THE EFFECT OF EXERCISE AND FISH OIL SUPPLEMENTS ON THE BLOOD LIPID LEVELS OF THE HAMSTER

by

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Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Human Nutrition and Foods

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July 1989

Blacksburg, Virginia

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(ABSTRACT)

The effect of aerobic exercise and fish oil supplements on plasma lipid parameters was investigated in the hamster. The hamsters were fed a basal hypercholesterolemic purified diet (0.1% cholesterol, 15% fat) to elicit an elevated lipid response. Fifty-six hypercholesterolemic hamsters were divided into four groups: control, swimming (trained up to one hour every other day), daily fish oil supplementation (.35g/kg/day omega-3), and a swimming/fish oil combination. The effect of these treatments on plasma TC, TG and pooled samples of plasma HDL-C, HDL_2-C, LDL-C and VLDL-C was studied over 12 weeks. All hamsters had a significant increase in body weight. Feed intakes increased at 6 weeks and then declined over the second 6 weeks, but remained above the beginning levels. Plasma TC levels were significantly decreased in all three treatment groups overall (-23 to 26%) and significantly lower than the control group at 12 weeks with fish oil producing the greatest decrease. Plasma TG levels were lower, though not significantly decreased in the swimming (-44%) and fish oil

(-12%) groups, but was significantly decreased in the swimming/fish oil combination (-86%) by week 12. The pooled samples had similar changes in plasma TC and TG in all treatment and control groups. An apparent increase in the pooled plasma HDL-C and HDL_2-C and a decrease in plasma LDL-C was observed in the swimming group. In the present study, it appeared that the fish oil was most effective in lowering plasma TC and the swimming was more effective in reducing plasma TG and raising plasma HDL-C in the hamster.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. Forrest Thye for his encouragement, patience and advice throughout the course of this study. I also wish to thank the following people:

Dr. Janette Taper for her encouragement and understanding.

Dr. Reed Humphrey for his assistance, sense of humor and willingness to pinch hit with little notice.

Dr. Janet Walberg for her advice.

and for helping with unfamiliar laboratory methods.

for his statistical interpretation of the data.

who endured my constant complaints and impatience and being such wonderful friends throughout this period of my life.

My most recent supporter, , I would like to thank for his patience and encouragement as my thesis neared completion.

I also want to thank, over and over, my wonderful family who has supported me throughout this experience with an open mind and an open heart.

Lastly, in loving memory, I would like to thank

. Without her guidance, expertise and moral support, I would have never made it through the experimental period of my study. She will be dearly missed by myself and all those fortunate enough to have worked with her.

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INTRODUCTION

Coronary heart disease (CHD) has been one of the leading killers in Western Civilization for over fifty years. CHD results in more than 500,000 deaths per year in the U.S. (McCunney, 1987). Many risk factors have been identified which contribute to CHD, most importantly hypertension, smoking and hyperlipidemia, most notably plasma total cholesterol (TC). It is generally agreed among researchers that a plasma TC level over 240 mg/dl markedly increases the risk for CHD and also exacerbates the effects of the other risk factors for CHD (McNamara, 1987). Current evidence suggests that plasma triglycerides (TG) are not independently associated with CHD death, but are positively associated with high levels of plasma TC (Criqui, 1986).

Research in the area of lowering plasma TC and altering the blood lipid profiles have progressed extensively over the past two decades. Recent research shows that the raising of plasma high density lipoprotein cholesterol (HDL-C) and lowering of plasma TC and low density lipoprotein cholesterol (LDL-C) can help to lower the risk of CHD (Grundy, 1986).

In the past twenty years CHD has decreased by 25% in the U.S. One of the contributing reasons to this decline in CHD has been improved medical care (Feinleib, 1984). A more

important factor may be the changing lifestyles of the American population with reductions in cigarette smoking in the male population, control of high blood pressure and reduced plasma TC (Kannel, 1983). The reduction of plasma TC, LDL-C, VLDL-C and TG as well as the increase of HDL-C will be the issues addressed in this study.

The use of animal models for cholesterol research has many advantages. It is much easier to manipulate an animal's lifestyle, environment and diet than it is with humans, all of which are important factors to control in cholesterol research.

Statement of the Problem

The effects of exercise and fish oil on plasma total cholesterol and triglycerides have been researched separately by a number of investigators. Cholesterol and triglycerides are carried in three different lipoproteins of which each has its own relationship with CHD. Plasma LDL-C comprise approximately 60-70% of plasma TC and has a strong positive relationship with coronary disease (Criqui, 1986). This would explain the association of plasma TC with CHD. The level of plasma HDL-C has been shown to be inversely related to the risk of CHD. The protective effect of plasma HDL-C is at least as strong as the atherogenic effect of plasma LDL-C and is independent of other risk factors (Kannel, 1983). For every 10 mg/dl of change in the plasma

HDL-C level, there is an association with a 50% change in risk (Kannel, 1983). Very low density lipoprotein cholesterol (VLDL-C) are strongly correlated with triglyceride levels, and like triglycerides, appear not to have an independent CHD association (Criqui, 1986).

A great deal of research has been done on the effects of intense endurance exercise on plasma TC and HDL-C. Research has shown that individuals who perform vigorous, large muscle dynamic exercise for extended periods have similar or slightly differing plasma TC concentrations than less active people (Haskell, 1984). An increase in plasma HDL-C has been reported in athletes or individuals who exercise regularily (McCunney, 1987). Moderate exercise has not been as thoroughly researched, but findings have shown decreased levels of plasma TG levels and increased levels in plasma HDL-C and HDL_2-C.

A number of studies have been done with fish oils rich in omega-3 fatty acids. The findings in these studies were related to the amount of eicosapentaenoic acid (EPA) & docosahexaenoic acid (DHA) used. The principal effect of fish oil supplements has been a decrease in plasma TG and VLDL-C levels (Leaf and Weber, 1988). The changes in plasma TC and LDL-C with the use of fish oil supplements have been inconsistent and unpredictable. Plasma HDL-C levels are usually unchanged or may be slightly increased (Mehta et al, 1987).

One of the major drawbacks to the use of the animal model in research is relating it to the human response. Rodents such as the rat have been commonly used for cholesterol research, however HDL-C can account for as much as 90% of the plasma TC. Unlike rats, hamsters have a relatively abundant LDL, with the ratio of cholesterol content between HDL and LDL being about 1:1 (Tsuda et al, 1983).

Hamsters and humans have a similar low rate of cholesterol synthesis as compared to other animals such as the rat (Spady and Dietschy, 1985). Because of the limited synthetic capacity in the liver of man, and particularly the male hamster, the liver is slow to readily adapt to changes in cholesterol flux and thus alter the LDL transport in response to diet (Spady and Dietschy, 1985). Singhal et al (1983) studied the effects of dietary cholesterol on cholesterol metabolism in the hamster. It was found with the addition of 0.25% cholesterol to the diet that the hamsters' plasma TC increased similar to humans fed a high saturated fat, high cholesterol diet. A second study done by Sicart et al (1986) had similar results with an increase in plasma TC in hamsters fed a diet with 0.1% cholesterol

The hamster model is appropriate for this research project because of the similar lipid profile to humans, and the ability to regulate the animals' environment and diet.

Research Hypothesis

Ho: There was no difference between groups of hamsters that received (1) the control diet only, (2) the control diet plus MaxEPA fish oil, (3) the control diet and a moderate swimming exercise regime, and (4) the control diet and a combined treatment of MaxEPA fish oil and moderate swimming exercise regime in plasma TC and TG values.

Ho: There was no difference between groups of hamsters that received (1) the control diet only, and the control diet and (2) MaxEPA fish oil, (3) moderate swimming exercise or (4) a combined treatment of MaxEPA fish oil and swimming exercise in plasma VLDL-C, LDL-C, HDL-C, HDL_2-C values.

Significance of the Study

Numerous studies have addressed the issue of aerobic activity and its effect on plasma TC, HDL-C and TG. Fewer studies have explored the effects of moderate exercise on these variables.

A good number of studies have been done using fish oil supplements to alter lipid profiles. However, many of the findings with fish oil supplements have been inconclusive. Generally, it has been found that fish oil supplements do consistently lower plasma TG and VLDL-C concentrations (Nestel et al, 1984). A few studies have found fish oil supplements to lower plasma TC and to increase HDL-C (Nestel et al, 1984; Sanders et al, 1983; Saynor et al., 1984). If

the results of the present study using MaxEPA fish oil supplements were to show a decrease in TC, LDL-C, or TG and/or an increase in HDL-C or HDL_2-C, this would suggest that moderate exercise, fish oil supplements or both may decrease the risk of heart disease.

The purpose of this study was not only to study these two variables separately, but to combine fish oil supplements and exercise to explore their combined effects. Since this combined program has not been addressed in the literature to date, the results of this study will seek to answer some of the existing research questions as well as identify new areas for further research.

The use of the hamster for cholesterol research with emphasis in heart disease is a recent development. If the findings of this research correlate to past findings with humans and other larger animals with similar blood lipoprotein patterns, the use of the hamster may become a feasible small animal model for the human.

Symbols

The following symbols will be used throughout the text:

1. CHD: Coronary Heart Disease
2. DHA: Docosahexaenoic acid
3. EPA: Eicosapentaenoic acid
4. HDL-C: High density lipoprotein cholesterol
5. HDL_2 -C: High density $lipoprotein_2$ cholesterol

6. LDL-C: Low density lipoprotein cholesterol
7. VLDL-C: Very low density lipoprotein cholesterol
8. TC: Plasma total cholesterol
9. TG: Triglycerides

Summary:

Coronary heart disease has been the number one killer disease over the past 35 years. Research has provided some answers to reduce the mortality rate from CHD and has achieved some success. It has been generally accepted that a decrease in plasma TC concentrations and in particular plasma LDL-C will reduce the risk of CHD (Criqui, 1986). It has also been substantiated that an increase in plasma HDL-C will decrease the chances of developing CHD (Criqui, 1986). Aerobic activity has been found to significantly increase HDL-C and slightly decrease plasma TC or leave it unaltered (Haskell, 1984). Fish oil supplements have been consistently found to lower TG and VLDL-C concentrations (Leaf and Weber, 1988). However, the results are inconclusive relative to the effects of fish oil on plasma TC, and whether fish oil supplements are beneficial in the fight against hypercholestemia is still undecided (Grundy, 1986). This study will examine the effects of a moderate aerobic exercise treatment, a fish oil supplement treatment and a combined moderate aerobic exercise and fish oil supplements treatment on plasma TC, TG, LDL-C, VLDL-C HDL-C and HDL_2-C.

REVIEW OF LITERATURE

Introduction

Coronary heart disease (CHD) remains the number one killer in the U.S. and Western Europe. There is little debate that an elevated plasma TC level is one of the three major risk factors of CHD (McNamara, 1987). It has been shown that lowering plasma TC levels by bile acid sequestrants and hypocholesterolemic drugs does reduce the incidence of fatal and non-fatal heart attacks (McNamara, 1987; Grundy, 1986). Drugs can be the answer for some with extremely high or uncompromisimg plasma cholesterol levels. However, alternative measures such as diet and exercise can significantly lower plasma TC and LDL-C and should be recommended for the majority of the population.(Grundy, 1986).

The Pathogenesis of Atherosclerosis

Atherosclerosis is the disease in which cholesterol and debri accumulate in the walls of the arteries. This can form a plaque which can inhibit the flow of blood until a clot is formed and completely blocks the artery leading to a heart attack or stroke (Brown & Goldstein, 1984). The cholesterol for plaque formation is acquired from plasma LDL-C circulating in the blood stream. The more plasma LDL-C present in the blood stream, the faster the athersclerotic lesions will develop (Brown & Goldstein,

1984).

The leading theory of atherosclerosis formation is based on the model of Brown & Goldstein (1984). Atherosclerotic plaques develop slowly, and are apparently initiated by damage to the thin layer of the endothelial cells which lines the arterial walls. The damaged cells become leaky and allow LDL particles and blood particles to penetrate. Hormones such as the platelet-derived growth hormone (PDGH) and platelet derived growth factor (PDGF) are released in response to the penetration, causing smooth muscle cells to proliferate and migrate to the damaged area. At the same time white blood cells, monocytes, are attaching to the area and are activated to macrophages which are scavenger cells. The smooth muscle cells and macrophages ingest and degrade the LDL particles forming foam cells. If the LDL concentration in the blood is very high the LDL particle itself can accumulate in the foam cells. The accumulated cells, debri and cholesterol, also known as an atheroma narrow the opening of the artery more and more over time. The final blockage of the artery results in a heart attack or stroke.

Ross (1986) suggested a subtler form of injury to the endothelium of the cell which may cause no morphologic alterations but may be enough to stimulate endothelial cells to form and secrete growth factors. Ross asked the question whether chronic hypercholesterolemia could alone induce this

change. If the endothelial cells are chronically bathed in elevated levels of LDL-C and VLDL-C, rapid cholesterol exchange occurs within the plasma membranes of the endothelial cell. This could lead to alterations in the viscosity of the membranes of the cell and to an increase in the attachment of monocytes which may serve as a stimulus for the subendothelial macrophages. In reponse to the stimulus, fibrous plaque formation and further progression of the lesion could occur. Thus, when the plasma LDL-C is more available in the blood from elevated plasma LDL-C levels, fatty streaks are thought to progress faster to lesions.

Further research is needed in the area of growth factor production and plaque formation. Direct information is lacking on how certain risk factors are associated with plaque production at the cellular level. Further research is necessary to identify the impact of the different risk factors.

