THE EFFECT OF MaxEPA™ ON SERUM CHOLESTEROL, HIGH DENSITY LIPOPROTEIN CHOLESTEROL, AND BLEEDING TIME, IN PATIENTS WITH CORONARY ARTERY DISEASE

By

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

The number one cause of death in the United States today is cardiovascular disease (CVD). This disease claims more than 500,000 lives annually, and is debilitating to another 500,000 (45). In addition to the many lives lost to atherosclerosis, it should be noted that a great deal of money and productive work time are lost to the disease as well. It is estimated that the cost of heart disease alone is equal to one-fifth of the Gross National Product (45).

Many risk factors have been associated with the development of atherosclerosis. Risk factors are categorized as non-modifiable and modifiable (15). Non-modifiable risk factors, those that can not be significantly altered through lifestyle changes, are; (1) genetic predisposition towards the disease, (2) age, and (3) gender. Risk factors that are modifiable through lifestyle changes are; (1) smoking, (2) stress, (3) maintaining ideal body weight, and (4) exercise. Two additional risk factors which play an important role in the propensity for developing coronary heart disease (CHD), which can be included in both categories, are hypertension and increased cholesterol levels in the blood. These are affected by lifestyle, but

can be dependent on genetic predisposition as well.

Physicians and health care professionals have long advocated that the fat content in the diet be decreased (29). Diets high in fat are a part of the American way of life. It is estimated that in the average diet 40% of the total kilocalories are derived from fat, 30% from carbohydrates, and 30% from protein (54). In contrast, the American Heart Association has recommended that these levels be changed to 30-58-12, respectively (1). For those patients that have already developed atherosclerotic lesions the recommendation is even lower, 20% total fat. It is easy to see why Americans gain an average of a pound to a pound and a half a year from the ages of 20 to 40. Fat contains 9 kcal. per gram while carbohydrates and proteins contain 4.5 kcal. per gram (8).

Consuming a diet high in fat, and particularly high in saturated fat, activates the body's mechanism to produce cholesterol (1,54). These high levels of cholesterol in the blood (hypercholesterolemia) cause the build up of atherosclerotic plaque in the intima of arteries, primarily in the heart, brain, and peripheral vessels (54). This build up continues over a period of time until the vessel becomes completely occluded, usually as a result of thrombus formation. When this occurs, distal blood flow from the lesion is compromised, and an infarction can occur. There is increased emphasis on the causal connection between hyperlipidemia and the development of CHD (22,26,29,41).

Although the clinical range of values for total cholesterol is from 100-300 mg./dl., the Center for Disease Control, as well as the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) and the National Heart Lung and Blood Institutes (NHLB) are now recommending that values do not exceed 200 mg./dl. for those over 40 years of age, and 180 mg./dl. for those under age 40 (2,39). Perhaps even more important in the prevention of CHD is the measurement of the levels of lipoproteins, the cholesterol-carrying molecules in the blood. Some authors consider these lipoproteins a more accurate predictor for the development of atherosclerosis than total cholesterol alone (10,11,26).

Given that there is a definite causal connection between the development of hyperlipidemia and CHD, how can this disorder be reversed? At the outset it is essential to accurately measure blood cholesterol levels in the general population. After individuals with adverse blood lipids are identified, there should be specific and uniform recommendations made on how to reduce these elevated levels (2).

The current thought is to initially recommend a diet low in fat (30%) and particularly low in saturated fat, with no more than a two to one ratio of polyunsaturated to saturated fat advocated (45). After having followed this stringent dietary regimen for a period of 3 months the hyperlipidemic patient should be retested. If the condition persists, the physician may choose to place the patient on

anti-hyperlipidemic drug therapy (22).

Although the above treatments are widely recognized as acceptable medical practice, a third alternative is possible. Many studies have demonstrated that decreased blood cholesterol levels can be achieved by supplementing the regular diet with increased amounts of eicosapentanoic acid (EPA) (28,36,37,40,42). This observation was first made among Greenlandic Eskimos who ingest a diet similar to those of carnivorous mammals, i.e., very little carbohydrate and high fat and protein. Despite the fact that this dietary regimen is contrary to the current recommendation, very low cholesterol levels and a low incidence of coronary heart disease are reported for this group (4-6,17-20).

This evidence strongly supports the idea that a third treatment may be possible. For instance, if supplementing the normal diet with fish oil (marine lipids having a high concentration of EPA and DHA, omega-3 fatty acids) produces the desired effects of lowering cholesterol levels, it may be possible to not only decrease cholesterol levels, but also to do so in a manner that is not disruptive to the individual's normal living patterns.

Additional research is warranted in this area as specific mechanisms of action, dosage levels, as well as over-all effectiveness in different populations are not clearly understood. Therefore, this investigation is intended to critically evaluate the effect of MaxEPA (tm) (a commercially prepared fish oil) on serum cholesterol, high

density lipoprotein cholesterol, and bleeding time in patients with a history of coronary heart disease. If the results of the investigation support the hypothesis that fish oils are beneficial, then it can be concluded that a low cost means for controlling lipid levels and thereby decreasing the incidence of CHD will have been found.

Statement of the Problem

This study investigated the effect of MaxEPA (tm) on serum cholesterol, high density lipoprotein cholesterol, and bleeding time in adult patients with hyperlipidemia and coronary heart disease.

Hypotheses

1. There will be no significant change in the total serum cholesterol level after dietary supplementation of 10 MaxEPA (tm) capsules (1.8 g.EPA) daily for a three week period.
2. There will be no significant change in high density lipoprotein cholesterol (HDL/C) after dietary supplementation of 10 MaxEPA (tm) capsules (1.8 g.EPA) daily for a three week period.
3. There will be no significant change in bleeding time after dietary supplementation of 10 MaxEPA (tm) capsules (1.8 g.EPA) daily for a three week period.

Limitations of the Study

1. All subjects were volunteers with documented histories of coronary artery disease.
2. No medications (including aspirin) were discontinued during testing.
3. The placebo used was an olive oil based placebo in capsule form furnished by the R. P. Shearer Co.

Delimitations of the Study

1. The subjects were limited to 9 males and 2 females chosen from a group of patients that had undergone cardiac rehabilitation at the Oklahoma State University Health and Fitness Center.
2. All subjects were hypercholesterolemic, i.e., had total serum cholesterol levels of 200 mg./dl. or above, or a total cholesterol to high density lipoprotein-cholesterol of 5:1 or greater.
3. Total serum cholesterol levels were measured using the Vision (tm) automated blood analyzer manufactured by Abbott Laboratories.
4. HDL/cholesterol was measured via the Sigma enzymatic reaction method and measured in the Vision (tm) automated blood analyzer.
6. Bleeding time was measured via Duke's method. A uniform puncture depth was accomplished by using the Autolet (tm) lancet.

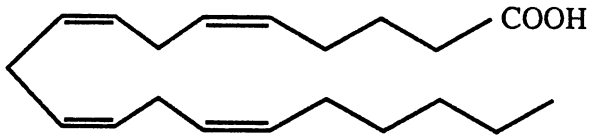
Assumptions

1. High density lipoprotein cholesterol (HDL/C) being considered "good" cholesterol, the remainder of total not being HDL/C is then, LDL/C, and VLDL/C, or "bad" cholesterol.
2. It is assumed that the protocol for ingestion of capsules, as well as adherence to dietary and exercise regimes, was maintained.

Definition of Terms

Conceptual

Arachidonic Acid (AA). (eicosatetraenoic acid, C20:4w-6) Used in the vessel wall and by platelets, the precurser to thromboxane A2 and prostaglandin I2. (4)



Atherosclerosis. A form of arteriosclerosis in which there are localized accumulations of lipid containing material (atheromas) within or beneath the intimal layer of blood vessels. (54)

Atherosclerotic Heart Disease. Atherosclerosis affecting the coronary arteries.

Atherosclerotic Lesion. An area of pathologically injured tissue due to the build up of cholesterol placque in the intima of a vessel.

