; and by limiting oils, fats, egg yolks, and other fried and other fatty foods. These other fatty foods probably include pastries, cakes, and cookies, most of which contain harmful cholesterol-raising fatty acids if produced commercially. This recommendation seems prudent except for the dietary cholesterol restriction of 300 mg/day for the general population. Research in this area of dietary

cholesterol is quite controversial, and as McNamara et al.(82) reported, some individuals are more sensitive to dietary cholesterol than others.

Thus, the results of this study show that much still remains unknown in the area of fatty acid intakes and serum lipid profiles. This however should not stifle further research, but should encourage further investigation into the topics discussed throughout this thesis.

APPENDIX 1
Typical Fatty Acid Composition of the Principal Vegetable Oils and Animal Fats with special reference to Saturated Fatty Acid Composition

Appendix 1. Typical Fatty Acid Composition of the Principal Vegetable Oils and Animal Fats with special reference to Saturated Fatty Acid Composition[a]

			SFA[b]						
Oil or Fat	PUFA[c]	MUFA[d]	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	total
Coconut oil	2	6	8	6	45	17	8	3	87
Palm kernel oil	2	11	3	4	47	16	8	3	81
Palm oil	9	37	-	-	.1	1	44	4	49
Butterfat	3	23	.9	2	2	18	21	10	54
Cocoa butter	3	33	-	-	0	.1	25	33	60
Beef tallow	4	42	-	-	.9	4	25	19	50
Lard	11	45	-	.1	.2	1	24	14	39
Olive oil	8	74	-	-	-	0	11	2	14
Peanut oil	32	46	-	-	-	.1	10	2	17[e]
Corn oil	59	24	-	-	0	0	11	2	13
Safflower oil	75	12	-	-	-	.1	6	2	9
Rapeseed oil[f]	28	62	-	-	-	-	3	1	6

[a]Data collected from United States Department of Agriculture Handbook No. 8-4, 1979.

[b]Composition of saturated fatty acids in percentage of total fatty acids.

[c]Percentage of fatty acids from polyunsaturated fatty acids.

[d]Percentage of fatty acids from monounsaturated fatty acids.

[e]Peanut oil also contains 1.6 percent C20:0 and 3.8 percent C22:0 as part of the total saturated fatty acid composition.

[f]Low erucic content (< 30%).

APPENDIX 2
Fatty Acid Composition of palm oil/palm kernel oil and BMCT fats as percent of total methyl esters

Appendix 2. Fatty Acid Composition of palm oil/palm kernel oil and BMCT fats as percent of total methyl esters.

	PALM	EXP
C8:0-C10:0 (MCFA)	3.4	50.0
C12:0-C16:0[a]	57.5	0.5
C18:0	4.1	0.9
Mono-unsaturated fat	28.4	0.0
Poly-unsaturated fat	6.2	0.0
C22:0	0.5	48.6

[a]Composition of C12:0, C14:0 and C16:0 are approximately 22, 8 and 27 percent of total methyl esters respectively.

APPENDIX 3
Names and codes of selected fatty acids

Appendix 3. Names and codes of selected fatty acids[a]

Name	Code
Acetic acid	C2:0[b]
Propionic acid	C3:0
Butyric acid	C4:0
Valeric acid	C5:0
Caproic acid	C6:0
Caprylic acid	C8:0
Capric acid	C10:0
Lauric acid	C12:0
Myristic acid	C14:0
Palmitic acid	C16:0
Stearic acid	C18:0
Arachidic acid	C20:0
Behenic acid	C22:0
Lignoceric acid	C24:0
Oleic acid	C18:1 (w-9,cis)
Elaidic acid	C18:1 (w-9,trans)
Linoleic acid	C18:2 (w-6,cis)
Linolenic acid	C18:3 (w-3,cis)
Eicosapentaenoic acid	C20:5 (w-3,cis)
Docosahexaenoic acid	C22:6 (w-3,cis)

[a]Data collected from Shils and Young text (see Reference number 108)
[b]For explanation of nomenclature, see respective section in Chapter II.