Cholesterol Transport

The relationship between the transport of cholesterol and CHD can be better understood through the lipoprotein transport system. Lipoproteins are composed of protein, triglycerides and phospholipids, and are classified according to size and density. The higher the ratio of lipid to protein, the lower the density. The transport of

cholesterol is by two pathways; an endogenous system (produced within the body) and an exogenous system (diet) (Brown & Goldstein, 1984). The liver is the primary producer of cholesterol (approximately 80%) while the diet supplies approximately 20%.

The exogenous pathyway begins with dietary cholesterol that is absorbed in the intestine and packaged with other dietary fats such as triglycerides and phospholipids into chylomicrons (Brown & Goldstein, 1984; Grundy, 1986). The chylomicrons first enter the lymphatic system, then blood stream and deliver TG mostly to adipose and muscle tissues. The chylomicron remnants are removed from the blood by the liver via a special receptor.

The endogenous pathway begins with the secretion of VLDL from the liver after the cholesterol from the chylomicron remnant is reabsorbed. The VLDL is composed mainly of TG with a small amount of cholesterol. The VLDL travels through the blood stream and TG are delivered to the body tissues. The VLDL remnant is an intermediate density lipoprotein compound known as IDL. About half of the IDL particles are removed quickly by the liver to make new VLDL and bile acids while the other half eventually lose the apoprotein E and become LDL particles. LDL particles circulate for an average of 2.5 days before binding with LDL receptors in the liver and other tissues (Brown and Goldstein, 1984). HDL particles are secreted from the liver

and intestine and entered into circulation independently of the secretion of triglyceride-rich lipoproteins (Havel, 1987). The metabolic function of HDL is to primarily transport cholesterol from peripheral tissues to the liver for excretion or synthesis of bile acids (Haskell, 1984).

Relationship between Plasma TC and CHD

It is known that there is a relationship between plasma TC and LDL-C and CHD. There is a progressive rise in risk of CHD as plasma TC increases (Castelli, 1983). Plasma LDL-C comprises approximately 60-70% of TC and is strongly associated with CHD (Criqui, 1986).

In the Framingham study, 5209 men and women were studied and 37 variables were identified as placing individuals at high risk for CHD. One of the top 3 factors was hypercholesterolemia (Castelli, 1983). The researchers found a relationship between plasma TC and risk of CHD, as plasma TC rises, so does the risk of CHD. It was estimated that for every 1% rise in cholesterol, the incidence of CHD increased by 2%. The investigators concluded that individuals with values of 200-220mg/dl had relatively constant rates of CHD. This does not suggest that levels below 200 mg/dl are not beneficial. A curvilinear relationship was seen between plasma TC and CHD in the Multiple Risk Factor Intervention Trial (MRFIT) (Stamler et al, 1986). 356,222 men of the ages 35 to 57 years were

studied for the relationship between CHD and serum cholesterol and other risk factors. It was found that the risk of CHD was a continuously graded one with plasma TC levels. Of all the CHD deaths, 46% were estimated to be deaths attributable to plasma TC >180 mg/dl. An individual with a plasma TC level of 264 mg/dl had a death rate 4 times higher than an individual with a plasma TC of 167 mg/dl.

The positive correlation between TC and CHD has led researchers to make recommendations for the general population. Many researchers think the plasma TC levels should be as low as possible, 150 to 180 mg/dl. Researchers do agree that plasma TC levels of 240 mg/dl or more clearly are strongly associated with increased CHD risk (McNamara, 1987). The AHA recommended that the plasma TC levels should fall below 200 mg/dl but several researchers suggested levels between 130-190 mg/dl (Grundy, 1986).

Castelli (1983) suggested that individuals who fall in the range of 200-220 mg/dl, are not safe to assume low risk of CHD. It was recommended to look at the TC/HDL-C ratio as a good indicator. A ratio below 4.5 appears most attractive and although not absolute safe, it suggested a direction in which to move. A TC/HDL ratio of 4.0 for women and 5.0 for men appeared to be standard risk and levels below these ratios should be obtained (Castelli, 1983).

Relationship Between HDL-C and CHD

Plasma lipoproteins have long been considered to be related to the development of CHD. Research over the past 25 years have suggested that some lipoproteins have atherogenic effects whereas others do not (Havel, 1979). Plasma HDL-C has received a great deal of attention over the years for its possible anti-atherogenic effect or removal mechanism.

The HDL particle is secreted by the liver and is considered to play a critical role in transport of cholesterol from peripheral and atherogenic tissues to the liver (Krishan & Kottke, 1987; Foster & Kottke, 1987). The transport mechanism is thought to deal with a key enzyme LCAT (lecithin-cholesterol acyl transferase) which is responsible for the synthesis of essentially all endogenous plasma cholesterol esters (Havel, 1987). The LCAT enzyme is loosely associated with plasma HDL-C, which esterifies all free cholesterol on the surface of the HDL particle. LCAT esterifies the cholesterol by transferring a fatty acid from lecithin to cholesterol, leaving lysolecithin as a by-product (Krishan & Kottke, 1987). The cholesterol esters are hydrophobic by nature and quickly migrate to the core of the HDL particle. The cholesterol binding sites will then be free to pick up additional free cholesterol from cell surfaces (Krishan & Kottke, 1987).

The increase in lipid content of the HDL particle

causes it to become larger and less dense, forming HDL_2-C. The denser HDL particles are referred to as HDL_3-C. The HDL_2 formed during active fat transport can be converted to HDL_3 by the enzyme hepatic lipase (Havel, 1987). The concentration of HDL_2 to HDL_3 tend to increase in persons with efficient lipolytic mechanisms associated with triglyceride transport (Havel, 1987).

A negative correlation between plasma HDL-C and CHD was first established in 1951 by Barr, Russ and Eder. Epidemiological studies have shown this to be true (Tan, 1980; Castelli et al, 1977; Gordon et al, 1977). In the Framingham study, it was found in each group studied that the plasma HDL-C concentrations were lower in persons with CHD as compared to those who did not have CHD. The data suggested an inverse relationship between plasma HDL-C and CHD and the incidence of CHD decreased as HDL-C rose above 45 mg/dl (Castelli et al, 1977; Gordon et al, 1977).

Several epidemiological studies have found associations between low levels of HDL and increased incidence of CHD using myocardial infarction and angina pectoris as indicators of CHD (Carlson et al, 1979; Robertson et al, 1977; Castelli et al, 1983; Gordon et al, 1977). However, these studies lacked the evidence to show any physiological change with low levels of plasma HDL-C such as atherogenic plagues. An investigation of 483 men and women looked at plasma HDL-C levels and the presence, severity and location

of anatomically proven coronary disease using coronary arteriography (Pearson, 1979). The results suggested that consistent and statistical trends of lower plasma HDL-C with increasing numbers of diseased coronary arteries were found in both men and women in both younger and older age groups. The researchers concluded that low levels of plasma HDL-C would be important risk factors for development of atherosclerosis and may be useful for identifying patients at high risk. A study by Tan et al (1980) found that among 104 patients who had a coronary angiopathy, those without CHD had a plasma HDL-C level of 53.9mg/100ml on the average. The average plasma HDL-C was 30% higher than those with abnormal coronary arteries.

In the Framingham study it was found that plasma HDL-C had the greatest impact on risk of CHD of the age group studied, 49 to 82 years (Gordon et al, 1977). There was a significant inverse association between plasma HDL-C and the incidence of CHD. However, until clinical trials can show that an increase in plasma HDL-C may reduce the risk of CHD, the relationship must remain tentative (Krishan and Kottke, 1987).

Relationship between triglycerides and CHD

The hypothesis that plasma TG are a cause of CHD has not yet been confirmed and is not universally accepted. Nonetheless, plasma TG levels strongly influence

the practice of preventive medicine (Hulley et al, 1980). Triglycerides have received attention over the years as a possible independent risk factor in CHD, but the findings are controverial at best and the biological basis for the involvement of plasma TG in CHD development has yet to be determined. It has been questioned whether plasma TG are a cause of CHD, or if elevated plasma TG and CHD are a consequence of a third variable (Hulley, 1980).

In the intestines TG synthesis is a direct function of absorption of dietary fat. TGs are also synthesized in the liver mainly by the phosphatidic acid pathways from fatty acids made in the liver or coming from the blood (Olsson, 1988). Newly synthesized TG may be incorporated directly into VLDL particles or they may be stored. In principle, when high levels of TG are secreted from the intestines or liver they are dealt with in two ways. More TG can be incorporated into each of the secreted lipoprotein particles or a larger number of lipoprotein particles can be synthesized (Olsson, 1988). Plasma TG are dynamic and can change levels very quickly because of variables such as diet, body weight, disease and drugs (Brown & Ginsberg, 1987).

Triglycerides were first proposed as a cause of CHD in 1959, by Albrink and Man. They found high serum levels of TG in men with a history of myocardial infarction (MI). Over the past two and half decades researchers have made attempts

at proving or disproving this theory. The controversy still exists as to whether serum TG are an appropriate target for efforts to prevent CHD (Hulley et al, 1980).

An independent association of TG to CHD had been reported in earlier studies, but has not been univerally accepted (Krishan & Kottke, 1987). Several studies have shown that high serum levels of plasma TG were not an independent risk factor when multivariate adjustment was done for major risk factors (Krishan & Kottke, 1987). The researchers in the Framingham study found that individuals with CHD had plasma TG levels approximately 21 mg/dl higher than those without disease. It was found however, when other serum lipids were taken into account TG had no significant effect on CHD (Castelli, 1977).

A study was conducted to compare Japanese men living in Japan, Hawaii and California. Higher levels of TG were seen with higher rates of CHD. After the influence of other risk factors such as blood pressure, weight and smoking were adjusted, it was found that TG had no independent effect on CHD (Robertson, 1977). Researchers investigated 50-year-old healthy men who were compared in two European cities, Naples and Stockholm. The mean serum TG levels did not differ between cities, although Stockholm had a higher risk of CHD mortality (Olsson, 1988).

Carlson et al (1979) did a fourteen year follow up of the Stockholm Prospective Study looking at the incidence of

myocardial infarction (MI) in a group of 3,846 men. Quintile analysis showed that the rate of new MIs increased more with higher concentrations of TG and TC. Triglycerides appeared to be a more important risk factor than TC (Carlson et al, 1979).

Several studies have shown there is an inverse relationship between HDL and TG (Havel, 1979). It appeared that any independent relationship that TG had with CHD was primarily due to the inverse relationship with HDL (Brown & Ginsberg, 1987).

The evidence for a positive relationship between plasma TG and CHD was limiting as compared to the evidence supporting the relation of high plasma TC to a higher incidence of CHD. These arguments against plasma TGs fall short of disproving the belief that lowering plasma TG can prevent CHD (Hulley, 1980). Therefore in the scope of preventive medicine lowering plasma TGs may be of some benefit for lowering the risk of CHD.

Aerobic Exercise and CHD

Personal characteristics and environmental factors such as gender, family history, age, body composition, dietary intake, cigarette smoking, medication and exercise influence the composition of plasma lipids and specific lipoproteins (Haskell, 1984). These factors interact with one another in a variety of ways, which makes a definitive statement about

independent factors difficult to obtain. Many studies have developed designs with factors such as diet, age, gender and medications being controlled, thus enabling the researcher to suggest strong correlations between exercise and plasma TC (Webb, 1987).

The Effect of Aerobic Exercise on Plasma TC

Studies have shown that between chronic endurance exercise and plasma TC, athletes have similar or slightly lower plasma TC than the sedentary controls of similiar ages. Several researchers have shown long distance runners to have plasma TC levels not significantly different from plasma TC levels in less active individuals (Moore, et al, 1983; Morgan et al, 1986; Rotkis et al, 1984; Thompson et al, 1983). Other studies have shown slightly lower plasma TC in endurance runners compared to sedentary controls (Wood et al, 1983; Clarkson et al, 1981) or slightly higher plasma TC (Lehotonen & Viikari, 1980). No differences were found in plasma TC in trained competitive swimmers as compared to sedentary controls (Smith et al, 1982).

A study was conducted comparing 15 national level oarsmen and a sedentary control group matched for age and alcohol consumption (Danner et al, 1984). The oarsmen were involved in a training program for seven months. The oarsmen trained for 10 hours per week the first four weeks, and 14 hours per week thereafter. At day 14 of the study

the plasma TC of the oarsmen had significantly dropped and continued to decrease at day 30. At the end of the study the oarsmen had a significant decrease in plasma TC, 145 mg/dl vs. 166 mg/dl. Cross sectional studies have shown a positive relationship between dietary saturated fat intake and plasma TC, however studies with one population yield inconclusive results. The oarsmen consumed a greater quantity of food during training but food composition remained identical between groups. The researcher suggested that diet in this population was not a factor in the plasma TC levels.

Plasma variations of TC have been suggested with different intensities of training. Gaesser and Rich (1984) investigated high and low intensity training on a cycle ergometer. Sixteen non-obese, non-smoking males were assigned to one of two exercise groups for 3 day/week for 18 weeks. The first group (n=7) exercised at high intensity (H), 80-85% VO_2 max for 25 min/session and the second group (n=9) exercised at low intensity (L), 45% VO_2 max, 50 min/session. Subjects were encouraged to continue with their normal dietary habits. VO_2 max, body weight, percent body fat were determined and blood samples were collected at three week intervals. A significant increase in VO_2 was seen in both training interval groups (19.% (H); 17.2% (L)). Body weight reductions were not seen in either group, although significant reductions in percent body fat were

demonstrated in both groups ($P<0.05$). No significant difference was found for plasma TC between either group from the starting plasma TC levels. The researchers suggested that changes were not seen because of the low starting plasma TC levels. This however, was not seen in the study by Danner et al (1984) where values were lower than those by Gaesser and Rich (1984) and plasma TC was lowered considerably. It may be possible that the intensity of this training was not enough to elicit a change in plasma TC. The fact that diet was not controlled in the study with different training intensities could have contributed to the results.