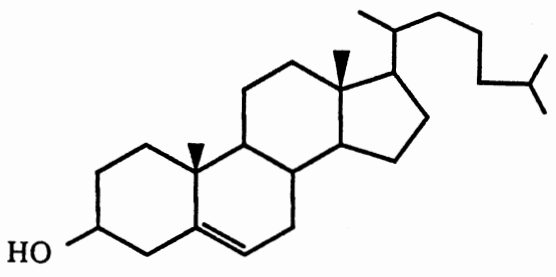
Bleeding Time. The time required for blood to stop flowing from a small wound. Normal bleeding time using Duke's method is 1-3 minutes. (56)

Carbohydrate. Foods containing only carbon combined with hydrogen and oxygen, in the ratio of two hydrogens for each carbon and oxygen, such as sugars, starch, and cellulose. (56)

Coronary Artery Disease. Atherosclerotic lesions affecting one or more of the coronary arteries. (57)

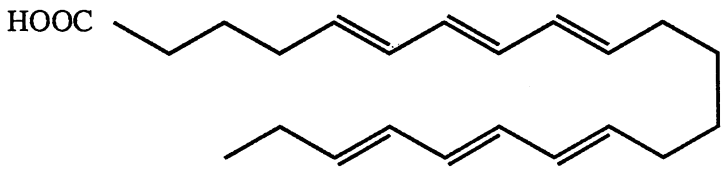
Cardiovascular Disease (CVD). All diseases affecting the cardiovascular system including coronary heart disease, atherosclerosis, high blood pressure, stroke, rheumatic fever, rheumatic heart disease. (57)

Cholesterol ($C_{27}H_{45}OH$). A sterol widely distributed in animal tissues and occurring in the yolk of eggs, various oils, fats, and nerve tissue. (54, 57)

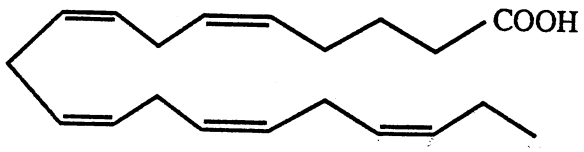


Chylomicron. The largest and lightest lipoproteins consisting of 80-90% exogenous triglyceride, 2-7% cholesterol, 3-6% phospholipid, and 1-2% protein. They are normally synthesized in the intestine and transport dietary triglyceride from there into the plasma. (54)

Docosahexaenoic Acid (DHA). (C22:6) An Omega-3 fatty acid the with first double bond 3 carbons away from the terminal CH3. (4-6)



Eicosapentanoic Acid (EPA). (C20:5) An Omega-3 fatty acid with the first double bond 3 carbons away from the terminal CH3. The major constituent in fish oil and considered the active structure. (4-6)

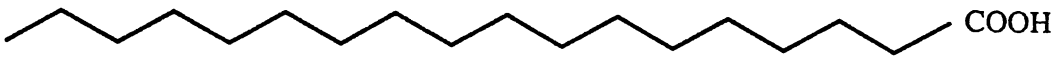


Erythrocyte. A red blood cell. (57)

Esterification. An organic reaction between an alcohol and an organic acid, such as when a fatty acid reacts with a cholesterol molecule. (55)

Fat. Triglyceride ester of fatty acids. The term lipid is applied in general to a fat or fatlike substance. Fats are insoluble in water. (54,57).

Fatty Acid. Usually found in natural triglygerides in foods and in human lipids and consisting of an even number of carbon atoms arranged in a straight chain-with a carboxyl group at one end. They may be saturated or unsaturated. (54)



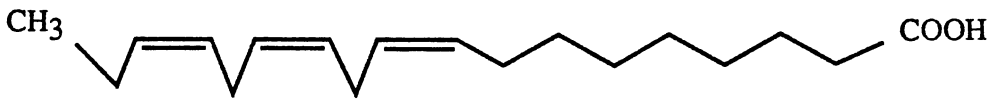
Granulocyte. A granular leukocyte. A polymorphonuclear leukocyte (neutrophil, eosinophil, or basophil). (57)

Herterozygous. Having unlike genes. In familial hypercholesterolemia having one affected gene. (57)

High Density Lipoprotein Cholesterol (HDL/C). The smallest of the lipoproteins, contains approximately 45-50% protein, 30% phospholipid, and 20% cholesterol and is a normal component of fasting plasma. The so-called "good cholesterol" due to its ability to act as an atherosclerotic placque scavenger in the circulatory system. (54)

Homozygous. Produced by similar gametes (a mature reproductive cell). In familial hypercholesterolemia having affected genes from both parents. (57)

Linoleic Acid. (C:18w6) A long chain fatty acid with the first double bond occurring nine carbons from the terminal COOH. The primary constituent of polyunsaturated vegetable oil. (36)



Lipids. Any one of a group of fats or fatlike substances, characterized by their insolubility in water. Most often, somewhere in the molecular structure of lipids is a fatty acid or a fatty acid derivative. Lipids include triglycerides, phospholipids, sterols and sterol esters, glycolipids and lipoproteins. (54, 57)

Lipoprotein. The carrier protein for lipids. The cholesterol lipoproteins are, HDL, LDL, VLDL, and chylmicrons. (57)

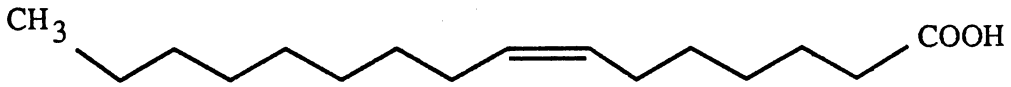
Low Density Lipoprotein (LDL). Partially or completely resulting from the metabolism of VLDL. It is about 50% cholesterol and 25% protein by weight and normally carries one half to two thirds of the total plasma cholesterol. The so-called "bad cholesterol" because of its greater athrogenic potential. LDL is slowly removed by the liver (2-5 days) and is what is mostly measured in a total cholesterol blood test. (57)

Lymphocyte. White blood cell without cytoplasmic granules. Normally 20-50% of the white cells. (57)

MaxEPA (tm). A commercially prepared food supplement of triglyceride marine lipids containing EPA (180 mg./gram capsule), DHA (120 mg./gram capsule), < 1 gram protein, < 1 gram carbohydrate, 15 mg. cholesterol, and 7 % vitamin E.

Monocyte. A large mononuclear leukocyte having more protoplasm than a lymphocyte. (57)

Monosaturated Fats (oleic acid). (C18:1,w-9) Having the maximum number of hydrogen atoms attached to only one carbon. A example of a monosaturated fat that is rich in oleic acid is commonly known as olive oil. (28)

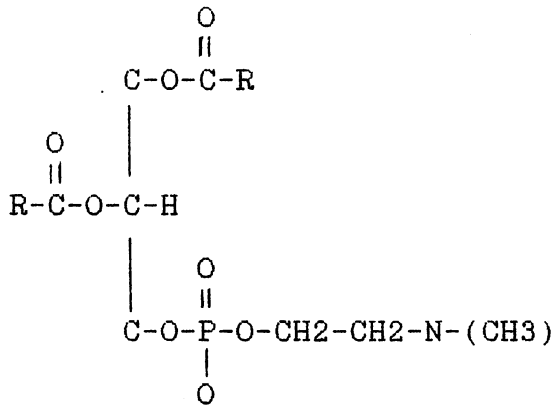


Omega-3 fatty acid (w-3 FA). A class of fatty acids so named because the first double bond is located 3 carbon atoms away from the terminal CH3. (4)

PgI2 (prostaglandin I2). A prostacyclin synthesized in the vessel wall from arachidonic acid having anti-aggregating effects on platelets. (4)

PGI3 (prostaglandin I3). A prostacyclin synthesized in the vessel wall from eicosapentanoic acid having anti-aggregating effects on platelets. (4)

Phospholipids. Usually derived from a glycerol. They contain two fatty acids, at least one of which is unsaturated, phosphate and a nitrogen base, most frequently choline. Since triglycerides are fat soluble and enzymes are water soluble, in order for enzyme-substrate interaction to take place, neutral fats (triglycerides) must be emulsified. Phospholipids in tissue and foods can act as emulsifying agents which aid in digestion of fats. (54)



(Lecithin, a phospholipid)

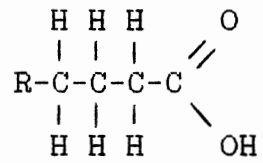
Plasma. The liquid portion of blood. (57)

Platelet. A round or oval disk found in blood. Important in blood coagulation, hemostasis, and blood thrombus formation. (57)

Platelet Aggregation. The ability of platelets to form clusters or stick to each other. (57)

Prostaglandins. A group of fatty acid derivatives present in many tissues. There are more than a dozen prostaglandins. They are extremely active biological substances which affect the cardiovascular system, smooth muscle, and stimulate the uterus to contract. (54)

Saturated Fat (fatty acid). (Cn H2n O2) A fat that is a solid at room temperature. Except for the terminal carboxyl group, both available bonds for each carbon in the chain are occupied by hydrogens. (8)



Serum. The watery portion of blood remaining after a clot has formed. (57)

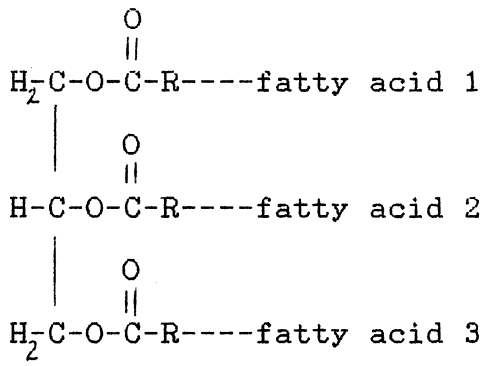
Thromboxane A2 (TxA2). Derived by platelets from arachidonic acid. A pro-aggregating agent. (4)

Thromboxane A3 (TxA3). Derived by platelets from eicosapentanoic acid. Has no aggregating properties. (4)