Appendix 4
Diet Study and Medical History Questionnaire

DIET STUDY

Last Name	First Name	Middle	Birth Date (Mo.Day. Yr)
Height	Weight	Marital Status	
Residence Address	City	State Zip	Residence Telephone
Business Address	City	Occupation	Business Telephone

GENERAL		Circle One		
1.	Has there been any change in your general health during the last year?	Yes	No	DK*
2.	Have you been examined by your physician within the last year?	Yes	No	DK
3.	Are you receiving any treatment by any doctor now?	Yes	No	DK
4.	Are you taking any medications now?	Yes	No	DK
5.	Has a physician or dentist ever told you that you you had a tumor or a cancer?	Yes	No	DK
6.	Have you had rheumatic fever, growing pains, or twitching of the limbs?	Yes	No	DK
7.	Have you had a stroke?	Yes	No	DK
8.	Have you ever had excessive bleeding following extraction of teeth or from a cut?	Yes	No	DK
9.	Are you sensitive to any particular medicine (Aspirin-Penicillin)?	Yes	No	DK
10.	Do you suffer badly from frequent severe headaches?	Yes	No	DK
11.	Do you have spells of dizziness?	Yes	No	DK

*Don't Know

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12.	Have you fainted more than twice in your life?	Yes	No	DK
13.	Have you ever had severe pains of the face or head?	Yes	No	DK
14.	Have you ever been treated for eye trouble other than corrective glasses?	Yes	No	DK
15.	Have you ever been treated for ear trouble?	Yes	No	DK
16.	Do you have hay fever?	Yes	No	DK
17.	Do you have sinus trouble?	Yes	No	DK
18.	Have you at times had bad nose bleeds?	Yes	No	DK
19.	Do you have frequent sore throat?	Yes	No	DK

CARDIOVASCULAR

20.	Has a physician ever said you had heart trouble?	Yes	No	DK
21.	Have you ever had rheumatic heart disease?	Yes	No	DK
22.	Have you ever had a heart attack?	Yes	No	DK
23.	Has a physician ever said your blood pressure was too high or too low?	Yes	No	DK
24.	Do you get out of breath easily?	Yes	No	DK
25.	Are your ankles often badly swollen?	Yes	No	DK
26.	Do you bruise easily?	Yes	No	DK

GASTRO-INTESTINAL

27.	Do you suffer from stomach trouble?	Yes	No	DK
28.	Have you ever had liver trouble?	Yes	No	DK
29.	Do you have frequent diarrhea?	Yes	No	DK
30.	Has a physician ever told you that you had ulcers?	Yes	No	DK
31.	Are there any foods you cannot eat?	Yes	No	DK
32.	Have you gained or lost weight recently?	Yes	No	DK
33.	Have you ever been jaundiced?	Yes	No	DK

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RESPIRATORY

34. Have you ever coughed up blood? Yes No DK

35. Do you have asthma? Yes No DK

36. Have you ever had tuberculosis? Yes No DK

37. Have you ever lived with anyone who had TB? Yes No DK

GENITO-URINARY

38. Are you thirsty much of the time? Yes No DK

39. Did a physician ever say that you had kidney or bladder trouble? Yes No DK

40. Do you have to get up every night to urinate? Yes No DK

ENDOCRINE SYSTEM

41. Have you ever had diabetes? Yes No DK

42. Have you ever taken thyroid tablets? Yes No DK

43. Do you get tired easily? Yes No DK

NERVOUS SYSTEM

44. Have you ever had a nervous breakdown? Yes No DK

45. Has a physician ever told you that you had epilepsy? Yes No DK

46. Do you consider yourself a nervous person? Yes No DK

SKIN

47. Have you ever been treated for a skin disease? Yes No DK

48. Do cuts on you skin usually stay open a long time? Yes No DK

49. Have you ever had hives or skin rash? Yes No DK

BONES AND JOINTS

50. Are your joints often painfully swollen? Yes No DK

51. Have you ever had more than one fracture? Yes No DK

52. Have you ever had more than one dislocation? Yes No DK

53. Do you have arthritis or rheumatism? Yes No DK

Diet Study
Page 4

Subject's Signature Date

Please explain any "Yes" answers:

APPENDIX 5
Consent to Investigational Treatment

THE OHIO STATE UNIVERSITY Protocol No. _____

CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE

I, _____, hereby authorize or direct Dr. Gordon Wardlaw associates or assistants of his choosing, to perform the following treatment or procedure (describe in general terms), Approximately weekly blood draws from an arm vein of 20 ml per week (about 1 ounce) on 8 occasions and 40 ml (about 1 1/2 ounces) on up to four occasions for a total of 320 ml (10 ounces, which is 1 1/4 cup); feed a nutritionally adequate diet for 9 weeks; which matches the typical American diet or is high in an experimental fat, called a structured fat. I will be randomly assigned using a flip of a coin to either diet.

_____ upon _____.
(myself or name of subject)

The experimental (research) portion of the treatment or procedure is: Use of blood samples and special diets containing palm oil and possibly a structured fat (if placed in that group) to see how blood cholesterol and other blood fats change as the type of fat in the diet changes. Again, I will be randomly assigned using a flip of a coin to a diet Neither I nor the researchers will know which group I am in until the study ends. The researchers will also not know which group I am in until that time. A third party will keep the record of who is in which group.

This is done as part of an investigation entitled: The relative effects of diets containing a structured triglyceride or palm oil/palm kernel oil on serum lipids and apolipoproteins in men.

1. Purpose of the procedure or treatment: To determine if the structured fat can lower blood cholesterol levels in comparison to palm oil. The palm oil diet should allow my cholesterol level to remain about the same (+/- 10% of current level).

2. Possible appropriate alternative procedures or treatment (not to participate in the study is always an option):
Not to participate. Neither participation nor non-participation in and of itself will affect my grades.

3. Discomforts and risks reasonably to be expected: 1) social limitations from eating most meals in the metabolic kitchen in the Division of Medical Dietetics, 1583 Perry Street, 2) fainting, bruising, infection, and pain from the weekly blood drawing are a possibility, 3) limitation of the use of medications to as little as possible during the study, 4) intestinal gas from meals is a possibility.

4. Possible benefits for subjects/society: I will learn about my usual blood cholesterol values, be paid $400, and receive free meals. Society will see if the structured fat can reduce blood cholesterol levels.

5. Anticipated duration of subject's participation (including number of visits:
9 weeks of diet study which includes daily visits to the study site.

I hereby acknowledge that _____ has provided information about the procedure described above, about my rights as a subject, and he/she answered all questions to my satisfaction. I understand that I may contact him/her at Phone No. 292-8142 should I have additional questions. He/She has explained the risks described above and I understand them; he/she has also offered to explain all possible risks or complications.

I understand that, where appropriate, the U.S. Food and Drug Administration may inspect records pertaining to this study. I understand further that records obtained during my participation in this study that may contain my name or other personal identifiers may be made available to the sponsor of this study. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the project director without prejudicing future care. No guarantee has been given to me concerning this treatment or procedure.

In the unlikely event of injury resulting from participation in this study, I understand that immediate medical treatment is available at University Hospital of The Ohio State University. I also understand that the costs of such treatment will be at my expense and that financial compensation is not available. Questions about this should be directed to the Human Subject Review Office at 292-9046.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date: _____ Time _____ AM PM Signed _____
(Subject)

Witness(es) if Required _____

_____
(Person Authorized to Consent for Subject, If Required)

I certify that I have personally completed all blanks in this form and explained them to the subject or his/her representative before requesting the subject or his/her representative to sign it.

Signed: _____
(Signature of Project Director or his/her Authorized Representative)

HS-028A (Rev. 4/89)

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