Morgan et al (1986) investigated the plasma TC of women in various lifestyles and found no significant differences. Twenty-seven women were selected and put into three groups. The first group was comprised of weight trainers who did various isotonic and isokinetic training methods with moderate to high resistance. The length of weight-training experience ranged from 9 to 39 months. The second group was comprised of endurance runners who averaged 25 to 59 miles/week during the preceding year. The third group was comprised of sedentary women who had not been involved in any form of weight or aerobic training for the past year. The plasma TC values for the three groups were 180 mg/dl, 186 mg/dl, 183 mg/dl, respectively. The levels of plasma TC were low in all three groups, however in looking at lipid

fractions, a considerable difference was seen in plasma HDL-C levels with the highest plasma HDL-C being in the high intensity aerobic group. Body weights and skinfold sums were found to be similar between the weight trainers and runners.

Based on variable results from different studies of measures before and after training as well as different types of training, investigators have not produced any clear consensus as to whether moderate or vigorous exercise has an independent effect of lowering plasma TC (Haskell, 1984). Investigators have not always controlled for dietary intake or assessed body weights in their analyses. Intensity and duration of exercise could also be variables contributing to inconclusive results. Another factor in the unchanged plasma TC could be the altering of the lipid fractions, with an increase in HDL-C and a decrease in LDL-C and VLDL-C (Morgan et al, 1986; Cook et al, 1986; Hicks et al, 1987). This would leave the plasma TC unchanged but the TC/HDL-C ratio decreased, and therefore result in a decreased risk of CHD (Kannel, 1983).

The Effect of Aerobic Exercise on Plasma TG

In contrast to aerobic exercise's limited effect on plasma TC, endurance-type exercise has been consistently associated with lower plasma TG concentrations (Haskell, 1984). Highly trained atheletes such as long distance

runners (Moore et al, 1983; Thompson et al, 1983) and oarsmen (Danner et al, 1984) all had low plasma TG concentrations when compared to sedentary controls. In cases where endurance athletes do not have lower plasma TG concentration when compared to sedentary controls, it is usually due to lower than average values for the comparison groups (Haskell, 1984). The training of speed and power athletes usually does not result in lower plasma TG levels (Lehtonen & Viikin, 1980). It would appear that the extreme leanness of the athletes may make some contribution to the lower plasma TG concentrations (Haskell, 1984).

Moore (1983) examined long distance runners (26 mi/wk), joggers (6 mi/wk) and sedentary controls and found the plasma TG concentrations were significantly lower in long distance runners as compared to the sedentary control (61 mg/dl vs. 79 mg/dl, Moore, 1983). The joggers did not have significantly lower plasma TG levels than the control (73 mg/dl vs. 79 mg/dl, repectively). Calorie intake did not differ significantly among the three groups. Significant differences were found in body weight and percent body fat. These findings indicated the inactive individual weighed significantly more than the long-distance runners ($P<0.05$), and the percent body fat of each group differed significantly from every other group. The percent body fat was the variable most highly correlated with plasma TG ($r=.270$).

Oarsmen (n=15) in a seven month high intensity training period and sedentary controls (n=21) were compared for serum TG levels (Danner et al, 1984). The oarsmens' serum TG levels continuously decreased over the seven month period as compared to the controls and at the seventh month the levels were .85 mmol/l vs. 1.26 mmol/l in the control group. One possible reason for the continuous decrease in plasma TG was that the intensity of the workouts became greater over the seven months. This was not discussed in the paper, however.

Ten obese men were put into a vigorous walking program for 16 weeks (Leon et at, 1979). They walked five days a week, expending 1100 kcal per session. No attempt was made to influence the diet. It was found that after the 16 week program the plasma TG levels were not significantly lower at 144 mg.dl as compared to the baseline at 165 mg/dl. Body composition measurements indicated a loss of 5.9 kg of body fat and a gain of 0.2 kg of lean tissue. Percent body fat was decreased from 23.3 to 17.4. The investigators suggested that when plasma TG are within normal ranges, they are unlikely to be significantly lowered by increased physical activity and/or weight reduction.

Studies have been conducted to look at moderate aerobic exercise and its influence on plasma TG levels. Huttenen et al (1979) investigated the effects of mild-to-moderate exercise with men ages 40-45 years. The exercise group

participated in a four month program which consisted of 3-to-4 sessions per week. The groups maintained previous exercise habits. For the first two months of the exercise program the men exercised mildly at 40% of their maximal heart rate (HR). The last two months the men exercised moderately at 66% of their maximal HR. A progressive statistically significant decrease in serum TG levels was seen after only two months in the exercise group. The plasma TG values were significantly lower than the control group at the end of the exercise treatment (1.27 mmol/l vs. 1.58 mmol/l, respectively). A small but significant reduction in body weight was seen in both the control and exercise group. The decrease in the plasma TG concentrations was not solely attributed to weight loss, but it may have been a factor. Subjects who maintained their weight during the exercise phase also had significantly lower plasma TG levels similar to the persons that lost weight. It was suggested that the reason for the decrease in the exercise group was a direct chronic effect of increased physical activity on VLDL synthesis or catabolism.

One study reported differing results with no significant change in plasma TG levels with high intensity aerobic exercise (Gaesser & Rich, 1983). High (80-85% of VO_2 max) and low (45% of VO_2 max) intensity exercise regimes were compared for lipid parameters in a 18 week exercise program. There were no significant changes in either group

in plasma TG concentrations. Body weight did not change in either group, but significant changes in percent body fat were seen in both groups. It was explained that the pre-training values for plasma TG values were initially low, which helps to explain why there were no significant decreases.

The mechanism for lowering plasma TG during aerobic exercise training has been of interest, but evidence has been lacking for a direct answer. There would be the possibility of decreased hepatic TG synthesis following exercise (Haskell, 1984). Both acute and chronic lowering of plasma TG concentrations have frequently been attributed to an increase in lipoprotein lipase (LPL) activity in the skeletal muscle and adipose tissue. LPL is the key enzyme for catabolism of TG-rich lipoproteins and contact between LDL and TG results in rapid free fatty acid production. The free fatty acids are then transported through the endothelial layer and assimiliated to storage TG in tissue cells. While LPL is found on the luminal surface of the capillary endothelium in extrahepatic tissues, the major action of LPL takes place in the skeletal muscle and adipose tissue (Haskell, 1984). Much of this is not well understood, but chronically low plasma TG levels of endurance athletes appeared to be related to low body fat and high skeletal muscle LDL activity, in relation to slow-twitch muscle fibers (Haskell, 1984). For

hypertriglyceridemic people, caloric restriction and vigorous exercise seem to be synergistic in lowering plasma TG levels; lower levels are possibly a result of decreased TG synthesis and increased catabolism (Haskell, 1984). Much of the increase in LPL occurred in the first week of training which suggested an acute response to exercise rather than a true training effect. Increases in LPL activity following exercise, however, have not been closely linked to a decrease in plasma TG concentrations (Haskell, 1984).

The Effect of Aerobic Exercise on Plasma HDL-C

Plasma lipoproteins have long been considered a major factor in relation to CHD. Plasma HDL-C has received considerable attention because of its potentially protective quality against CHD (Haskell, 1984). Increased levels of HDL-C have been associated with age, gender, alcohol, body composition, medication use, cigarette smoking and exercise (Haskell, 1984; McCunney, 1987). Depressed levels of plasma HDL-C have been shown to be an independent risk factor for CHD and are associated with male gender, high body weight, cigarette smoking and sedentary lifestyles (Haskell, 1984; McCunney, 1987).

Many investigators have endorsed the use of physical activity in preventing or slowing the onset of CHD. Generally most studies have found an increase in plasma

HDL-C with the advent of physical activity. The time required to produce an increase in plasma HDL-C is dependent on several factors: (a) the initial plasma HDL-C level; (b) the intensity of the exercise training; (c) the duration of the training session; (d) and the frequency of the training (Hartung, 1984).

Cross-sectional studies which compared active individuals to sedentary populations have generally agreed that the active groups had significantly higher plasma HDL-C than the inactive groups. Plasma lipids in groups of women, aged 24-59 years old, were compared among different levels of exercise (Moore et al, 1983). One group ran 26 mi/week, a second group jogged 6 mi/week and a third group was sedentary. The plasma HDL-C levels in the running group were significantly higher than in the jogging or sedentary control group. The plasma TC was not significantly different in any of the groups which suggested the plasma LDL-C was lower as well in the running group.

A study was conducted comparing the blood lipids and food intake of male long distance runners to a sedentary control group (Thompson et al, 1983). The plasma HDL-C was significantly higher in the runners (66 mg/dl) as compared to the sedentary group (46 mg/dl). The plasma TC levels were not significantly different, but the plasma LDL-C levels were approximately 10 mg/dl higher in the sedentary controls.

Several studies have been done comparing aerobic exercise at different intensities of training. Smith et al (1982) compared competitive swimmers who swam 6 days/week, averaging 8000 yards in 2 hours to synchronized swimmers who swam 3 days/week for 2 hours averaging 1500 yards and to sedentary controls. Thirty-four female undergraduates aged 19 to 20 years were involved. The plasma HDL-C was signficantly higher in the competitive swimmers (82 mg/100 ml) as compared to the synchronized swimmers (70 mg/100 ml) and control (67.2 mg/100 ml). The results of this study demonstrated that women who exercise vigorously have elevated plasma HDL-C. Women at moderate intensity exercise had similiar plasma HDL-C levels as the control group, however all plasma HDL-C levels were high.

Longitudinal studies have generally agreed that physical aerobic exercise does increase plasma HDL-C levels. However, in studies where an increase in plasma HDL-C was not found it was attributed to already high plasma HDL-C levels before exercise training (Haskell, 1984). Several researchers had reported a decrease in plasma HDL-C with training. But in some of these, no control group was used. Explanations for the variation in the findings of these studies are not easily identified, but differences in training regimes, laboratory procedures, baseline plasma HDL-C levels and personal habits may help explain some of these differences (Haskell, 1984; Gaesser and Rich, 1984).

Exercise intensities seen in long distance runners would not be expected in the average individual. High intensity exercise and long duration would discourage the beginning exerciser. Leon et al (1979) involved 6 obese men in a 16 week vigorous walking program of 90 minutes, 5 days/week. The plasma TC and TG levels were significantly unchanged. The plasma HDL-C concentrations progressively increased to 15.6% above pretraining levels. This caused the HDL-C/LDL-C ratio to increase in the men from .27 to .34 by the end of the study. Significant reductions were seen in body weight and percent body fat. Changes in body composition comprised of a decrease of 5.9 kg of body fat and and increase of 0.2 kg of lean tissue. The authors suggested that the initial levels of plasma TC and TG were within normal ranges and it would be unlikely for the levels to decrease significantly with increased physical activity and/or weight reduction.

The effects of a mild-to-moderate physical exercise training on serum lipid levels was investigated (Huttunen, 1979). One hundred middle-aged men were assigned to a 4 month, 3-to-4 days/week group or to a sedentary control. The exercise subjects were assigned an individualized training program that consisted of walking, jogging, swimming, skiing or cycling. The plasma HDL-C levels increased from 1.27 mmol/l to 1.41 mmol/l ($p<.01$) in the exercise group during the 4 month training period. No

change was seen in the control group. A small but significant decrease in the body weight was observed in the exercise and control group over the 4 month period, however no significant differences in body weight were seen between the two groups throughout the study. These results indicated that a much lower intensity and frequency of exercise can influence the level of plasma HDL-C.

Twenty-six obese male subjects were randomly assigned to either a dietary weight loss group or an aerobic exercise group, for a 3 month period (Schwartz, 1987). The walk/jog exercise group trained for 40 minutes 2-to-3 supervised periods per week. The diet group was placed on a 1200 kcal/day diet. Both groups had a significant decrease in body weight, but the weight loss was greater in the diet group. Significant reductions in plasma TC and TG were observed in the diet group only. A significant increase in plasma HDL-C was seen in the diet group (4 mg/dl) and exercise group (3 mg/dl) but the Apo A-I increased only in the exercise group (7 mg/dl). The authors concluded that dietary weight loss appeared to mainly affect the transport of cholesterol and TG to the cells as VLDL and LDL lipoproteins. The changes in the aerobic exercise group appeared to be more directly related to the proposed HDL-mediated reverse cholesterol transport system with increases in both plasma HDL-C and Apo A-I.

A study conducted by Cook et al (1986) investigated 35

active postal carriers. Miles walked per day were reported and lipoproteins were assessed every three months for a 1 year period. Reported miles walked/day (5.3) was significantly correlated with plasma HDL -C (r=0.50, P=0.003) and plasma HDL-C approached a significant correlation (r=.29, P=0.06). The exercise intensity of these postal carriers was much lower than in other studies. This suggested that an increased plasma HDL-C was the result of chronic low intensity physical activity.

Low plasma HDL-C levels are considered by many researchers to be an important risk factor for CHD. Plasma HDL-C levels may help to explain why some populations are at higher risk than others and provide the biochemical link for the benefits of exercise (Brooks and Fahey, 1985). The ratio of plasma TC to HDL-C seems to be an important indicator in the development of CHD. In the Framingham study it was shown that the average ratio of male heart attack victims was 5.4. The recommended ratio was below 4.5 with the optimal ratio being 3.5 (Castelli, 1983; Kannel, 1983).

Endurance exercise has been shown to increase plasma HDL-C levels as well as LCAT and lipoprotein lipase. This should result in an increased elimination of cholesterol in the bile and may be the principle mechanism by which exercise reduces the risk of CHD (Brook and Fahey, 1985). Variables such as intensity, frequency and duration of the

aerobic training have been addressed, and appear to be important factors in the elevated response of plasma HDL-C.