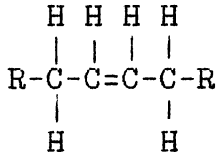
Thromboxane B2 (TxB2). A metabolite of TxA2 and excreted in the urine. (50)

Thromboxane B3 (TxB3). A metabolite of TxB3 and excreted in the urine. (50)

Triglyceride. Also called neutral fats, are fatty acid esters of glycerol. The physical state of the triglycerides depends on the chain length and degree of unsaturation of their fatty acid components-the more unsaturated the fatty acids and the shorter the chain length, the lower the melting point of the fat. A component of lipoproteins and highly associated with VLDL. (57)



Unsaturated Fat (fatty acids). A liquid at room temperature. One hydrogen atom is missing from each of two adjacent carbons, which then share a double bond. (8)



Very Low Density Lipoprotein (VLDL). The second lightest lipoprotein next to the chylomicrons. They are glyceride-rich (60-80%). They carry endogenous triglycerides (which appear to originate predominantly in the liver) from the liver to peripheral sites in muscle and adipose tissue. Once in the plasma, VLDL are quickly attacked by the same lipases that act on chylomicrons and in a few hours become an intermediate lipoprotein form divested of much triglyceride and some protein. Within 2-6 hours it is further depleted of triglyceride and becomes LDL. (54)

Xanthoma. A slightly elevated, soft rounded placque or nodule, usually on the eyelids. A characteristic finding in familial hypercholesterolemia. (54)

Functional

Hypercholesterolemic Subject. A subject having a total cholesterol greater than 200 mg./dl.

Subject with Coronary Heart Disease. A subject that has experienced a myocardial infarcton, coronary artery bypass graft surgery, or coronary balloon angiography.

Description of Instruments

Vision (tm) Automated Blood Analyzer. An instrument used to measure different components of the blood. A self contained wet-chemistry blood analyzer. This instrument is fully automated.

Spring Actuated Lancet. A lancet attached to a mechanical device that punctures the skin to a uniform depth.

IEC Clinical Centrifuge. A variable speed (1,500 to 2,000 revolutions per minute), table top centrifuge.

CHAPTER II

A SELECTED REVIEW OF LITERATURE

As early as 1971 Bang and Dreyerberg noted that Greenlandic Eskimos had a low rate of mortality from coronary heart disease (4). They tested the blood of 130 Eskimos for total cholesterol, triglycerides, phospholipids, and lipoproteins (VLDL, LDL, HDL, and chylomicrons) and the results were compared to a control group comprised of healthy Danes and Eskimos living in Denmark. Greenlandic Eskimos were found to have a much lower level of pre-beta-lipoprotein (VLDL), and consequently a lower triglyceride level. The fact that low mortality rates for the Greenlandic Eskimos correlated with low triglyceride levels coincided with the idea prevalent at the time that high triglyceride levels were thought to have a direct causal effect on the development of CHD (32).

In 1978 Bang and Dryerberg reported the results of another study conducted on similar populations in which a correlation was found to exist between the lower incidence of coronary events for Greenlandic Eskimos and increased bleeding times (4). It was believed that this finding might have a bearing on the frequency of CHD development which they sought to explain.

From the first study the Eskimo diet was determined to be very high in fat (280 gm./day) and protein (135 gm./day), low in carbohydrates, since their primary food was fish. Ironically, this was not consistent with what was known about diet and coronary heart disease, however, it was hypothesized that diet was probably the key consideration. It should be noted that neither genetic predisposition, nor exercise were regarded at this point in their investigations. Additionally, they alluded to a possible role for the recently discovered compounds called prostaglandins which appeared to have many functions within the human body (4).

Prostaglandins are hormone like compounds, synthesized in various tissues from arachidonic acid (C20:4w-6) (AA). Among their many functions they are known to regulate reactions in the inhibition of gastric secretion, decrease blood pressure, and interact with other hormones (6). Bang and Dryerberg thought that these new compounds might contain the key to confirming a causal relationship between the lower lipids levels, longer bleeding times, and decreased CHD mortality (4).

Bang and Dryerberg found that one of the major components of the fish in the Eskimo diet was eicosapentanoic acid (C20:5w-3) (EPA), an omega-3 polyunsaturated fatty acid, which they proposed successfully decreases platelet aggregation and thereby increases

bleeding times (5,6). In order to do this, EPA (present in quantity) must compete against arachidonic acid in its reactions to produce thromboxane A2 (TxA2), a pro aggregating substance that also causes vasoconstriction. EPA, using the same enzyme, cyclooxygenase, used by AA, reacts to produce the biologically inert thromboxane A3 (TxA3). Both thromboxanes in further reactions produce prostacyclins PGI2 and PGI3 respectively, both of which are vasodilators and platelet aggregation inhibitors. So the one step of value in the EPA reactions is the elimination of TxA2 the vasoconstrictor and platelet aggregator, therefore increasing bleeding times (4).

Bang and Dryerberg proceeded then to measure the percentage of total fatty acids in the lipid fractions of blood in the Eskimos and Danes (4). Greater percentages of EPA were indeed found in the Eskimo lipids than in the Danes as well as lower plasma cholesterol and triglyceride levels. These findings were so significant that a separate study based upon the same arguments was reported in the 1979 Lancet (5).

Investigations into the effects of fish-oil continued through the early 1980's (24,35,50,58). Recognition was eventually given to the work by the Editorial board of the The New England Journal of Medicine, when a section of three articles devoted to the promotion of the hypothesis that an increase in dietary fish consumption could decrease the

incidence of CHD appeared in the May 1985 issue (40,42,46).

In one of these, a study of the inverse relationship of mortality from CHD in those that consumed fish over those that did not, left little doubt a diet high in marine lipids could decrease the number of deaths attributable to CHD (42). The two remaining articles addressed the problems associated with the identification of the process or processes by which fish oil affected the metabolism. Results from the work of Phillipson et al. (46) favored the changes that marine lipids had on triglyceride metabolism, while Lee et al. (40), suggested that a diet containing fish-oil polyunsaturated fatty acids altered the function of monocytes and their interaction with the endothelium.

It was clearly the empirical view of these authors that an increased dietary consumption of fish-oil (EPA) would decrease the incidence of CHD by decreasing cholesterol and triglyceride levels, increasing HDL, and increasing bleeding time (23). It was also evident that more research was needed in order to fully understand what mechanism was involved and what dosage regimen was needed to produce the desired effects.

At this point in time, there is little doubt that a decrease in cholesterol will decrease the incidence of CHD (10,11,26,41). In fact, it is noted that for each 1% reduction in cholesterol, there is a corresponding 2% decrease in CHD mortality (39,48). So of the possible

effects of EPA in the Eskimo diet, the one of primary significance may be the lowering of total cholesterol. Most studies in which the Eskimo diet has been simulated by supplementing the normal diet with fish-oils have provided evidence that a reduction in total cholesterol occurs (4-6,44,52). The results are encouraging enough to stimulate additional research.

Decreasing the dietary intake of total fat in addition to increasing the amount of polyunsaturated fat are but two ways in which the incidence of CHD can be decreased. Weiner et al. (60) proposed four ways in which atherosclerosis is initiated and perpetuated: (1) monocyte adherence and migration; (2) endothelial injury; (3) platelet aggregation and the release of platelet secretory products; and (4) lipid accumulation. Prostaglandin metabolism may be important in many or all of these processes and modifying prostaglandin metabolism may influence the development and progression of atherosclerosis. It has been proposed that platelet membranes rich in EPA alter prostaglandin synthesis and metabolism, thereby EPA may indirectly influence the development of CHD.

Weiner et al. (60) found that feeding hyperlipidemic swine a diet rich in cod-liver oil (a marine lipid rich in EPA) decreased the development of atherosclerotic lesions. The proposed reason for this finding was in the decrease of platelet arachidonic acid and increase of platelet

eicosapentanoic acid thereby altering the prostaglandin metabolism. Through this process, platelet aggregation was decreased as well as lipid accumulation, and therefore decreases in atherosclerotic lesions were noted.

Lee et al. (40) noted that a diet high in polyunsaturated fatty acids altered the function of monocytes. In the early stages of hypercholesterolemia, monocytes adhere to the atrial endothelium then migrate to the intima. They are then transformed in macrophages, acting not only as lipoprotein and cholesterol lesion scavengers, but also they may release certain chemicals which are growth factors stimulating the proliferation of arterial smooth muscle (40). Monocytes containing increased amounts of EPA may decrease the amount of leukotriene B4 produced. Leukotrienes are the product of arachidonic acid via the 5-lipooxygenase pathway. Leukotriene B4 has been shown to aid in the adhesion of monocytes to the vessel walls, therefore, as EPA increases within the cell membrane less leukotriene B4 is produced and monocytes are less adhesive (40).