Fish oil and CHD

Epidemiological studies have shown a much lower prevelance of CHD in Greenland Eskimos and other Artic populations as compared to Western cultures (Mehta et al, 1986). The major food consumed was fish and the most significant differences in the fatty acid content of the Eskimo diet was they were much higher in EPA and DHA than Western diets (Mehta et al, 1986). Studies done on other populations such as Japanese men with an intake of fish of 1000 grams per day appeared to have a protective effect against CHD as well (Nutrition Reviews, 1986). Eicosapentaenoic acid and docohexanoic acid belong to the omega-3 family which means that one double bond is three carbon atoms away from the methyl end of a 20-carbon chain (Mehta et al, 1986). In comparison, linoleic acid which is higher in the western diet, belongs to the omega-6 family of the polyunsaturated fatty acids.

The plasma and platelet lipids of the Eskimos were unusual because they contained high levels of EPA and DHA. The omega-3 fatty acids had to originate from the diet because we cannot synthesize them in the body. Omega-3 fatty acids, like arachidonic acid can be converted into prostaglandins and may influence haemostasis (Saunders &

Roshanani, 1983). Omega-3 fatty acids, unlike arachonidic acid may actually inhibit platelet aggregation, which may prevent CHD by reducing atherosclerosis (Saunders & Roshanani, 1983).

A triglyceride lowering effect has been consistently observed in fish oil trials which may have clinical implications (Nutrition Revews, 1988). A significant decrease in plasma VLDL-C is generally associated with a decrease in plasma TG. Reported findings in other plasma lipids such as plasm TC, LDL-C and HDL-C have been inconclusive, however.

The Effect of Fish oil on Plasma TC

The feasibility of using fish oil preparations rich in omega-3 fatty acids for treating hypercholesterolemia has not yet been established (Nestel, 1986). Plasma TC levels have been shown to be significantly reduced by fish or fish oil consumption, but the majority of the reductions occurred in the plasma VLDL-C fraction. Also at very high intakes of fish oil (90-120 grams per day) both plasma LDL-C and LDL apo B are reduced by a possible decrease in the rate of LDL synthesis (Sanders, 1987). At lower intakes (15 grams/day) an increase in LDL-C and LDL apo B is seen. A possible explanation for this is that a moderate intake of fish oil decreases hepatic TG synthesis so smaller VLDL particles are formed. These small particles are known to be more readily

converted to LDL (Sanders, 1987).

Demke et al (1988) conducted a study on 31 hypercholesterolemic patients using a diet rich in omega-3 fish oils. Thirteen of the patients took 5 grams of encapsulated fish oil (MaxEPA) daily and 18 patients (control) took 5 grams of encapsulated safflower oil daily. Diet and exercise patterns were kept as constant as possible. During the study each one gram capsule of fish oil contained 18.6 % EPA and 15.8% DHA. In the fish oil group the plasma TC increased 39 mg/dl(14%) from baseline to day 29 (P=0.0001). One month following the end of the study, the plasma TC values had returned close to baseline values in the fish oil group, thus being only 10 mg/dl higher. The plasma LDL-C also significantly increased (P=0.003) by 31 mg/dl(16%) to day 29. After one month from the finish of the study the plasma LDL-C values had decreased closer to baseline values, being only 7 mg/dl higher. The placebo group had small insignificant decreases in plasma TC and LDL-C. Neither the fish oil or placebo group had significant changes in plasma VLDL-C values. The results of this study call into question the benefits of fish oil as a lipid lowering agent at lower doses.

Several researchers have obtained conflicting results as compared to the above study. Nestel (1986) investigated six subjects who were hospital inpatients for seven weeks. There were 3 dietary periods; a habitual diet (1 wk (P/S

0.47, cholesterol 710 mg/dl), a fish oil enriched diet (3 wk) [40 g/day Max EPA, P/S 1.62, cholesterol 190 mg] and a fish oil and cholesterol supplemented diet (3 wk) [fish oil and egg yolk P/S 1/62, cholesterol 940 mg/dl]. The diets were given in order as reported. The change from habitual diet to fish oil diet caused a significant fall in all lipoprotein lipid measurements. Both fish oil (40 gram MaxEPA, cholesterol 190) diets had a reduction in percent calories as fat (4% kcal) which might account for the decrease in serum LDL-C and serum TC.

A similar study was conducted with five male subjects, 18-31 years, consuming 5, 10 and 20 grams of MaxEPA fish oil/day in random order for three week periods (Sanders et al, 1983). The doses of MaxEPA provided .83, 1.67, and 3.33 grams of EPA and .8, 1.61, and 3.22 grams of DHA each day. Each period was separated by a break of six weeks. Measurements of plasma TC, TG and HDL-C were made at the beginning and at the end of each period. Plasma TC levels were lowered at the 20g/day dose from 4.21 mmol at the control period to 3.82 mmol (P=.05). This did not occur at the lower doses. The small sample size per treatment of this study and the lack of a control group should be considered before making any substantial conclusions.

Several studies have had findings where serum TC was not affected by varying levels of fish oils rich in omega-3 fatty acids. Bronsgeest-Schoute et al (1981) examined 52

healthy volunteers and looked at different levels of omega-3 fatty acids daily over a period of four weeks. Doses of 1.4, 2.3, 4.1 and 8.2 grams of omega-3 fatty acids, EPA & DHA preparation were administered daily. There were no significant changes of serum TC values observed during the intake of the omega-3 fatty acid oils at any of the different levels. A considerable significant decrease, 60 mg/dl was seen in serum VLDL-C levels in the 8.2 gram dosage group. The serum LDL-C fraction increased, especially in the 8.2 gram dosage diet, final values increasing 51 mg/100ml from baseline values. Although a change in serum TC was not seen there was an alteration of the lipid fractions. While serum VLDL-C was lowered, serum LDL-C was increased. The researchers suggested a dose-response effect, specifically since no significant changes were observed with the lower doses.

Investigators studied six volunteers taking supplements of 10-40 ml cod liver oil daily for 5 months (von Schacky et al, 1985). Serum TC did not change significantly during the study and individual lipid fractions were not investigated. The researcher concluded that with a maximum dose of 8.2 grams per day of omega-3 fatty acids (EPA and DHA) in normocholesterolemic volunteers, a lowering of serum cholesterol could not be expected possibly due to the already low plasma TC values. Nevertheless, it was suggested that omega-3 fatty acids are as potent as omega-6

fatty acids in lowering serum cholesterol levels.

The use of omega-3 fatty acid fish oils have very inconsistent results in plasma TC reponse. The results that have been found do not appear to have beneficial results for serum lipids aside from TG. The occasional increase in serum TC and serum LDL-C is a negative aspect in any omega-3 therapy. The use of omega-3 supplements should be carefully considered until actual conclusions are drawn on plasma cholesterol lipids if CHD is already present.

The Effect of Fish oil on Plasma TG

It is now well established that the use of fish oil will lower plasma TG concentrations in hypertriglyceridemic subjects. This is thought to be accomplished by reducing the synthesis of both TG and Apolipoprotein B in the plasma VLDL. These effects have been attributed to EPA and DHA as well as other long chain polyunsaturated fatty acids. (Sanders, 1986). The role TG-lipoproteins play in atherosclerosis is still being debated.

The lipid lowering mechanism of fish oils rich in EPA and DHA on elevated and normal plasma lipids is not well understood. A study by Wong et al (1984) compared the effects of fish and safflower oils on the metabolism of isolated rat livers perfused with undiluted rat blood. The rats were fed either a standard commercial chow diet alone or the standard chow modified with the addition of 0.5%

cholesterol and 15% fish oil or safflower oil by weight. The total fat of the modified diet was 15% fat with 63.5% omega-6 fatty acids in the safflower oil and 20.2% omega-3 fatty acids in the fish oil. After two weeks of dietary modifications, the rats were anaesthetized and their livers were perfused with undiluted rat blood from male rats fed a standard chow diet.

Supplementation of the diet with both oils produced a marked inhibition of newly synthesized fatty acids in the liver and VLDL. Rates of secretion of VLDL rich in TG was significantly diminished in the group receiving the modified diet and fish oil. Plasma TG levels were significantly lower in the rats fed fish oil than those fed safflower oil 38 mg/dl vs. 63 mg/dl, respectively. Ketogenesis (higher fatty acid oxidation) was significantly higher in the group receiving the modified diet and fish oil.

The potent plasma TG-lowering effect that fish oil has may be one of the best dietary means for treating hypertriglyceridemia (Nestel, 1986). Six healthy normolipidemic volunteers were involved in a seven week study (Nestel, 1986). Three dietary period were involved: habitual diet (one week), followed by a fish oil diet for 3 weeks and a fish oil and cholesterol supplemented diet for 3 weeks. The fish oil (MaxEPA) provided approximately 33% of EPA and DHA. Forty grams of MaxEPA, given in one gram capsules, were taken daily during the fish oil periods.

There were very substantial falls in the plasma TG levels when the subjects changed from the habitual diet to the fish oil diet only, 95 mg/dl at baseline to 33 mg/dl ($P<.001$). This study reconfirmed the well established TG-lowering effect of dietary omega-3 fatty acids.

A study investigated by Simons et al (1985) looked at the effects of 6 grams per day and 16 grams per day of fish oil on hyperlipidemic subjects in a randomized double-blind crossover study. Each period lasted three months. The MaxEPA contained a minimum of 19.8% EPA and 12% DHA. Each patient ingested 1.82 grams EPA and DHA on the 6 grams per day diet and 4.82 grams EPA and DHA on the 16 grams per day diet, daily. The data indicated a maximum therapeutic effect after 1 month of using MaxEPA. A dose relationship was apparent with a 33% decrease in plasma TG in the 6 grams per day group and 58% decrease in plasma TG in the 16 grams per day group after one month of treatment. Further significant decreases in plasma TG were not seen after the first month of treatment. The investigators concluded that the plasma TG levels could be significantly lowered with as little as 1.82 grams of EPA and DHA daily.

An investigation with similar findings used 52 heatlhy volunteers to study the effects of different amounts of omega-3 fatty acids on blood lipids (Bronsgeest-Schoute et al, 1981). Doses of 1.4, 2.3, 4.1 and 8.2 grams of omega-3 fatty acids (EPA and DHA) were administered to the

volunteers daily for four weeks. Significant decreases were seen in the serum TG values ($P<0.01$) only in the group taking 8.2 grams omega-3 fatty acids daily. The researchers suggested that lower concentrations of EPA and DHA may be more effective on elevated serum TG levels, whereas higher doses of EPA and DHA may be required to decrease normal TG levels.

The effects of fish oil were studied on plasma TG levels of patients with elevated plasma lipids (Demke et al, 1988). Thirteen patients took 5 grams per day of encapsulated fish oil and eighteen patients took 5 grams/day encapsulated safflower oil as a control for 28 days. The total daily dose of EPA and DHA was 1.79 grams/day in the fish oil group. The patients were asked not to modify their diet or exercise habits during the study. The plasma TG levels fell 51 mg/dl (24%) from baseline to day 29 in the fish oil group. The plasma TG levels of the placebo group fell only 5 mg/dl (4%). Although the fall from baseline in the fish oil group was large, the change was not statistically significant. This study supported the plasma TG lowering effect of fish oil, even at relatively lower doses. (Demke et al, 1988).

Doses as low as 5 grams have been shown to lower serum TG levels when they were originally elevated, however a statistical significance was not found in the study done by Demke et al, (1988). Decreases in plasma TG levels have

been seen in healthy normolipidemic individuals taking as little as 8.2 grams/day of fish oil. At levels as high as 40 grams/day the TG lowering effect is substantial. However at such a high intake there is an increase in fat calories as well as cholesterol.

Fish oil supplements can be effective in the treatment of hypertriglyceridemia. However, multivariate analysis of several epidemiological studies have suggested that plasma TG concentrations are not an independent risk factor for CHD, but are associated with elevated levels of plasma TC (Sanders, 1987).

The Effect of Fish oil on Plasma HDL-C

The use of fish oil to increase plasma HDL-C is not a common practice. Controversial findings of the effects of fish oil on plasma HDL-C are quite present. It has been seen that Greenland Eskimos have moderately higher concentrations of plasma HDL-C as compared to Eskimos living on a Western diet (Sanders, 1987). An increase in plasma HDL-C has been seen in individuals taking high amounts of fish oil (Simons, 1985). If increases were observed in plasma HDL-C in feeding trials, it appeared the levels tended to fall toward baseline with the longer duration of supplementation.

An encapsulated fish oil (MaxEPA) was administered to hyperlipidemic patients to establish the effect of dietary

omega-3 fish oil supplements on plasma lipoproteins (Simons et al, 1985). Thirteen patients took six grams per day and 12 patients took 16 grams per day of fish oil in a randomized double-blind crossover study, whereby each subject took fish oil supplements for three months or olive oil as a control. The fish oil capsules contained 30% of omega-3 fatty acids. The MaxEPA treatment was associated with a significant increase in plasma HDL-C level which was possibly a dose related 4.9% (1.07 mmol/liter) increase on 6 grams per day and 7.3% (1.18 mmol/liter) increase on 16 grams per day vs. the baseline, 1.02 mmol/liter.

A study by Sanders and Roshanai (1983) had similiar findings of an increase of plasma HDL-C, but at a higher dose. Five male subjects took 5, 10 and 20 grams MaxEPA daily for 3 weeks with a break period of at least six weeks each between each experimental period. The dose of MaxEPA provided 1.63, 2.28 and 6.55 grams of EPA and DHA, respectively. The plasma HDL-C concentration was significantly higher at the 20 grams/day, (1.51 mmol/liter) vs. the baseline value (1.15 mmol/liter), ($P<0.05$). Plasma HDL-C values were not elevated at the lower doses. The study concluded that further work needed to be done to see if MaxEPA actually raised plasma HDL-C levels.