Although most research studies have found EPA to be beneficial in raising platelet EPA, decreasing cholesterol and triglyceride levels and increasing HDL cholesterol, some authors have not found this to be true (27,49). This finding however appears to be related to at least four reasons: (1) the dosage of EPA administered; (2) the

duration of therapy; (3) the population sampled; and finally, (4) the type of placebo administered.

Sanders et al. (50) found no change in total cholesterol in 10 healthy subjects, however, the duration of administration was only two weeks and the placebo used was a mixture of 10 g./day of olive oil and vegetable oil. Both olive oil, a monosaturated fatty acid, and vegetable oil, a polyunsaturated fatty acid have been found to decrease plasma cholesterol and triglyceride levels (28,30). Therefore, it is possible that there was not a significant difference in the treatment and control groups lipid levels because the control group placebo also lowered lipid levels.

Green et al. (27) found no change in total cholesterol, HDL cholesterol, or bleeding time in 11 stroke patients after having administered 10 capsules of MaxEPA (1.8 grams EPA daily) for a period of six weeks. Again the major constituent of the placebo was olive oil. It is possible that the mechanism for cholesterol synthesis and metabolism in patients with documented cardiovascular disease may be different from that of healthy subjects. It is known that hypercholesterolemia is a familial disease process and exists as a result of a heterozygous or homozygous gene (53). The heterozygous type is the least severe, however each type exhibits distinct clinical manifestations, the most severe of which is the early development of cardiovascular disease.

Familial hypercholesterolemia (FH) has been classified according to different phenotypes: Type I (excessive chylomicrons); Type IIa (increased LDL with normal VLDL); Type IIb (increased LDL and VLDL); Type III (increased intermediate lipoproteins); Type IV (increased VLDL); and Type V (increased VLDL and chylomicrons).

Phillipson et al. (46) administered three different diets to 10 subjects with Type IIb (mean total cholesterol (TC) 337 mg./dl. and mean triglycerides 335 mg./dl.) and 10 subjects with Type V (mean TC 514 mg./dl. and mean triglycerides 2874 mg./dl.). The diets consisted of a control diet (typical low-fat, polyunsaturated to saturated fat ratio [P:S] of 1:4), a diet high in fish-oil (20-30% kcal. from fish-oil), and a vegetable oil diet (20-30% kcal. from vegetable oil). The Type IIb group had reductions in both plasma cholesterol and triglyceride levels of 27% and 64% respectively when fed the fish-oil diet. The Type V group had even greater decreases of 45% and 79% respectively. The vegetable oil diet produced decreases in the lipid levels, however they were not as significant (46).

Because of the differences between these groups it has been hypothesized that the mechanism for lipid metabolism is different for different types of familial hypercholesterolemia. Cuthbert et al. (14) attempted to test for these effects by administering the drug mevinolin to subjects with different phenotypes. Mevinolin disrupts

the synthesis of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), the necessary enzyme in LDL metabolism. It was found that those subjects with the heterozygous form of FH could benefit most from the administration of mevinolin. Thus the theory that different phenotypes respond at different levels to treatment was borne out.

The dosage of EPA required to produce the desired effects, as well as the duration of administration, has been a point of controversy. The true Eskimo diet contained a daily amount of 7 grams of omega-3 fatty acids.* In the various investigations, as much as 10 g./day (36) and as little as 1.1 g./day have been used (53). Similarly broad limits to the length of administration have been used, as little as 2 weeks on the one hand (50), and as much as 2 years on the other (47). Peak effectiveness appears to lie in dosage levels of 2.9 g./day and above and in treatment intervals of a minimum of three weeks. However, in those studies in which there was a decrease in lipid levels following administration of EPA, lipid levels returned to pre-treatment levels soon after administration was discontinued.

It is evident that additional research is needed with respect to the choice of controls, the population effects,

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* Taken from the Sept. 26, 1985 issue of The New England Journal of Medicine, Letters to the Editor, pp.823.

dosage levels, and length of therapy employed in the administration of EPA.

Lipids and Lipid Metabolism

In order to fully understand cholesterol, its importance to the body, and how it could be altered by the administration of dietary fish-oil, it is necessary to have an understanding of lipids in general, and the chemical reactions taking place.

Cholesterol

Cholesterol is a principal member of a class of organic compounds known as steroids, however it is often classified as a lipid because of its insolubility in water (55). Cholesterol is present in all animal tissue and is synthesized primarily in the liver and intestine (8). If synthesized within the body it is described as endogenous, and exogenous if it is ingested as part of the diet. The rate of total cholesterol absorbed by the body is approximately 1 gram per 24 hour period. Of that the exogenous cholesterol intake averages approximately 600 mg. per day, 300 mg. of which is the maximum which can be absorbed. Approximately 1 gram of sterol is excreted daily in the feces. The net result is that an approximate steady state for cholesterol exists in the healthy body (54).

Cholesterol is essential to the body for vitamin D

synthesis, hormone production, cell membrane formation, and it is the precurser to cholic acid produced as bile salts which aid in digestion (54,8). Cholesterol is so vital to cellular function that cells will retain cholesterol at the expense of other needed compounds.

Lipids

In broad terms the word lipid is used to describe a variety of biologically important molecules which are water insoluble. Included in these groups are the triglycerides, phospholipids, steroid and steroid esters, glycolipids, and lipoproteins (54). Fatty acids form the skeleton of the lipid molecule. They generally consist of an even number of carbon atoms with a carboxylic acid group at one end. Often the acid group has reacted with an alcohol to form an ester. These fatty acids may be saturated, monosaturated or polyunsaturated. If saturated, all the carbon-carbon bonds are single. If monosaturated, one carbon-carbon double bond is present, and if polyunsaturated, two or more double bonds are present. The majority of the cholesterol in the body is esterified by reacting with a fatty acid, e.g., linoleic acid to give cholesteryl linoleate (8,54,55).

Fatty Acids

Fatty acids are used by the body for energy production (oxidation) and for energy-consuming processes (reduction).

Fatty acids are available in the liver where they originate from synthetic processes involving excess dietary triglyceride, hydrolysis of chylomicron triglycerides, and through the mobilization of fatty acids from adipose tissue (54).

Some fatty acids are known as essential fatty acids because they can not be manufactured by the body and are required in some way for the metabolism of cholesterol and other lipids. Without them abnormal accumulations of cholesterol and triglycerides form in the liver. Essential fatty acids are also the precursers to the prostaglandins. Among the most important of these are linoleic acid and arachidonic acid. Linoleic is the most abundant of the fatty acids that combine with cholesterol to form cholesteryl esters. Fatty acids are classified according to the number of carbon atoms in their chain (8,54).

Triglycerides

Triglycerides (true fats) are formed by combining three fatty acid molecules (usually different from one another) with a single glycerol molecule to form a tri-ester. Triglycerides are identified by the fatty acid component. The shorter the fatty acid chain, the lower the melting point of the fat. Triglycerides are the principle lipids of foodstuffs, as well as adipose tissue. They are well suited in the role of energy production as 9 kcal of energy is

yielded per gram of fat. In addition, these fats are non-polar and can be stored efficiently within a cell (8,54).

Phospholipids

Contained within the phospholipid molecule is glycerol, two fatty acids (one saturated) which form carboxylate esters, one phosphate ester and a nitrogen base. Phospholipids are present in food and in tissue and act as emulsifying agents which help digest food by breaking down fats (emulsifying) into colloidal particles for easier transport. Because triglycerides are water insoluble and the enzymes needed for these reactions are water soluble, the triglycerides must first be finely dispersed (emulsified) with the aid of phospholipids for more intimate mixing before the metabolic reactions can occur (54).

Lipoproteins

Lipoproteins are long chain fatty acids with a protein molecule attached. Lipoproteins are classified as chylomicrons (the largest and lightest), very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). They are critical to the transport process for insoluble materials in a subtle three-tier system. The water soluble proteins bond with the water insoluble lipid to form lipoproteins. Then the cholesterol and other lipids associate with this complex to be

transported to the cells where they are needed (54).

Chylomicrons

The chylomicrons contain 80 to 95% exogenous triglyceride, 2 to 7% cholesterol, 3 to 6% phospholipid and 1 to 2% protein. These molecules are synthesized in the intestine and carry most of the dietary cholesterol to the plasma where the fatty acids and glycerols are broken away and either used or stored by the cells. The remnants are cleared by the liver (54).

VLDL

VLDL contains 60-80% glyceride and carry endogenous (synthesized primarily in the liver) triglycerides. These triglycerides are transported from the liver to muscle and adipose tissue where it is either used or stored (54).

LDL

LDL originates at least partially from the VLDL. It is composed of about 50 to 75% cholesterol and 25% protein. The LDL remnants are very slowly removed from the liver (2-5 days), and because of the high concentration of cholesterol are thought to have the greatest athrogenic potential (54).

HDL

HDL contains about 50% protein, 30% phospholipid and 20% cholesterol and is the smallest of the lipoproteins. The exact function of HDL is not known, however, because it is composed of greater amounts of protein and phospholipids than cholesterol it is often termed "good" cholesterol (54). Some researchers also claim that HDL circulates in the blood stream acting as a "scavenger" for excess cholesterol remnants adhering to the arterial walls and carrying them back to the liver where they can be metabolized, formed into bile salts, or excreted in the feces (11,13).