Demke et al (1988) conducted an investigation to study the effects of a fish oil supplement on plasma lipoprotein levels in 31 hypercholesterolemic patients. Thirteen

patients took 5 grams of fish oil and 18 patients took 5 grams of encapsulated safflower oil (control) per day for 28 days. The daily dose of EPA and DHA was approximately 1.7 grams. The fish oil group had a significant increase in plasma HDL-C of 13% ($P<0.015$) and plasma HDL_2-C of 36% ($P<.001$). Plasma HDL-C significantly increased 8 mg/dl or 13% from baseline to day 29. This was associated with a 9 mg/dl or 36% increase in the plasma HDL_2-C, but no significant change in the HDL_3-C fraction was observed.

The plasma HDL-C increase could be beneficial for reducing the progression of atherosclerosis. However, after the fish oil supplementation ended there was a definite trend toward baseline values. It is important to note, however, that these were hypercholesterolemic subjects. It has been suggested that an increase in plasma HDL-C may be attributed to the increased intake of cholesterol from the fish oil. An increase in plasma HDL-C concentrations has even been seen in other feeding trials when cholesterol was increased in the diet (Nestel et al, 1984).

The results of several studies conflict with the findings of Demke et al (1988), Simons et al (1985) and Sanders and Roshanai (1983). A decrease in plasma HDL-C seen in several feeding trials using fish oil supplements was generally lower, but not significantly. Seven subjects received up to 30% of daily needs from fish oil (MaxEPA) or safflower oil (Nestel et al, 1984). Approximately 30% of

the fish oil contained EPA and DHA. Each diet lasted from 2 to 3.5 weeks. The plasma HDL-C concentration was lower in the fish oil supplemented group at 33 mg/dl as compared to 40 mg/dl in the safflower oil group. The mechanism for the reduction in plasma HDL-C, as seen with omega-6 fatty acids, has been attributed to diminished synthesis since the fractional removal is unaltered. This may apply to omega-3 fatty acids as well.

A second study by Nestel (1986) again found a significant decrease in plasma HDL-C values with a fish oil supplemented treatment. This study tested the capacity of MaxEPA oil to modify the rise in lipoprotein cholesterol during cholesterol-rich diets. Six subjects were tested with 3 diets in a crossover design: 1) habitual diet which was representative of the subjects' preadmission eating habits, (P/S 0.47, cholesterol 710 mg/dl); 2) fish oil (40 grams/day MaxEPA, P/S 1.62, cholesterol 190 mg/dl); 3) fish oil (40 grams/day MaxEPA)and egg yolk (P/S 1.02 and cholesterol 940 mg/dl). The test period lasted for seven weeks. Approximately 33% of the MaxEPA oil consisted of EPA and DHA omega-3 fatty acids. Changing from the usual diet to the fish oil diet significantly decreased plasma HDL-C values from baseline value, 34 mg/dl vs. 45 mg/dl ($P<0.001$). The apparent decrease in plasma HDL-C did not change from the fish oil diet to the fish oil diet with high cholesterol intake.

Several researchers have found no significant change in plasma HDL-C concentrations when omega-3 fish oil supplements were used. Harris et al (1983) investigated the use of a salmon oil diet rich in omega-3 fatty acids in comparison to a control diet and a vegetable oil diet. All diets contained 40% of the total calories as fat and 500 mg cholesterol. Twelve healthy adults participated in the crossover study and were fed each diet randomly for a four week period. Twenty percent of the fish oil diet was rich in omega-3 fatty acids. The plasma HDL-C concentration was not affected by the salmon oil diet as compared to the control diet and the vegetable oil diet.

A study with similar findings conducted by von Schacky et al (1985) supplemented 6 volunteers with 10-40 ml of cod liver oil/day. Every four weeks the dose of fish oil was changed from 10 ml, to 20 ml, to 40 ml, and again to 20 ml. The cod liver oil contained 9.4% EPA and 13.8% DHA. There were no significant changes over the entire study in the plasma HDL-C concentrations, from time 0 to 40 weeks.

The results of the last four studies were in definite contrast to the earlier studies presented in this section. The high doses of fish oils of the last two studies had no significant effect on plasma HDL-C concentrations. Specifically at higher doses of as much as 40 grams per day, conflicting results were seen by an increase, decrease or no signficant change in the HDL-C concentration. The effects

of fish oil on plasma HDL-C concentrations have too many varying results in research feeding trials, however it appears that even with a significant change in plasma HDL-C the levels tend to fall towards baseline on studies of longer duration (Haskell, 1984).

Animal Models

The use of animal models for cholesterol research has many advantages. In considering the effects of variables such as diet, environment and activity on cholesterol metabolism, the use of an animal model would be quite practical. Unfortunately, animals that are often used for cholesterol research possess differing proportions of plasma lipoprotein with HDL-C being the major lipoprotein in the rodent such as the rat. (Spady and Dietschy, 1983).

One rodent, the hamster, has a relatively high LDL level as compared to other rodents with a ratio of 1:1 of LDL to HDL (Tsuda, 1983). Hamsters and humans have a similarly low rate of cholesterol synthesis in the liver as compared to the rat (Spady and Dietschy, 1985). Spady and Dietschy (1988) have concluded that the hamster is a good human model for cholesterol research since its concentration of plasma LDL-C responds to changes in dietary lipid intake in a similar manner to that seen in the human.

Singhal et al (1983) studied the effects of dietary cholesterol on the cholesterol metabolism in the hamster. It

was found with the addition of .25% cholesterol to the diet, the plasma TC of the hamster increased similarly to humans fed a high saturated fat, high cholesterol diet. A second study explored the effect of feeding hamsters a high cholesterol diet (0.1%) on plasma TC (Sicart et al, 1986). A significant increase was seen in the plasma TC, but the percent change was seen mainly in the plasma VLDL-C.

Spady and Dietschy (1985) reported that the use of saturated fatty acids was associated with a significant increase in plasma LDL-C concentration in the hamster. In addition, the feeding of cholesterol (0.12%) plus saturated fatty acids significantly reduced the receptor-dependent LDL uptake of the liver. In a later study, Spady and Dietschy (1988) examined the effects of dietary cholesterol and saturated or unsaturated fatty acids on the rates of receptor-dependent and receptor-independent LDL-transport in the liver of the hamster. In hamsters fed diets with the addition of 0.1, 0.2 or 1% cholesterol for one month, receptor-dependent LDL-transport in the liver was significantly suppressed, 43, 63 and 77%, respectively. There was a reciprocal increase in plasma LDL-C concentrations. With the addition of 20% coconut oil the LDL-C concentrations were magnified in each of the three cholesterol enriched diets. The addition of 20% polyunsaturated fatty acids (safflower oil) caused a plasma LDL-C reduction in all three cholesterol enriched diets.

Fish oil and animal models

The lipid lowering benefits of polyunsaturated fatty acids, specifically omega-3 fish oils is limiting in hamster research. Other studies using animals with similar lipid profiles as the hamster, such as the monkey and rabbit, have been conducted.

Theiry and Seidel (1987) investigated the influence of fish oil on cholesterol induced atherosclerosis in rabbits. Twenty-four rabbits were randomly assigned to three groups. Group I was fed a cholesterol free basal diet. Group II was fed the basal diet plus 1.5% cholesterol. In addition to the cholesterol-enriched basal diet, Group III received 2 ml of MaxEPA fish oil every day. The composition of the fish oil was 18% EPA and 12% DHA. The cholesterol content of the fish oil was 12 mg/gram. The researchers reported a comparable increase in plasma TC in Group II and Group III, 1404 mg/dl and 1639 mg/dl vs. Group I, 73 mg/dl. Group III had elevated plasma TG levels 118 mg/dl vs. 63 mg/dl in Group II. Aortic atherosclerosis as measured by planimetry sudanophilic lesions indicated a significant increased blockage in Group III as compared to Group II, although plasma TC levels were similiar.

A study by Vas Dias et al (1982) examined the effects of four different oils (linseed, corn, fish or coconut) on platelet aggregation and platelet and aortic fatty acid composition in rabbits. Each group had 60 g/kg of the

specified oil. The level of plasma TC in rabbits fed coconut oil was significantly higher than the linseed and corn oil, but not significantly higher than the plasma TC level of those rabbits given fish oil. There were no significant differences in serum TG levels among the four groups.

The influence of dietary mackerel oil on blood lipid composition in the young growing pig was investigated (Ruiter et al, 1978). Twelve pigs were randomly assigned to two groups and received either 10% mackerel oil or 10% olive oil. The pigs received 96 grams per day of the oils for 4 weeks. Plasma TG concentrations were significantly lower in the mackerel oil group, 33 mg/dl vs. 58.7 mg/dl in the olive oil group. No significant differences were seen in plasma TC and this may have been due to the fact that olive oil may have plasma TC lowering tendencies as well. Plasma VLDL-C was significantly decreased in the fish oil group, but there were no significant changes between groups for plasma HDL-C or LDL-C.

Parks et al (1987) investigated the effects of a menhaden fish oil diet as compared to a lard diet in African green grivet monkeys. Forty percent of the kcal were supplied from one of the two fats for a period of 10 months. At 8 months, blood samples were taken. The fish oil group had significantly lower plasma TC and HDL-C concentrations, both 33% lower, than the lard group. The plasma LDL-C

concentration of the fish oil group was 34% lower than the lard group, but this difference was not statistically significant. The plasma VLDL-C and IDL values were lower in the fish oil group. The fish oil group had a significant reduction in the protein (46%) of HDL subfractions of intermediate size compared to the lard-fed group. This response to fish oil supplements is distinctly separate from that of humans and non-human primates fed omega-6 polyunsaturated fatty acids, which results in a decrease in concentration of the larger, less dense HDL subfractions.

Weiner et al (1986) investigated the inhibition of atherosclerosis by cod liver oil in a hyperlipidemic swine model. The swine were fed a high cholesterol, high fat diet for a period of 8 months. The study group also ingested 30 ml of cod liver oil per day, which contained 18% EPA and 12% DHA. Both groups of swine had severe hyperlipidemias during the study. The cod-liver oil supplementation had no significant effect on plasma lipid levels (TC, HDL-C, LDL-C, VLDL-C, TG) as compared to the control group. Though the plasma TG levels were less in the fish oil group, this difference was not significant. Sections of the coronary arteries of the swine were examined and there was significantly less arterial calcification seen in the sections of artery from the animals fed cod liver oil than in the control group ($P<0.001$).

Researchers investigated a diet rich in omega-3 fatty

acids and found a significant decrease in plasma TG in the rat (Yamazaki et al, 1987). There were three test groups, with six rats in each group; fish oil diet, safflower diet and a control diet low in fat. The oil supplemented groups received 15% by weight of the diet from the oils. Approximately 30% of the fish oil (MaxEPA) was rich in EPA and DHA. There were no significant differences in plasma TC. The fish oil diet produced a significant decrease in plasma TG as compared to the safflower oil and control groups (505 mg/dl vs. 586 mg/dl vs. 646 mg/dl, respectively) ($P<0.01$).

Studies of the effect of a fish oil diet rich in omega-3 fatty acids in animal models have varying results. The plasma TG lowering effect has been demonstrated in human models. The animal model studies had shown inconsistent changes in plasma TG, TC, HDL-C, LDL-C and VLDL-C. This could be partially due to the different amount of fish oil supplement used per kg of body weight. Also different metabolic factors could be involved in the animal models as compared to humans. No conclusions have been reached on the effects of fish oil on plasma lipids and lipoproteins in humans except for plasma TG and animal studies may help to pinpoint a specific mechanism involved.

Exercise and animal models

Aerobic exercise has been suggested to have the

potential to alter the risk of CHD by favorably altering the plasma TC lipid profile. Exercise has been shown to be effective in increasing plasma HDL-C and reducing plasma LDL-C and VLDL-C levels which should help to reduce the risk of CHD (Haskell, 1984).

Moderate exercise in monkeys eating an atherogenic diet was investigated (Kramsch et al, 1981). Two groups of nine monkeys per group were studied for 35 months and one group of nine monkeys was studied for 42 months. The three groups began on a chow diet for one year, two groups then received an atherogenic diet of 0.1% cholesterol and 10% butter by weight. One of the groups that received the atherogenic diet had been on an exercise training program during the first twelve months. This group was studied for 42 months. The exercise was done on motorless treadmill on wheels. The third group remained on the control diet.

The mean serum TC rose sharply from values of 102 mg/dl in the sedentary control group to 620 mg/dl in both sedentary and exercise groups receiving the atherogenic diet. These increases were mainly due to increases in plasma LDL-C and VLDL-C. There was a small but significant ($P<0.01$) increase in plasma HDL-C in the exercise group as compared to the sedentary atherogenic diet. The mean serum TG levels, LDL-TG and VLDL-TG were significantly lower in the exercise group ($P<0.01$). As compared to the sedentary atherogenic diet group, the plasma TG in the exercise group

rose, but to a significantly lesser extent. One monkey was considered to be a poor exerciser and its plasma lipoprotein levels were similar to those of the monkeys in the sedentary atherogenic diets (Kramsch et al, 1981).

Borer et al (1979) examined the effects of exercise and dietary protein levels on somatic growth, body composition and serum lipid levels in adult hamsters. The design of the study was factorial with physical activity (exercise or sedentary) and dietary protein (casein) levels (18 or 39%) as the treatments. A significant increase in plasma TC was seen in the high protein, exercise treatment group. No changes were seen in percent of body fat or protein. Plasma TG levels were unchanged in any of the four groups.