This completes the discussion of the lipids and the particular interactions between them and cholesterol. If one focuses on the role of LDL as the principle culprit in the athrogenic process, one learns that considerable information is known about its function.

The LDL Receptor

Goldstein and Brown won the Nobel Prize in 1985 for their work with LDL and LDL receptors (9). The LDL receptors remove cholesterol from the vascular system by a process known as receptor mediated endocytosis. There are two types of lipoprotein receptors: (1) LDL receptors, those that bind the lipoproteins carrying endogenous cholesterol derived from non-intestinal sources; and (2) chylomicron remnant receptors, those that bind lipoproteins

containing exogenous cholesterol absorbed from the intestines.

LDL receptors are extremely important to cell metabolism and are present on the surface of all mammalian cells. They provide the cells with needed cholesterol for growth by mediating the uptake of plasma LDL. Most of the receptors are located in the liver where they are needed to supply cholesterol for bile secretion, for conversion to bile acids, and for resecretion into the blood stream to be transported by newly formed lipoproteins (9).

Located on the cell membrane are so-called "coated pits" that have the responsibility for regulating a number of receptor-bound molecules in the process of receptor-mediated endocytosis. The LDL binds to the receptor by attaching to a protein in the coated pit, then within a few minutes the pit invaginates into the cytoplasm releasing its cholesterol into the cell (9).

The process, like many life processes, is controlled enzymatically. There appear to be three mechanisms which control cholesterol levels in the cell. First, if the enzyme (HMG-CoA) is suppressed, the cell will preferentially use exogenous cholesterol derived from the receptor mediated uptake of LDL. However, if this too is not available, it will stimulate the cell to produce its own cholesterol. Secondly, an excess of endogenous cholesterol activates an enzyme that re-attaches a fatty acid to the excess

cholesterol to form an ester that can be stored in droplets in the cytoplasm. The third cholesterol regulating mechanism by the cells is called a "feedback" system and is perhaps the most important. Excess cholesterol inside the cell causes it to shut off the synthesis of any new receptors so that new cholesterol can not be taken into the cell. Through this mechanism the cell is able to regulate the intake of cholesterol. When cholesterol is needed, such as in times of growth, it can be taken in by some 40,000 receptors per cell. Then, when growth ceases, the receptor sites can be shut down to approximately 36,000. The discovery of the LDL receptor shed new light on familial hypercholesterolemia and the treatment thereof (9).

Although fats have many useful functions within cells, there are detrimental aspects of fats to be considered and these should be remembered when considering the beneficial effects purported to occur for EPA. An increased intake of saturated fats has been shown to increase serum cholesterol levels (54). Although the mechanism for this is not well understood, two theories have been proposed. It is possible that an increased consumption of saturated fatty acids decreases bile acid secretions resulting in less efficient metabolism of cholesterol and an increase in the build up of the sterol. Secondly, it has been proposed that increasing the ratio of polyunsaturated to saturated fatty acids in the diet transfers the amount of cholesterol from the vascular

system to the muscular tissues of the body (54). In addition, it is the hypothesis of this author that because the lipoprotein carriers are at least partially composed of lipids, as dietary intake of fats increases more of the lipoprotein carriers are occupied resulting in more cholesterol, triglycerides, and phospholipids being circulated throughout the body. It is also conceivable that more of the LDL receptors on the cells are engaged.

In reviewing the literature regarding the effects of the oral administration of fish-oil, it is the purpose of this work to conduct a study in which the dosage level, duration of therapy and population type are optimized in a controlled double blind study.

CHAPTER III

METHODS AND PROCEDURES

This study investigated the effects of MaxEPA (tm), a commercially prepared fish-oil (supplied by the R. P. Shearer Co., and Dr. Desmond Davies), on total cholesterol (TC), high density lipoprotein cholesterol (HDL/C), and bleeding time (BT). The methods and procedures were submitted to and approved by the Oklahoma State University, Institutional Review Board.

A total of eleven subjects participated in this cross over design study which lasted 6 weeks. The subjects were required to orally ingest 10 capsules daily of either MaxEPA (tm) or an olive oil placebo for the duration of the study. Blood samples were taken at the beginning, at cross over, and at the conclusion of the study. Standardized clinical methods were used for analyses.

Selection of Subjects

The 11 subjects in this study, 9 males and 2 females, were volunteers chosen from a group of subjects that had participated in the Cardiac Rehabilitation Program at the Oklahoma State University Health and Fitness Center. The subjects had documented CHD in the form of atherosclerotic

heart disease. The ages of the subjects ranged from 40 to 71 years (mean 58.5). In order to be selected for the study an individual's total cholesterol had to exceed 240 mg./dl, or the total cholesterol to high density lipoprotein ratio (TC/HDL) had to exceed 5:1, or both. Medical approval for the selection and permission to participate in the study were obtained for each subject by way of a telephone conversation with their personal physician before the program began.

The subjects were randomly assigned to one of two groups. Group I began the study by taking the placebo then crossed over to the treatment. Group II began by taking the treatment then crossed to the placebo.

Personal Data

Upon arrival at the Cardiac Rehabilitation laboratory, each subject was asked to fill out a brief health history (see Appendix A) which was updated from the personal history already on file since these subjects had been involved in the rehabilitation program in the past. Included on the form were questions regarding name, age, sex, address, telephone number, smoking history, alcohol consumption history, exercise history, family history of heart disease, personal history of heart disease, lipid levels, and current medications (see Appendix A).

Additionally, each subject was required to read and

sign an informed consent for the testing procedure and a disclaimer for bleeding disorders. The forms were thoroughly explained to the group by the investigator and questions were answered to the satisfaction of each of the subjects (see Appendix A).

All subjects were requested to write down on a diet diary (see Appendix A) the exact amounts and types of foods consumed on two week days and one weekend day and return it to the laboratory upon completion.

All the procedures were then carefully explained to the subjects, instructions given regarding how the capsules should be taken, what time of day they should be taken, and when the subjects should return for the next series of tests. The subjects were also reminded that normal exercise and diet regimens should not be altered while participating in the study.

For each of the test periods that followed, this same protocol was used.

Testing Procedures

All subjects reported to the Cardiac Rehabilitation laboratory having fasted for at least 12 hours previous to their arrival (they were instructed that they could drink only water, see Appendix A). Personal histories were collected, the proper forms completed (see Appendix A), and height and weight were measured and recorded.

At the outset, total cholesterol (TC), high density lipoprotein-cholesterol (HDL/C), and bleeding time (BT) were obtained on two consecutive days to determine a steady baseline for each. After the second day, the subjects were randomly assigned to one of two groups. One group of subjects was given a 21 day supply of an inert placebo and the other group the MaxEPA (tm) capsules. All of the capsules were bottled in the same manner and neither the investigator nor the subjects knew which treatment they were receiving, yielding a double blind design. The subjects were instructed to take by mouth, 10 MaxEPA (tm) capsules daily (1.8 g. EPA) for the prescribed period. The subjects were thoroughly briefed in regard to the protocol of the study and instructed to return on the proper date.

In 21 days the subjects returned to the Cardiac Rehabilitation laboratory, again having fasted for at least 12 hours. The same measurements (TC, HDL/C, BT) were obtained and recorded on two consecutive days. Weights were also measured and recorded to insure dietary compliance. After the second day of laboratory testing the subjects were given another 21 day supply of capsules. The placebo group was given the MaxEPA and the MaxEPA group was given the placebo. The subjects were again reminded of the proper protocol and instructed on when to return for final testing.

In another 21 days (42 days after initial screening) the subjects arrived back at the laboratory for the final

series of testing, again in a fasting state. The blood samples for TC, HDL/C, and BT were again obtained on two consecutive days, and body weights were measured and recorded. Another 3 day dietary recall was completed by each subject to further insure compliance to dietary instructions.

Clinical Laboratory Procedures

Total Cholesterol and HDL/Cholesterol

In order to determine the total cholesterol (TC) and HDL/Cholesterol (HDL/C) levels in the blood a venous blood sample was drawn from the brachial fossa of either the right or left arm of each of the subjects. Standard aseptic venipuncture technique was employed with the tourniquet being released prior to removal of the #21 gauge needle. All subjects were in the sitting position during venipuncture. Vacutainer (tm) red stoppered tubes (serum separation tubes, SST) were used in venous collection. These have a floating gel to aid in separation of the red cells from the serum. One tube per subject was collected (approximately 10 ml.).

All venous samples were allowed to stand at room temperature for a minimum of 30 minutes and a maximum of 1 hour 30 minutes until a clot was formed in the tube. The samples were then centrifuged at a speed of 5,000 revolutions per minute for five minutes in a table top

clinical centrifuge (International Equipment Co., model #428-17108, Needham, Ma.). The gel then separated the red cells from the serum. The supernatant serum was aspirated using a disposable transfer pipette and pinch bulb and transferred into a new glass test tube and stoppered. At this point the serum was ready to be analyzed.

Total cholesterol was analyzed in the Vision (tm) automated blood analyzer by placing two drops of each subject's serum into the Abbott Laboratories "cholesterol kits" (furnished by the Oklahoma State University, Health and Fitness Center) (3).

HDL cholesterol was analyzed using a manual separation technique in which magnesium phosphotungstate is added to precipitate low density lipid fractions (3). The HDL in the resultant supernate was analyzed in the same manner as described for cholesterol.

Both TC and HDL/C samples were analyzed within 4 hours of being collected. Prior to each set of measurements the Vision (tm) blood analyzer was calibrated with Abbott controls.

Bleeding Time

Bleeding time was determined using Duke's Method (59). A uniform puncture depth of 3 m.m. was accomplished by using the Autolet (tm) lancet. Bleeding time was measured in 30 second time intervals by blotting the puncture with a piece of filter paper. The wound was allowed to clot with no pressure being exerted on it. The same finger and the same site was used in each of the test periods to insure uniformity.

Apparatus and Equipment

The essential pieces of equipment used in this study were the Vision (tm) automated blood analyzer (Abbott Laboratories, Dept. 99F, North Chicago, Il., 60064), Abbott Laboratories dry chemistry cholesterol kits, the Autolet (tm) lancet, and the clinical centrifuge (International Equipment Co., 300 Second Ave, Needham Hts., Mass.)

Statistical Treatment

This study was placebo controlled, double blind, and employed a cross-over research design. In this design each subject was allowed to act as his own control (12,43).

The data were analyzed using the 2 x 2 repeated measures analysis of variance with a grouping factor at two levels (group 1 vs. group 2), and a trial factor at two levels (period 1 vs. period 2). The following dependent variables were each analyzed separately: (1) total cholesterol; (2) high density lipoprotein cholesterol; and (3) bleeding time. All statistical tests were performed using the .05 level of significance. Statistical analysis was done through the Oklahoma State University Computer Center.

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this study was to determine if MaxEPA had a beneficial effect on total cholesterol, high density lipoprotein cholesterol, and bleeding time in 11 patients with a documented history of coronary heart disease. The experimental design was a double blind cross-over utilizing two groups with each subject acting as their own control. Groups were controlled by administering a placebo.

Of the 11 subjects (9 male and 2 female), all had documented histories of CHD in varying degrees of severity. Eight of the 11 subjects had undergone coronary artery bypass graft surgery (CABG), one had had an inferior myocardial infarction (MI), and two had undergone balloon angioplasty on one or more coronary arteries. Additionally, one subject had had an MI, then CABG, then angioplasty and one subject had had an MI prior to CABG surgery. All subjects were at least 12 weeks and not more than 3 years post coronary event. All of the subjects were on prescribed low fat diets (below 30%), and all exercised a minimum of 20 minutes, three times per week. One of the subjects continued to smoke cigarettes. Phenotyping histories were not available to the investigator. Descriptive information

is depicted in Table I.

All of the subjects that began completed the study. The supplement was tolerated well by all subjects, however, there were instances in which mild gastric distress was noted.

Results

Pre test results are given in Table II. Experimental data are given in Table III.

Results of Total Cholesterol Analysis

The results of the total cholesterol analysis by group and by period are given in Table IV. There was no change in total serum cholesterol levels at the .05 level of significance.

Results of HDL-Cholesterol Analysis

The results of the HDL/C analysis by group and by period are given in Table V. There was no change in high density lipoprotein cholesterol levels at the .05 level of significance.

Results of Bleeding Time Analysis

The results of the bleeding time analysis by group and by period are given in Table VI. There was no change in bleeding time at the .05 level of significance.

TABLE I

DESCRIPTIVE DATA

Subject	Group	Sex	Age	Weight	Percent Dietary Fat	History of CVD
1	1	F	48	108	17.7	CABG
2	2	M	71	153	36.0	MI, CABG
3	2	M	52	183	30.7	CABG
4	1	M	50	169	21.7	ANGIO
5	2	M	71	172	31.7	CABG
6	2	M	64	208	35.7	CABG
7	1	M	59	189	28.3	CABG
8	1	F	61	156	32.7	MI
9	1	M	40	184	44.7	MI, CABG ANGIO
10	2	M	68	146	13.3	CABG
11	2	M	59	184	16.3	ANGIO
$\bar{X}$	-	-	58.5	168.4	28.1	
SD	-	-	10.0	26.9	9.7	

CABG= Coronary artery bypass graft surgery
MI= Myocardial infarction
Angio= Coronary artery balloon angioplasty

TABLE II

PRE TEST DATA

GROUP I

Subject	Age	Sex	Starting Points T.C. (mg./dl.)	HDL/C (mg./dl.)	B.T. (min.)
1	48	F	321.9	36.2	3.0
4	50	M	236.9	37.3	5.0
7	59	M	266.5	28.0	4.5
8	61	F	266.4	44.3	3.5
9	40	M	198.5	34.0	4.5
$\bar{X}$	51.6	-	258.0	36.0	4.1
SD	8.6	-	45.3	5.9	0.8

GROUP II

Subject	Age	Sex	Starting Points T.C. (mg./dl.)	HDL/C (mg./dl.)	B.T. (min.)
2	71	M	232.5	40.8	4.0
3	52	M	236.9	38.4	3.0
5	71	M	245.8	50.2	1.5
6	64	M	237.8	38.9	3.0
10	68	M	202.3	39.5	6.0
11	59	M	304.9	36.8	7.5
$\bar{X}$	64.2	-	243.4	40.8	4.2
SD	7.5	-	33.7	4.8	2.2

T.C.= total cholesterol
HDL/C= high density lipoprotein cholesterol
B.T.= bleeding time

TABLE III

EXPERIMENTAL DATA

GROUP I

Subject	Treatment T.C. (mg/dl)	Treatment HDL/C (mg/dl)	Treatment B.T. (min)	Placebo T.C. (mg/dl)	Placebo HDL/C (mg/dl)	Placebo B.T. (min)
1	340.5	47.0	7.0	306.8	46.1	4.75
4	204.1	38.8	5.5	225.5	32.7	6.75
7	264.0	33.2	5.0	276.6	35.0	4.25
8	235.5	50.3	3.5	231.0	30.2	4.5
9	215.3	36.6	3.5	198.8	35.0	5.75
$\bar{X}$	251.9	41.2	4.9	247.7	35.8	6.1
SD	54.5	20.0	1.5	43.3	6.1	1.0

GROUP II

Subject	Treatment T.C. (mg/dl)	Treatment HDL/C (mg/dl)	Treatment B.T. (min)	Placebo T.C. (mg/dl)	Placebo HDL/C (mg/dl)	Placebo B.T. (min)
2	246.2	45.5	5.5	231.0	48.0	4.0
3	224.8	39.7	3.75	218.6	43.4	4.0
5	264.6	60.6	3.5	245.4	59.4	2.75
6	220.4	33.9	6.5	211.3	40.0	8.5
10	211.6	47.9	8.25	194.5	45.7	9.5
11	324.5	45.5	4.75	265.2	41.1	4.25
$\bar{X}$	248.8	45.5	5.4	227.7	46.3	5.5
SD	41.8	9.0	1.8	25.2	7.1	2.8

Group I began the study ingesting the placebo, Group II began by ingesting the MaxEPA (tm) capsules.

TABLE IV

ANALYSIS OF VARIANCE FOR
TOTAL CHOLESTEROL

SOURCE	SS	d.f.	M.S.	F*
Group	736.39	1	736.39	0.23
Error a	29406.12	9	3267.35	-
Period	505.44	1	505.44	2.38
Group x Period	863.56	1	863.56	4.06
Error b	1913.10	9	242.57	-
Total	33424.61	21		

*Critical Value for F at .05 level of significance = 5.12

TABLE V

ANALYSIS OF VARIANCE FOR HIGH DENSITY LIPOPROTEIN CHOLESTEROL

SOURCE	SS	d.f.	M.S.	F*
Group	208.29	1	298.29	3.28
Error a	818.81	9	90.98	-
Period	45.10	1	45.10	2.11
Group x Period	29.02	1	29.02	1.36
Error b	192.36	9	21.37	-
Total	1293.58	21		

*Critical Value for F at .05 level of significance = 5.12

TABLE VI

ANALYSIS OF VARIANCE
FOR BLEEDING TIME

SOURCE	SS	d.f.	M.S.	F*
Group	0.82	1	0.82	0.13
Error a	57.02	9	6.34	-
Period	0.03	1	0.03	0.02
Group x Period	0.25	1	0.25	0.21
Error b	10.70	9	1.19	-
Total	68.82	21		

*Critical Value for F at .05 level of significance = 5.12

Discussion of Results

The interaction between eicosapentanoic acid (EPA, the major constituent of MaxEPA) and the blood platelets is not at present clearly understood. There are conflicting results in the literature as to whether EPA is in fact a beneficial treatment for hyperlipidemia. Clearly, in this study, using this population, at this dosage level, and using an olive oil placebo for a 3 week period, no beneficial outcome was derived.