Sandretto and Tsai (1988) examined the effects of fat intake on body composition and hepatic lipogenic enzyme activities of hamsters shortly after exercise cessation. Forty-two, eight week old golden hamsters were divided into two groups, exercise or sedentary. The running activity of the hamster was recorded as revolutions per day (RPD). The animals were fed a low-fat diet containing 5% fat for the first 30 days. Water and food were provided ad libitum. After 30 days the exercise variable was ended. The groups were then divided into two groups, a high-fat diet which contained 15% fat or the original low-fat diet. The exercise group had a significant increase in weight gain (43% more, $P<0.05$) and food intake (28% more, $P<0.05$) at 30

days. The high-fat diet significantly increased weight gain ($P<0.05$). Serum TG and TC levels were slightly reduced with exercise but the effects were not significant. The plasma HDL-C values were slightly but not significantly higher among the high-fat diet hamsters. The length of the high-fat diet (7 days) was short and it is unknown whether plasma HDL-C and TC values would continue to increase or if a plateau would be reached over a longer time span.

Several studies found similar tendencies in hamsters involved in an exercise regime. Tsai et al (1982) examined the metabolic alterations by voluntary exercise and discontinuation of exercise in hamsters. Thirty-two hamsters were divided into four groups. One group served as a sedentary control and the other three groups had access to voluntary disc running during a 35-day period at different points of the 76-day experimental period. There was a trend toward a decrease in voluntary activity with increase in age in each exercise group. Hamsters gained less weight/day during the exercise period and consumed a greater (10 to 20%) quantity of food. Serum TC concentrations were not significantly changed by exercise or retirement. Plasma HDL-C values were slightly increased in 2 of the exercise groups and significantly higher in one of the exercise groups as compared to the sedentary group. Voluntary running did significantly reduce plasma TG concentrations ($P<0.05$), however, this effect disappeared at 41 days after

the discontinuation of exercise.

Tsai et al (1981) investigated the somatic, endocrine and serum lipid changes during detraining in adult hamsters. Thirty-five hamsters were divided into a sedentary and exercise group. The exercise group had access to voluntary running on horizontal discs for the first 42 days of the experimental period. The level of activity was decreased slightly with age. Voluntary running resulted in a 10 to 20% increase in food consumption however body weights remained similar in both the exercise and sedentary groups. Disc-running significantly decreased percent body fat (7.6% vs. 18.5%) and serum TG levels (205 mg/dl vs. 287 mg/dl) as compared to the control group. Serum TC values were not altered by exercise. Discontinuation of the exercise resulted in a reversal of exercise effects on percent body fat and serum TG levels.

In several studies there has been a consistent trend toward reductions in serum TG levels in hamster with voluntary exercise. A change in plasma TC and HDL-C levels was not consistently observed in the studies. It has been demonstrated by several researchers that there is a similar exercise response in plasma lipid levels in humans, hamsters and other animal models.

JOURNAL ARTICLE

THE EFFECT OF EXERCISE AND FISH OIL SUPPLEMENTS ON THE BLOOD LIPID LEVELS OF THE HAMSTER

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(ABSTRACT)

The effect of aerobic exercise and fish oil supplements on plasma lipid parameters was investigated in the hamster. The hamsters were fed a basal hypercholesterolemic purified diet (0.1% cholesterol, 15% fat) to elicit an elevated lipid response. Fifty-six hypercholesterolemic hamsters were divided into four groups: control, swimming (trained up to one hour every other day), daily fish oil supplementation (.35g/kg/day omega-3), and a swimming/fish oil combination. The effect of these treatments on plasma TC, TG and pooled samples of plasma HDL-C, HDL_2-C, LDL-C and VLDL-C was studied over 12 weeks. All hamsters had a significant increase in body weight. Feed intakes increased at 6 weeks and then declined over the second 6 weeks, but remained above the beginning levels. Plasma TC levels were significantly decreased in all three treatment groups overall (-23 to 26%) and significantly lower than the control group at 12 weeks with fish oil producing the greatest decrease. Plasma TG levels were lower, though not significantly decreased in the swimming (-44%) and fish oil

(-12%) groups, but was significantly decreased in the swimming/fish oil combination (-86%) by week 12. The pooled samples had similar changes in plasma TC and TG in all treatment and control groups. An apparent increase in the pooled plasma HDL-C and HDL_2-C and a decrease in plasma LDL-C was observed in the swimming group. In the present study, it appeared that the fish oil was most effective in lowering plasma TC and the swimming was more effective in reducing plasma TG and raising plasma HDL-C in the hamster.

INTRODUCTION

Coronary heart disease (CHD) remains as the major cause of death in the United States and other western cultures. In the U.S. alone, coronary heart disease accounts for more than 500,000 deaths per year (1). Risk factors such as cigarette smoking, high blood pressure and elevated plasma lipids have been strongly associated with CHD. Researchers have agreed that a plasma total cholesterol (TC) level greater than 240 mg/dl is related to an increased risk of CHD and enhances the negative effects of other risk factors (2). Research has shown that plasma LDL-C levels comprise approximately 60 to 70% of plasma TC and are positively associated with coronary disease (3). A protective effect of plasma HDL-C against CHD has been suggested and is at least as strong as the atherogenic effect of plasma LDL-C; both are independent risk factors (4). The subfraction plasma HDL_2-C has also been associated with a decreased CHD incidence (5). It has been suggested that plasma triglyceride (TG) levels are not an independent risk factor for CHD but have a positive relationship with elevated plasma TC (3, 6). Exercise has been implied to provide a protective effect against the development of CHD by lowering plasma LDL-C, VLDL-C and TG and increasing plasma HDL-C and HDL_2-C (5, 7, 8). The use of fish oil supplements to lower plasma TC and TG has received a great deal of attention from

the research community and the media. The principal effect of fish oil supplements has been a decrease in plasma TG and VLDL-C levels (5, 9, 10). Researchers have reported inconclusive findings of elevations, reductions or no significant changes in plasma TC (11-13), LDL-C (11, 14, 15) and HDL-C (11, 16, 17) with the use of fish oil supplements.

The objective of this study was not only to investigate these two variables, exercise and fish oil consumption, separately, but also to combine these two to explore their combined effects on plasma lipid levels in the hamster. The use of the hamster for cholesterol research with emphasis on CHD is a recent development. If the findings of this research are in accordance with past findings on humans and larger animals with similar plasma lipoprotein patterns, the hamster may become a feasible small animal model for cholesterol research.

METHODS

Animal Model

Seventy-five, 9-11 week old, Golden Syrian male hamsters (102-121 grams) were purchased from Sprague Dawley* Company. The hamsters were housed individually in plastic tubs with wire tops which were lined with corn cob bedding.

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* Sprague Dawley Company, Indianapolis, Indiana

The hamsters remained in a temperature-humidity controlled room having equal 12 hour periods of light and dark. Upon arrival in the laboratory, the hamsters were quarantined for one week and fed a standard chow diet. The hamsters were transferred to the research room for the second week and were continued on the standard chow diet. Records of food consumption and body weight were recorded every other day.

Diet

At the end of the second week, all hamsters were switched to a purified, basal (hypercholesterolemic) diet for the remainder of the study. The basal diet consisted of 71.5% carbohydrate, 10% crude protein, 15.5% fat (2:1:3.3, P:M:S ratio) and 0.1% cholesterol as shown in Table I. Food and water were fed ad libitum throughout the course of the study. The diet was prepared in a single batch, sampled for analysis and stored in the freezer until feeding time. The hamsters were fed using feeding cups that remained in the cages.

Design

Blood samples were drawn from the orbital sinus of the hamsters prior to the introduction of the basal hypercholesterolemic diet to establish normal plasma TC levels. At the end of the third week of the basal

TABLE I

Composition of Experimental Diet

Component	% Dry Weight	% Moisture
Protein (Casein)[1]	10.0*	5.6
Carbohydrate	71.5*	
Cornstarch	36.5	10
Dextrose[2]	28	
Alphacel[3]	4	3.3
Cholesterol[4]	0.1	
DL-Methionine[5]	0.1	
Vitamin Mix[6]	1.0	
Mineral Mix[7]	3.06*	
Choline Chloride[8]	0.3	
Fat	15.5*	
Butter	5.3	15
Puritan Oil	3.2	
Mazola Oil	1.5	

[1-5 8]Casein, Vitamin Free; Dextrose; Alphacel non-nutritive bulk; Cholesterol U.S.P.; DL-Methionine U.S.P.; Choline Chloride; ICN Biomedicals Inc., Costa Mesa, CA.

[6]Vitamin Mix composed of (mg/kg mix) the following: Thiamine hydrochloride 600; Riboflavin 600; Pyridoxine Hydrochloride 700; Nicotinic Acid 3; D-Calcium Pantothenate 1.6; Folic acid 200; D-Biotin 20; Cyanocobalamin 1; Retinyl Palmitate 1.6; DL-alpha-Tocopherol Acetate 20; Cholecalciferol 250; Menaquinone 5; Sucrose 972, ICN Biomedicals Inc, Costa Mesa, CA.

[7]Mineral Mix composed of (gm/2 kg mix) the following: Calcium Phosphate Dibasic 500; Sodium Chloride 74; Potassium Citrate Monohydrate 220; Postassium Sulfate 24; Magnesium Oxide 24; Manganous Carbonate 3.5; Ferric Citrate 6; Zinc Carbonate 1.6; Cupric Carbonate .3; Potassium Iodate .01; Chromium Potassium Sulfate .55; Sucrose 118; ICN Biomedicals Inc, Costa Mesa, CA.

*Values from proximate analysis

hypercholesterolemic diet another blood sample was drawn and tested for any possible elevated cholesterol response. An estimated 20% nonresponder rate was observed. The remaining 56 hamsters were ranked by plasma TC level and randomly assigned to one of four treatments, with 14 hamsters to a treatment group. One group served as the control group receiving only the basal hypercholesterolemic diet, a second group received the basal diet and an exercise component of swimming, a third group received the basal diet and fish oil supplements, and the fourth group was a combination of the second and third, receiving the basal diet, exercise component, and fish oil supplements The experimental design is summarized in Table II.

Exercise treatment

For 12 weeks the hamsters assigned to one of the exercise groups were involved in a swimming program. The swimming was introduced gradually beginning with 5 minutes every other day and increasing to one hour every other day by the eighth week. The swimming apparatus consisted of two large plastic tubs, approximately half filled with lukewarm water. Heating lamps were used to dry the animals after swimming and before placing them back into their individual cages.

TABLE II

Experimental Design

Treatment	---------Week of Blood Samples----------					
	-3	0	3	6	9	12
Control	0	0	0	0	0	0
Swimming	0	0	Y	Y	Y	Y
Fish oil	0	0	X	X	XX	XX
Swimming/Fish oil	0	0	XY	XY	XXY	XXY

Y - swimming regime
X - fish oil supplement received
XX - fish oil supplement doubled

Fish oil treatment

The fish oil supplement (MaxEPA) group received 50 microliters (ul) daily for the first 6 weeks of the treatment period. The fish oil was delivered by a Hamilton microliter syringe and stainless steel feeding tube. The MaxEPA fish oil provided approximately 14 ul of EPA and DHA, which provided 0.375 ml/kg of fish oil daily and 0.175 g/kg of EPA and DHA fatty acids together. At week 7, the fish oil treatment group received 100 ul daily for the remainder of the study. This provided 0.75 ml/kg fish oil and 0.35 g/kg of EPA plus DHA daily. The MaxEPA fish oil was provided by R.P. Scherer Company*. Fatty acid analysis of the fish oil was conducted in the R.P. Scherer Company laboratory and in this laboratory using capillary gas chromatography (18). Peroxide values were also determined to determine the rancidity of the fish oil (19). (See Appendix B).

Swimming/Fish oil treatment

A fourth group received a combination treatment of swimming and MaxEPA fish oil supplementation at the same level as the swimming only and fish oil only treatment groups.

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* R.P. Scherer Company, Troy, Michigan

Analytical Procedures

Blood samples were drawn before the introduction of the basal hypercholesterolemic diet and then every three weeks throughout the remainder of the study. Blood samples (approximately 1.5 ml) were drawn from the orbital sinus of the 12-hour fasted hamsters after the administration of the anesthetic ketamine (20). Plasma samples were obtained by reduced temperature centrifugation at 3000 rpm for 30 minutes. Aliquots of 500 ul of each plasma sample were pooled for later lipoprotein fraction analysis.

Plasma total cholesterol (TC) was determined using the Liebermann-Burchard colormetric reaction (21). Plasma triglyceride (TG) were determined by a quantitative, fully enzymatic colorimetric method using glycerylphosphate oxidase supplied by Stanbio* TG kit #2000-75 (22). The remaining plasma (3.5 ml/sample) was pooled, spun and separated by ultracentrifugation at hydrated density 1.006 for 18 hours (23). The plasma high-density lipoprotein cholesterol (HDL-C) was separated by precipitation of plasma low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) using a heparin manganese chloride solution (23). After ultracentrifugation, the combined HDL-C and LDL-C

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*Stanbio Company, San Antonio, Texas

concentrations were determined in the supernatant and the LDL-C and VLDL-C concentrations were calculated as follows: TC - (HDL-C + LDL-C) = VLDL-C; (HDL-C + LDL-C) - HDL-C = LDL-C. Plasma HDL_3-C was determined in the supernatant obtained after precipitation of HDL_2-C by a dextran sulfate solution (25). Plasma HDL_2-C was determined by difference: HDL-C - HDL_3-C = HDL_2-C.

Statistical Analysis

A general linear model (ANOVA) with split plot repeated measures (week 0, 6 and 12) was used to test for overall differences among the four groups ($P<0.05$). A student t-test was used to locate differences among the means of the four groups ($P<0.05$).

RESULTS

Several hamsters died during the course of the study for various reasons. Four of the hamsters had to be sacrificed because of a condition known as "wet-tail disease". Three hamsters did not appear tolerate the bleeding procedures and four hamsters did not tolerate the swimming component. Throughout the 15 week study we lost a total of eleven hamsters. This resulted in a reduced number of animals in the three treatment groups as shown in Table III.

Table III. Average daily feed intake of hamsters.

	WEEK 0	WEEK 6	WEEK 12
GROUP	----------grams----------		
CONTROL (n=14)	18.4[a] (0.59)	27.5[b] (1.28)	21.1[c 1] (0.71)
SWIMMING (n=9)	17.0[a] (0.70)	25.9[b] (1.51)	19.8[c 2] (0.84)
FISH OIL (n=12)	17.9[a] (0.62)	26.9[b] (1.33)	20.3[c 12] (0.73)
SWIMMING/ FISH OIL (n=9)	17.9[a] (0.74)	27.5[b] (1.59)	18.9[c 2] (0.88)

Values expressed in parenthesis are SEM.
Values expressed in (n=) are the hamsters left in each group at week 12)
[1-2]mean values with different numbers are significantly different in columns ($P<0.05$).
[a-c]mean values with different letters are significantly different in rows ($P<0.001$).

Feed Intake

The differences in feed intake of hamsters within and between the four groups are presented in Table III. All four groups had a significantly greater mean feed intake at week 6 than at week 0 and week 12 ($P<0.001$). At week 12, the swimming/fish oil group had a significantly smaller feed intake than the control group ($P<0.05$) (18.9 vs. 21.1g). The swimming and fish oil groups ate less feed than the control group, but this was not significant.

Weight Gain

Weight gain was noted in all hamsters, most of which could be attributed to growth. The body weight of the hamsters among the four groups is presented in Figure 1. All four groups gained a significant amount of weight from week 0 to week 12 ($P<0.05$). However, a significant loss was seen in the swimming group from week 6 to week 12, from 141 to 135g, not observed in the other three groups ($P<0.05$). At week 12, the swimming group at 135 grams, was significantly lighter in body weight than the control, (147 g) and the fish oil group, (154 g, $P<0.05$). The swimming group weighed less than the swimming/fish oil group also, but this was not significant. The swimming/fish oil group had a similar body weight pattern to the control group.

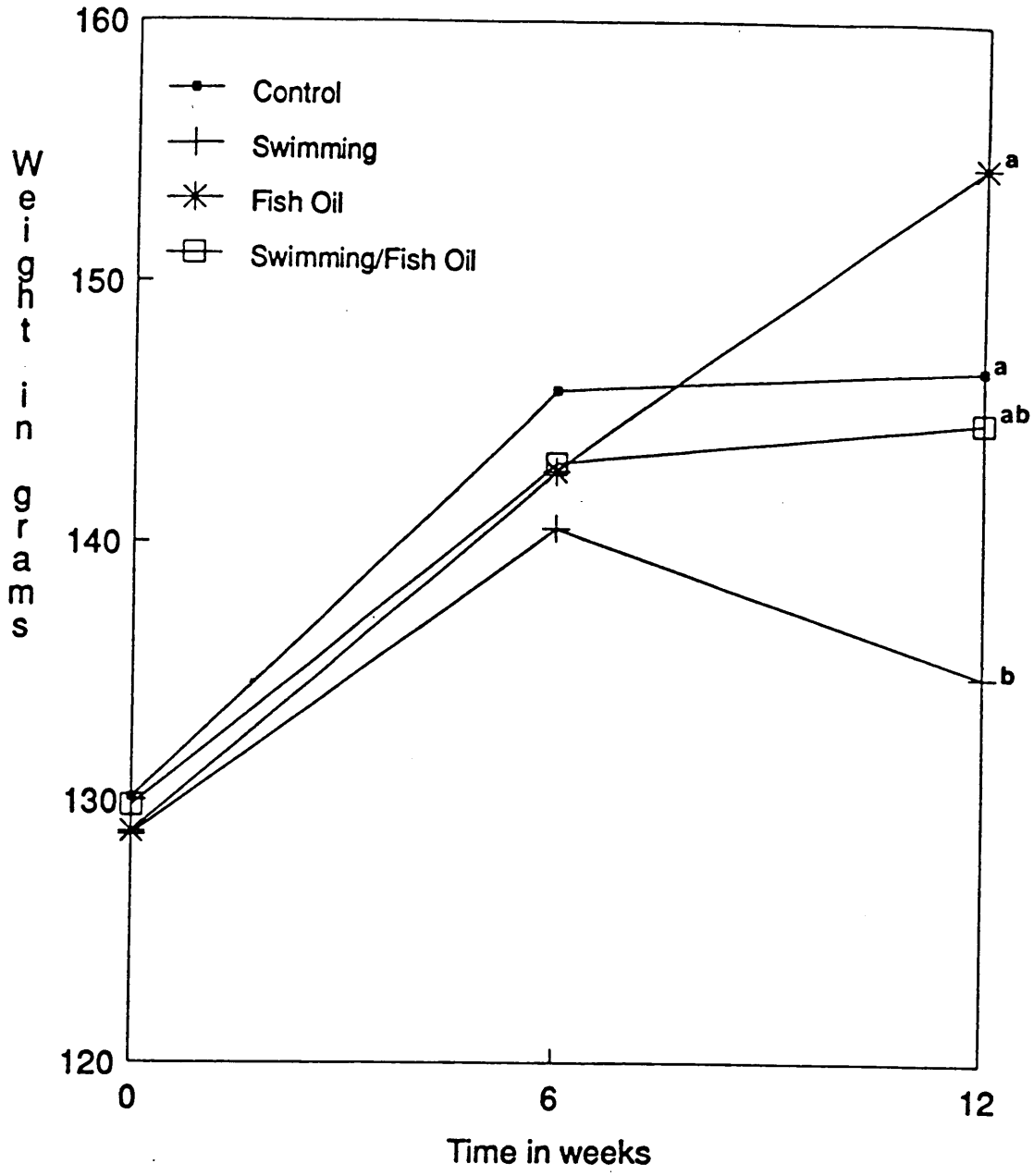

Figure1. Average body weights of hamsters.
$^{a-b}$ mean values with different letters are significantly different at $P<0.05$.

Plasma Total Cholesterol

Plasma TC levels (see Table IV) were significantly increased in all groups from week 0 to week 6 ($P<0.001$). At week 12 the control group remained at the same level as at week 6, 230 mg/dl. The swimming group had a significant decrease in plasma TC, from 252 mg/dl to 195 mg/dl ($P<0.001$) (57 mg/dl, 23%) from week 6 to week 12. The fish oil group had a significant reduction in plasma TC, from 226 mg/dl to 167 mg/dl ($P<0.001$) (59 mg/dl, 26%) from week 6 to week 12. A similiar reduction of plasma TC was seen in the swimming/fish oil group, 248 mg/dl to 189 mg/dl ($P<0.001$) (59 mg/dl, 24%) from week 6 to week 12. From week 0 to 12 a significant decrease in plasma TC was observed in the three treatment groups. At week 12 the swimming, fish oil and swimming/fish oil treatment groups had plasma TC levels significantly lower than the control group ($P<0.01$). Furthermore the fish oil treatment group (167 mg/dl) was significantly lower than the swimming (195 mg/dl) and swimming/fish oil (189 mg/dl) treatment groups.

Plasma Triglycerides

The changes in plasma TG between and within the treatment groups are presented in Table V. At week 0, the swimming/fish oil group started the experimental treatments with a significantly greater plasma TG value (220 mg/dl)

Table IV. Average plasma total cholesterol levels of hamsters.

	WEEK 0	WEEK 6	WEEK 12
GROUP	----------mg/dl----------		
CONTROL	211[a] (5.9)	230[b] (9.4)	231[c1] (9.3)
SWIMMING	214[a] (6.1)	252[b] (11.1)	195[c2] (11.9)
FISH OIL	209[a] (7.0)	226[b] (9.8)	167[c3] (9.6)
SWIMMING/ FISH OIL	217[a] (7.4)	248[b] (11.7)	189[c2] (11.6)

Values expressed in parenthesis are SEM.
[1-3]average values with different numbers are significantly different in columns ($P<0.01$).
[a-c]average values with different letters are significantly different in rows ($P<0.001$).

Table V. Average values of plasma triglycerides of hamsters.

	WEEK 0	WEEK 6	WEEK 12
GROUP	----------mg/dl----------		
CONTROL	137[a] (23.7)	199[b] (32.4)	98[a2] (11.1)
SWIMMING	139[a] (28.1)	227[b] (38.3)	78[a1] (13.2)
FISH OIL	124[a] (24.6)	249[b] (33.6)	109[a2] (11.1)
SWIMMING/ FISH OIL	220[a] (29.6)	353[b] (40.4)	118[c2] (13.9)

Values expressed in parenthesis are SEM.

[1-2]average values with different numbers are significantly different in columns ($P<0.05$).

[a-c]average values with different letters are significantly different in rows ($P<0.004$).

than the other two treatment groups, as well as the control group ($P<0.05$). All four treatment groups had a significant increase (about 40%) in plasma TG from week 0 to week 6 ($P<0.004$). At week 6 the swimming/fish oil group remained considerably higher in plasma TG at 353 mg/dl than the other two treatment groups, and the control group ($P<.05$). At week 12 all four groups had a significant drop (about 61%) in plasma TG values from week 6 ($P<0.004$). At week 12, the swimming group had the lowest plasma TG level 78 mg/dl and was significantly lower than the swimming/fish oil group at 118 mg/dl ($P<0.05$). An overall significant decrease from week 0 to week 12 was seen in the swimming/fish oil group, from the initially high 220 mg/dl to 118 mg/dl ($P<0.004$). However, the final plasma TG level for the swimming/fish oil group was similar to the other treatment groups and the control.

The changes in plasma TC and TG seen above were similar to the feed intake levels. Although a trend was apparent, no significant correlations were determined.

Lipoprotein Cholesterol Analysis-Pooled Samples

In order to do separate plasma lipid fraction analysis, a minimum of 3.5 ml was necessary for the equipment available in the laboratory. Only 1.5 ml of blood could be taken from each hamster at each bleeding time. In order to

have adequate amounts of plasma each treatment group was divided into two pooled samples to do the plasma lipid fraction analysis. With only two observations per treatment it was not feasible to do statistical analysis. The plasma lipid fractions of the pooled samples for each of the four groups are presented in Table VI. At week 0, the plasma TC values were similar among the four groups. This would be expected as they were ranked and randomly assigned by blocks of four. The plasma TC values of the four groups continued to increase (21%) to week 6 similar to the means of the individual analysis. From week 0 to 12 there was a reduction in plasma TC of 7% for the control, 12% for the swimming, 17% for the fish oil and 12% for the swimming/fish oil groups.

The plasma HDL-C values of the pooled samples increased 20% in the control group, 26% in the swimming group, 13% in the fish oil group and 32% in the swimming/fish oil group. The percentage of plasma HDL-C in the TC was considerably higher (74%) in the the swimming group at week 12. The percentage of plasma HDL_2-C in the TC was 60% in the swimming group which was also considerably higher than the other two treatment groups and the control. The other two treatment groups had values similar to the control.

The plasma LDL-C fractions of the pooled samples had results similar to plasma TC. From week 0 to week 12 there

Table VI. Cholesterol levels in lipoproteins of pooled samples of hamsters expressed in mg/dl.

		Control	Swimming	Fish oil	Swimming/ Fish oil
WEEK -3	TC	71			
	HDL	37 (52)			
	HDL_2	26 (37)			
	LDL	29 (41)			
	VLDL	5 (7)			
	TC/HDL	1.90			
WEEK 0	TC	209	202	193	206
	HDL	99 (47)	96 (48)	92 (48)	80 (39)
	HDL_2	94 (45)	93 (46)	87 (45)	77 (37)
	LDL	99 (47)	83 (41)	81 (42)	90 (44)
	VLDL	11 (5)	24 (12)	20 (10)	36 (18)
	TC/HDL	2.10	2.10	2.09	2.58
WEEK 6	TC	236	268	250	266
	HDL	163 (69)	166 (62)	123 (49)	140 (53)
	HDL_2	113 (48)	138 (52)	88 (35)	111 (42)
	LDL	57 (24)	43 (16)	70 (28)	37 (14)
	VLDL	26 (11)	59 (22)	57 (23)	89 (34)
	TC/HDL	1.45	1.61	2.03	1.90
WEEK 12	TC	194	176	160	182
	HDL	123 (63)	130 (74)	106 (66)	118 (65)
	HDL_2	78 (40)	106 (60)	82 (51)	75 (41)
	LDL	59 (30)	32 (18)	46 (29)	48 (26)
	VLDL	12 (6)	14 (8)	8 (5)	16 (9)
	TC/HDL	1.58	1.35	1.51	1.54

Values in parenthesis are percentages of the lipid fraction of plasma TC.

was a reduction in plasma LDL-C of 4% in the control group, 61% in the swimming group, 43% in the fish oil group and47% in the swimming/fish oil group. The swimming group had a considerably lower plasma LDL-C level as well as the percentage of plasma LDL-C in the TC than did the other two treatment groups and the control group.

From week 0 to week 12 there was a reduction in plasma VLDL-C of 40% in the swimming group, 60% in the fish oil group, 56% in the swimming/fish oil group and a 8% increase in the control group. The final plasma VLDL-C values were similar in all four groups.