Although the reasons for this outcome can not be explained totally because the mechanism of action can not be explained, there are several possible interpretations. One primary area of concern which has been noted in recent literature is the reliability of automated blood analyzer results (7,56). Superko et al. (56), reported that of 130 clinical laboratories that participated in a study to test the accuracy of HDL/C results, 52% varied from the overall mean by more than 7.5%. In clinical numbers this variation represents approximately 34.5 mg./dl. In another 40% of the laboratories surveyed the results varied about the mean by greater than 10%.

The January 1988 issue of Clinical Chemistry reported that the Center for Disease Control surveyed 5,000 laboratories to analyze the accuracy of total cholesterol testing (7). The results revealed that 47% of those labs surveyed were greater than plus or minus 5% of the control

value. The control value was a "true value" traceable to the Center for Disease Control and the National Bureau of Standards. Additionally 16% of the 47% were greater than plus or minus 10% of the control value (7). Although daily controls for the Vision (tm) blood analyzer that were run prior to each test period were acceptable, there is still a possibility of deviation in both the total cholesterol and HDL/cholesterol values.

In the Abbott dry chemistry kits which were used in the analysis of both TC and HDL/C, a red color is produced when reacted with cholesterol in human serum. The color intensity is determined from the amount of light absorbed from an internal light source within the Vision (tm) machine. Because the color produced is red there is the possibility that the color could be enhanced by such interfering substances as hemolyzed blood cells. Although the machine does have an internal safeguard to print an error message if such an event occurs (3), slight changes in color are not sufficient to activate the error message. The resultant TC or HDL/C level could be affected by these subtle changes in the red color. In addition, the light source used within the Vision (tm) blood analyzer is filtered to produce a single wavelength at 500 n.m., which is where the red color absorbs. Measurement at a single wavelength light source is minimal at best. A more accurate method would be to read the sample using several different

wavelengths and then average the readings to determine the TC and HDL/C levels. This problem is not confined to the Vision (tm) blood analyzer, but is a problem for all clinical laboratories in which a single wavelength is used.

Among the other factors which have recently been cited as having an effect on cholesterol readings are: (1) body position; (2) time of year; (3) coffee consumption; and (4) how long the tourniquet is on the arm before removing the sample (56). It is certainly difficult, if not impossible to control these variables even for an individual subject which evidently produces unreliabilities in TC and HDL/C levels.

The majority of the literature in which a statistical decrease in TC and HDL/C has been shown from the administration of fish-oil, has been for healthy populations (37,40,46). In the study by Green et al. (27), in which 1.8 gm. EPA was administered daily for 6 weeks in stroke patients, no significant decrease in TC, HDL/C, or bleeding time was found. This leads one to suspect that perhaps there is a genetic deficiency in those with atherosclerotic diseases that perhaps somehow affects the action of EPA. Although the LDL receptor-mediated endocytosis theory appears to be well documented in healthy individuals (9), there is much needed research still to be done regarding the atherosclerotic patient and the LDL receptor.

Bleeding time was measured in this study using Duke's

method (59) in which the subject's finger is pricked with a small lancet to a uniform depth, and the time is measured until bleeding ceases. Although this method is widely accepted, an alternative that may have produced more reliable results would have been to use Ivy's method (59) in which the forearm is pricked instead of the finger. The forearm is generally less calloused than the fingers, therefore the blood supply is closer to the surface yielding results that are more reproducible from subject to subject.

Other factors that possibly affected the results for the present study, were: (1) choice of placebo; (2) length of therapy; and (3) dosage level. The placebo used in this case was one of an olive oil base. Recent literature has shown that diets rich in monosaturated fats (particularly olive oil) may have an effect on lowering serum cholesterol levels (28). If this is true, the administration of an olive oil placebo may effect the cholesterol level as much or more than the MaxEPA (tm) treatment. It was the intent of this study to gain insight as to whether 1.8 gm. of EPA (10 MaxEPA,tm, capsules) given daily would sufficiently alter blood lipid levels enough that they could be recommended to CVD patients. It is now known that MaxEPA (tm) given in this dose for a three week period is not sufficient to produce the desired results in subjects with documented CVD.

Finally, the results for the present study might well

have been changed by using a larger sample. Because of the nature of this study, i.e. attempting to make inference to patients with some form of cardiovascular disease, it was difficult to solicit volunteers that met the required criteria. The cost of a larger study was also prohibitive.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

In recent years the public has been inundated with information about elevated cholesterol as a risk factor for developing heart disease. As public awareness has risen, increasing pressure has been put on physicians and pharmaceutical companies to produce a low cost, effective means by which cholesterol levels can be lowered without the public having to make drastic dietary changes. Because of the early studies involving Greenlandic Eskimos, fish-oil appeared to possess properties that would actually allow an individual to reduce serum cholesterol and still enjoy a high fat, high protein diet. Consequently, the market-place became flooded with fish-oil supplements that could be bought over the counter making claims that cholesterol levels could be significantly reduced.

With this in mind, it was the question of this investigator that if fish-oil supplements did in fact lower serum cholesterol levels in a healthy population could it also lower cholesterol levels in individuals with coronary heart disease.

Therefore, the purpose of this study was to determine if the oral ingestion of 10 MaxEPA capsules daily for a

period of three weeks would have the desired effect of decreasing total cholesterol, increasing high density lipoprotein cholesterol, and increasing bleeding time in subjects with documented histories of CHD.

This study was conducted as a double-blind cross over, using a 2 x 2 repeated measures analysis of variance with a grouping factor at two levels (group 1 vs. group 2), and a trial factor at two levels (period 1 vs. period 2).

Conclusions

Based on the hypothesis stated and the limits of this study, the following conclusions were made:

1. The oral ingestion of 10 MaxEPA (tm, 1.8 gm. EPA) capsules daily for a period of 21 days has no significant effect on total cholesterol.

2. The oral ingestion of 10 MaxEPA (tm, 1.8 gm. EPA) capsules daily for a period of 21 days has no significant effect on the high density lipiprotein-cholesterol.

3. The oral ingestion of 10 MaxEPA (tm, 1.8 gm. EPA) capsules daily for a period of 21 days has no significant effect on bleeding time.

Recommendations

Based on the data collected in this study, it is evident that additional research is needed to establish the exact dosage level of MaxEPA, as well as, the proper duration of therapy which is required to produce the desired effect of decreasing total cholesterol while increasing HDL cholesterol. Additional research is also required to focus on what population, if any, fish-oil would benefit.

When making these investigations it would be beneficial to include a large enough sample size so that the random error in cholesterol testing would not be a factor. It would also be advantageous to analyze multiple samples for each individual subject and average the resultant cholesterol level so that instrument variation would not be a factor.

In light of new research regarding triglycerides and the propensity for development of coronary heart disease (11), it would be beneficial for future studies to include a full lipid profile, including triglyceride levels, as well as, low density lipoprotein-cholesterol, and very low density lipoprotein-cholesterol.

Finally, and ideally, studies conducted in this area should be longitudinal in nature, spanning several years, with samples being analyzed at different times of the year. Then data collected could truly reflect the relationship of

the development of cardiovascular disease with the routine ingestion of fish-oil.

Additional research is warranted in this area to derive possible benefits from the oral administration of fish-oil.

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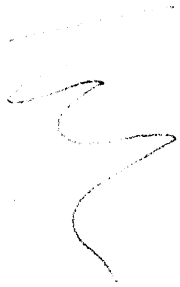
APPENDIX



January 20, 1987

MEMO: To Cardiac Rehab Patients
FROM: Robin
RE: FISH OIL STUDY

We will begin the fish oil study this Thursday, January 22. If you are interested in participating please be at the lab sometime between 7 and 8 a.m. so we can draw the first series of blood tests. PLEASE eat or drink nothing (except water) from 7:00 p.m. the night before.

The experiment will be as follows: Take a venous blood sample to test your cholesterol and HDL, and do a finger stick to test for bleeding time on both Thursday and Friday mornings. This will be done on two consecutive days so that we can find a stable baseline. Then you will begin on the fish oil or a placebo (you won't know which one you are on). You will be required to take 10 capsules each day for three weeks. I would like for you to take them all at once in the evening. Then on February 16th you will return to the lab between 7 and 8:00 a.m. for the same series of blood tests, receive your next round of capsules and again take them the same way you did before for 3 more weeks. On March 9th you will return to the lab for the final series of blood tests. The experiment will take a total of 6 weeks.

It is my thought according to what I know about "fish oil" that taking these capsules will lower your total cholesterol and hopefully increase your HDL. All the results will be made available to you at the conclusion of the study. In order to be eligible to participate you must have a cholesterol of 240 mg./dl. or above or an HDL of 20% or lower. This will be determined on the first day of testing. Also, if you are on any other cholesterol lowering substance you will not be eligible.

The possible risks associated with taking this food supplement are increased bleeding time, some pain or bruising associated with venipuncture and fingersticks, and some people may experience a certain degree of indigestion.

I hope to have at least 10 people in this first study. Thanks for your help!!!

HEALTH AND FITNESS CENTER
Oklahoma State University

The following information is needed for our records and in assessing your current health and fitness status. By providing as much of this information as possible in advance, time will be saved during the evaluation. All information provided will be held in strict confidence.

NAME _____ DATE _____

ADDRESS: Street _____ City _____ State _____ ZIP_____

HOME PHONE _____ EMPLOYER _____

OCCUPATION _____ BUSINESS ADDRESS _____ PHONE _____

AGE LAST BIRTHDAY _____ BIRTH YEAR _____ Does your job require physical activity? _____

Do you currently smoke? _____ If so, what? _____ number/day _____
If not, have you ever smoked?_____ If yes, what? _____ no/yrs_____ yrs. quit_____

Do you ever drink alcoholic beverages? _____ If yes, approx. no.: less than 1/day _____
1-2 per day _____ 3 or more per day _____

Do you currently participate in any form of exercise on a regular basis?_____
Indicate no. of times/weekly of participation: walking _____ jogging _____ swim _____
golf _____ basketball _____ handball/racquetball _____ tennis _____ other (name)_____
If you walk, job or swim, please indicate distance and time covered each session and approximate pace _____

What is your estimate of your current medical condition? ex.__ good__ fair__ poor__
What is your estimate of your current physical fitness? ex.__ good__ fair__ poor__

Circle the number of blood relatives (parents, grandparents, brothers, sisters, that have been diagnosed as having some form of heart disease:
Under 60 years of age: 1 2 3 4 5 6 7 8 9 Over 60 years of age: 1 2 3 4 5 6 7 8 9

Have you ever been told that you have any form of heart disease? _____
Have you ever been told that you have diabetes? _____
Do you have blood relatives with diabetes? _____ If so, how many? _____

Do you consider yourself to be overweight? _____ If so, approx. how many lbs.? _____
Do you have any medical conditions (other than heart disease or diabetes) that might affect your exercise performance? _____ If so, please list _____

Who is your family physician? _____ City _____
Address, if known _____ date last medical exam _____
Would you like your stress test records sent to this physician? _____
If you would prefer to have your records sent to another physician, please list name and address _____

Are you currently taking any kind of medication? _____
If yes, is it non-prescription? _____ If so, name _____
If yes, is it prescription? _____ If yes, give name if possible _____

Have you ever been told that you had high cholesterol or high triglyceride levels in the blood? Cholesterol: yes__ no__ Triglyceride: yes__ no__

If you know your cholesterol and/or triglyceride levels, please list

Cholesterol _____ Triglyceride _____

OKLAHOMA STATE UNIVERSITY
HEALTH AND FITNESS CENTER
MAXEPA STUDY

INFORMED CONSENT

EXPLANATION OF TEST

The tests you are about to undergo are a part of a research study involving the oral ingestion of a product called eicosapentanoic acid (EPA). The purpose of this study will be to monitor its effects on serum lipid levels and bleeding times. EPA could have possible benefits in the reduction of serum lipids and in increasing bleeding times in some patients. Since these two factors have been strongly linked to the incidence of atherosclerotic heart disease, it is possible that the ingestion of EPA will decrease the risk of developing heart disease.

It will be required of you to take 2 grams of the substance daily for a period of three weeks, and then a placebo for a period of three weeks. You will not know at which time you are taking which substance. At the beginning of the series of tests it will be required that we take two blood samples two days apart to establish a baseline serum cholesterol level. At the end of the first three week treatment period another blood sample will be drawn, and at the end of the second three week period.

Only trained personnel will be involved in the extraction of blood from the vein at the bend of the arm. In addition, a registered nurse will be in attendance during all procedures. The traditional venipuncture method will be employed.

Bleeding time will be determined using a small finger prick. Again, only trained personnel will be administering the treatment.

Further information regarding any procedures or complications can be obtained from the Health and Fitness Center. The numbers to call if you have questions or suffer from a medical problem are: 624-7556 (days), or 377-5283 (nights).

POSSIBLE RISKS

The potential risks that have been associated with MaxEpa are as follows: (1) increased bleeding time or decreased coagulation time; this could cause you to bruise more easily or to bleed easily. (2) Alteration of serum lipids; this should cause no untoward effects. (3) Decreased red blood count; this should not be sufficient to cause

anemia. Although the above risks are potentially dangerous, MaxEpa in the dosage recommended should not cause harm.

The potential risks associated with venipuncture are: (1) Venipuncture may cause some pain or discomfort. The exact amount, if any, will be dependent upon individual preconceptions and pain threshold levels. (2) Possible hematoma (bruising) at the venipuncture site following the procedure. The occurrence or non-occurrence will be dependent upon bleeding/coagulation times and adherence to instructions pertaining to holding a cotton ball against the venipuncture site, with pressure, for five minutes following extraction of the needle. (3) Slight risk of infection. Any break in the integrity of the skin is associated with a small degree of risk of infection. However, if directions are followed the risk is very little.

CONSENT BY SUBJECT

The information which is obtained will be treated as privileged and confidential and will not be released or revealed to anyone without your express written consent. Information will be used for research purposes only as a part of group data. Results will be given to you following the completion of the study.

I have read the foregoing, I understand it and any questions which may have occurred to me have been answered to my satisfaction. I understand I may withdraw from and discontinue my participation at any time that I feel it necessary.

DATE_____

SUBJECT_____

WITNESS_____

OKLAHOMA STATE UNIVERSITY
HEALTH AND FITNESS CENTER
MAXEPA STUDY

To my knowledge, I do not have ulcers, frequent nose bleeds, hemophilia, or any other bleeding disorders. If I develop any abnormal bleeding I will contact the Health and Fitness Center immediately, and discontinue use of the Maxepa.

SIGNED_____

WITNESS_____

DATE_____

SUBJECT INFORMATON SHEET
EPA STUDY

1. Do not change any of your dietary habits during the course of this study.

2. Do not change any of your normal exercise routines during the study.

3. On the day prior to having your blood drawn, do not eat or drink anything after midnight the night before.

4. Do not exercise the morning that you are having your blood drawn. (You may exercise after)

5. Do not take any aspirin or aspirin containing products during the study. (Anything containing acetlysalicylic acid)

6. If you are prone to any bleeding disorders (ulcers, Hemophilia, etc.) please advise one of the Health and Fitness Center employees.

7. If you have any medical problems that might be associated with the taking of EPA please call Robin at 624-7556 or Frank at 624-6753.

8. You are to return to the Physiology of Exercise Lab (room 120, Colvin Center) on:

_____at_____
for your next set of blood work.

9. If you can not adhere to the above instructions please advise the Health and Fitness Center.

10. Take all medicatons involved in the study in the evening before bedtime.

Oklahoma State University
HEALTH AND FITNESS CENTER
DIET DIARY for Weight Loss/Control Clinic

Name_____Date_____Day of Week_____
Tel._____Address_____

(Indicate seperate snacks)

BREAKFAST — Time you eat. When you eat. Who you eat with. What you are doing. How you feel. — CALORIES

Food, Description, Amount_____

Total Breakfast Calories _____

LUNCH

Total Lunch Calories _____

DINNER

Total Dinner Calories _____

TOTAL CALORIES FOR DAY _____

VITA 2

Robin Brown Purdie

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF MaxEPA (tm) ON SERUM CHOLESTEROL, HIGH DENSITY LIPOPROTEIN CHOLESTEROL, AND BLEEDING TIME IN PATIENTS WITH CORONARY ARTERY DISEASE

Major Field: Health, Physical Education, and Recreation

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Education: Graduated from Deer Creek High School, Edmond, Oklahoma, in May 1972; received Associate Degree of Nursing from Northern Oklahoma College, Tonkawa, Oklahoma, in May 1978; received Bachelor of Science Degree from Oklahoma State University, Stillwater, Oklahoma, in May 1985; completed the requirements for the Master of Science Degree at Oklahoma State University, Stillwater, Oklahoma in May, 1988.

Professional Experience: Staff Nurse, St. Anthony Hospital, Intensive Care, Oklahoma City, Oklahoma, from May 1977, to August 1978; Charge Nurse, Franklin Memorial Hospital, Intensive Care, from August 1978, to May 1979; Charge Nurse, St. Joseph Medical Center, Intensive Care, Ponca City, Oklahoma, from May 1979, to May 1980; Charge Nurse, Stillwater Medical Center, Intensive Care, Stillwater, Oklahoma, from May 1980, to September 1982; Manager, Cardiac Rehabilitation, Oklahoma State University, Health and Fitness Center, Stillwater, Oklahoma, from September 1982, to present. Member AACVPR, ACSM, OAHPERD.