The TC/HDL ratio is also presented in Table VI. The TC/HDL ratio decreased in all four groups from week 0 to week 12. At week 12, the swimming group had a much lower TC/HDL ratio of 1.35 than the other two treatment groups and the control. The two treatment groups had TC/HDL ratios similar to the control group at week 12.

DISCUSSION

Although hamsters have the most abundant plasma LDL-C among rodents, with a ratio of cholesterol content between plasma HDL-C and LDL-C being about 1:1 (26), the use of the hamster as a small animal model for cholesterol research has been limited. It had been reported that hamsters responded to a hypercholesterolemic diet in a manner similar to that

observed in humans who consumed a diet high in saturated fat (27). Spady and Dietschy (28) found that the hamster exhibited a sterol synthesis rate comparable to that found in human liver biopsies. Thus it was concluded that the hamster was a good model to study cholesterol research and in particular, the LDL-C regulation mechanisms since the hamster responded to changes in the diet in the same manner as humans (27, 29).

After three weeks on the hypercholesterolemic diet (0.1% cholesterol), an elevated response was seen in the plasma TC and TG in the hamsters of this study (See Tables IV & V). This was similar to elevated responses in the hamster when varying amounts of cholesterol were added to the diet (0.1%, 0.15%, 0.25% and 1%) in other studies (27, 28, 30). After three weeks (week 0) an increase in the plasma lipoprotein fractions was observed in the pooled samples, especially in plasma LDL-C and HDL-C after the introduction of the hypercholesterolemic diet as shown in Table VI. Tsuda (26) had similar results after one week on a 1% cholesterol diet in which the hamster plasma lipoprotein fractions, LDL-C and HDL-C, increased also. A considerable increase in plasma HDL_2-C was also observed in the present study, with the majority of the plasma HDL-C being found in HDL_2-C.

All four treatment groups had significant increases in

plasma TC from week 0 to week 6 ($P<0.001$) (See TableIV). This may have been associated with a continued elevated plasma TC response to the hypercholesterolemic diet along with the increased feed intake (34% increase) which resulted in greater total cholesterol consumption for the hamsters from week 0 to week 6. However, a significant correlation between the feed intake and plasma TC was not observed. A similar continual rise in plasma TC was observed when hamsters consumed a basal hypercholesterolemic diet for a 30 day period (27, 28, 30).

Although the four groups had a similar significant increase in plasma TC from week 0 to week 6, major significant decreases were observed from week 0 to 12 in the three treatment groups. As observed in the swimming treatment group, several human exercise studies have had significant decreases in plasma TC, all of which were accompanied by an alteration of the lipoprotein profiles (31, 32). In addition to the exercise, the significant decrease in plasma TC may have been partially due to a significant decrease in body weight and feed intake from week 6 to 12. The drop in body weight and feed intake appeared to begin as the hamsters started to approach one hour of swimming at week nine of the study. To maintain one hour of swimming was impossible for all but five of the hamsters in the swimming group and one of the hamsters in

the swimming/fish oil group after week nine. A possible relationship between forced and/or exhaustive exercise and weight loss and a decrease in plasma TC may have been involved. A classical study by Mayer et al (33) suggested an exhaustion curve in mice where both body weight and caloric intake dropped off as the length of exercise increased to an exhaustive effort. The drop in body weight may be involved, but it should be pointed out that although the feed intake decreased significantly for the other three groups also, body weight did not decrease as it did for the swimming treatment. A comparable decrease in plasma TC occurred in the swimming/fish oil group, a greater decrease was seen in the fish oil group and there was no change in the control group. Though all but one of the hamsters in the swimming/fish oil group was not able to maintain the one hour swimming period, a significant decrease in plasma TC was observed. In several animal studies where voluntary exercise was involved, plasma TC levels were not significantly decreased (34, 35). One study investigated the effect of hamsters doing voluntary disk running for 35 days and found no significant effects on the plasma TC (34).

Fish oils are widely promoted as hypocholesterolemic, antiatherogenic agents, however, for these results to be observed, very high doses of fish oil supplements must be consumed (12). The fish oil group (.35g/kg omega-3 fatty

acids/kg body weight) in this study did have a significant decrease in plasma TC from 0 to 12 weeks which was significantly lower than the other two treatment groups and the control at week 12. Similar significantly lower plasma TC values have been observed in nonhuman primates receiving 40% of the calories from menhaden fish oil (36). Different results were found with young pigs receiving 100 grams of fish oil (.5 g omega-3 fatty acids/kg body weight) per day where no significant changes in plasma TC were observed (37). The plasma TC of the fish oil group did decrease significantly although a continual increase in body weight was observed throughout the study and feed intake was similar to the control group. Although the dose of EPA plus DHA at .35g/kg (100 ul dose during weeks 6 to 12) was lower than observed in young pigs, the fish oil appeared to have a hypocholesterolemic effect in this study. As discussed earlier, the swimming/fish oil group also had a significant though not as much of a decrease in plasma TC as the fish oil treatment from week 0 to week 12. It is unclear if the hypocholesterolemic effect seen in the swimming/fish oil group was elicited by the swimming treatment or the fish oil treatment. The effects were not additive, however.

No significant decreases in plasma TG were observed in the swimming, fish oil and control groups from week 0 to 12. A significant decrease in plasma TG was observed in the

swimming/fish oil combination group from week 0 to 12. At week 6, both feed intake (See Table III) and plasma TG (See Table V) were significantly higher than week 0 in the four groups. However, from week 6 to 12 there was a significant decrease in plasma TG and feed intake in the four groups. When tested, however, there were no significant correlations between feed intake and plasma TG in any of the experimental groups. Although a significant decrease from week 0 to 12 was not observed in the swimming group, a 44% decrease was observed (-61 mg/dl). The swimming group did have a significant decrease in body weight, in addition to the decreased feed intake, from week 6 to 12 which may have contributed to the decrease in plasma TG. Several human studies have reported that weight loss appeared to be related to lower plasma TG concentrations in endurance athletes (38). Reductions in plasma TG have been associated with physical exertion in humans (38). Since the fish oil treatment did not significantly lower plasma TG and given the substantial change seen in the swimming group it would appear the swimming component of the swimming/fish oil group played a role in the significant decrease in plasma TG levels.

Fish oil supplements have been reported to consistently reduce plasma TG levels in humans (39). A significant decrease in plasma TG was not observed from week 0 to 12 in

the fish oil group even though the dose was doubled (100ul/day) to .35 g omega-3 fatty acids/kg body weight. Similar results have been observed in rabbits receiving a fish oil supplement, where a TG-lowering effect was also not observed (40). Young pigs receiving fish oil supplements (.5 g omega-3 fatty acids/kg body weight) had significant decreases in plasma TG (37). The dose of fish oil given to the pigs is higher than the dose the hamsters received in the present study. It appeared that the dose given to the hamsters in this study did not promote a TG-lowering effect as similar decreases were observed in the control. The overall lack of a TG-lowering effect in the hamsters may have been dose related. A dose response was observed with a decrease of plasma TG levels with 5 (14% decrease), 10 (23% decrease), and 20 grams (32% decrease) MaxEPA fed humans for three weeks (41).

Although this group did have a significantly higher starting value, the swimming/fish oil treatment produced a 102 mg/dl drop to levels similar to the other treatments. It would be reasonable to suggest a TG-lowering effect was related to the combined effects of the swimming and fish oil group treatments.

An interesting observation of this study was the overall increases at week 6 and decreases at week 12 of plasma TC and TG and feed intake of the three treatment

groups and the control group. The hamster may have the ability to metabolically adapt to dietary or environmental changes. The precise mechanism by which dietary sterol regulates plasma TC, TG, LDL and other lipoprotein pathways is not known. It was thought that increased intake of dietary cholesterol may lead to a larger cholesterol pool and, in turn, suppress the synthesis of necessary enzymes for cholesterol synthesis and the LDL-C receptor (27). A stabilizing effect was noted in the hamsters that received a 0.5% cholesterol (w/w) addition to the diet. A plasma LDL-C increase was noted in four days and a decrease in LDL-receptor uptake was observed. By day 30, the LDL-receptor uptake had returned to its original rate (27).

Similar changes over the 12 week period were observed in the pooled lipoprotein fractions of the three treatment groups and the control group. The only exception was observed in the swimming group with higher levels of plasma HDL-C and HDL_2-C and apparently lower plasma LDL-C from week 0 to 12. Several human studies have reported results with chronic exercise that did not significantly change plasma TC, (42, 43) but instead altered the lipoprotein fractions of plasma LDL-C and HDL-C. Monkeys that experienced a moderate treadmill exercise had a significant increase in plasma HDL-C and a significantly lower plasma LDL-C (35), although no significant changes in plasma TC were found.

The swimming/fish oil group did not have an increase in plasma HDL-C and HDL_2-C nor a decrease in LDL-C as seen in the swimming group. The fact that only one hamster was capable of maintaining the one-hour swimming period over the 12 weeks, may have contributed to this difference.

The TC/HDL ratio has been suggested to be a good indicator of CHD risk. In humans a ratio below 4.5 appears to indicate a low risk of CHD and as the ratio decreases there is a smaller risk of CHD (44). As presented in Table VI, the TC/HDL ratio decreased in all four groups from week 0 to 12. However, the swimming group had a much lower TC/HDL ratio (1.35) at week 12 than the other two treatment groups and the control. Even though the plasma TC levels of the two swimming groups were the same, the swimming/fish oil combination group's TC/HDL ratio (1.54) did not decrease as much as the swimming group did. It is possible that there was less of an exercise effect because only one out of nine hamsters maintained the one hour exercise period while others reduced their swimming times in the swimming/fish oil group. In contrast, five out of nine hamsters in the swimming group maintained one hour of exercise throughout the rest of the project. Although the fish oil group had a significantly lower plasma TC at week 12 than the control and the two treatment groups, the TC/HDL ratio (1.5) was not as low as the swimming group.

In summary, the results of the present study showed a significant decrease in plasma TC in the three treatment groups from week 0 to 12. It appeared that the fish oil group had a greater impact on plasma TC in the hamster and values in this group were significantly lower than the swimming and swimming/fish oil treatments as well as the control at week 12. No significant changes in plasma TG were observed in the swimming and fish oil treatment groups from week 0 to 12. A significant decrease in plasma TG was observed in the swimming/fish oil group. When combined with the considerable drop in plasma TG seen in the swimming group from week 0 to week 12 it appears exercise was effective in lowering plasma TG in hamsters. The changes in the plasma TC and TG levels may have been associated with the changes in feed intake in the hamsters though plasma TC did not change in the control group at week 12. The pooled lipoprotein fractions had similar increases and decreases in the three treatment groups and in the control group. There appeared to be higher plasma HDL-C, though not significant, found mostly in the HDL_2-C in the swimming group and a lower plasma LDL-C and TC/HDL ratio.

In the present study, it appeared that the swimming, fish oil, and the swimming/fish oil treatments had beneficial results in reducing CHD risk variables by significantly lowering plasma TC. A significant decrease in

plasma TG was also observed in the swimming/fish oil combination group. There was the suggestion that the plasma HDL-C increased in the swimming group, specifically the HDL_2-C, when the pooled plasma lipoprotein samples were analyzed. The use of the hamster for small animal model research provided some valuable information relative to the effect of exercise and fish oil supplements on blood lipid levels. However, more research is needed to substantiate these results and to further elucidate the effects of the swimming and fish oil treatments on plasma lipid levels in the hamster.

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SUMMARY AND CONCLUSIONS

CHD is the major cause of death in the U.S., with over 500,000 deaths attributed to heart disease yearly. Several risk factors have been independently associated with CHD such as cigarette smoking, high blood pressure, and elevated plasma lipid levels. Many researchers have agreed that levels of plasma TC greater than 240 mg/dl are related to an increased risk of CHD. The major plasma lipoprotein in humans is plasma LDL-C which comprises approximately 60-70% of plasma TC and is positively associated with CHD. Another plasma lipoprotein fraction, plasma HDL-C and its subfraction plasma HDL_2-C, have been suggested to have a protective effect against CHD.

Lowering certain lipid variables has been shown to decrease the incidence of CHD such as plasma TC, LDL-C, VLDL-C and TG, as well as increasing plasma HDL-C and HDL_2-C. Specifically, exercise has been shown in several human trials to provide a limited plasma TC lowering effect, but more importantly, to alter the plasma lipoproteins fractions of plasma TC by lowering plasma LDL-C and raising plasma HDL-C. The principal effect of fish oil supplements appeared to be a decrease in plasma TG and VLDL-C. Inconclusive results have been reported with the effect of fish oil supplementation on plasma TC, LDL-C and HDL-C.

The hamster was used in this study because of a higher plasma LDL-C than in other rodents, with a ratio of plasma LDL-C to HDL-C of 1:1. When consuming a diet with added cholesterol, the hamster has shown an elevated plasma TC and LDL-C response, as did humans consuming a diet high in saturated fat.

The present study was conducted to evaluate the effects of exercise (swimming) and fish oil supplements and a swimming/fish oil combination treatment on plasma TC, TG, and pooled plasma lipoprotein fraction levels of 56 hamsters. Prior to the beginning of the study, the hamsters were fed a chow diet for two weeks. They were then were introduced to a basal hypercholesterolemic diet (0.1% cholesterol) for the remainder of the study. After three weeks on the basal hypercholesterolemic diet, blood samples were drawn to determine an elevated lipid response. The 56 hamsters were randomly divided into four groups of 14 hamsters each; control, swimming, fish oil and swimming/fish oil combination groups. Blood samples were drawn (1.5ml) from the orbital sinus of the fasted hamsters prior to the introduction of the basal hypercholesterolemic diet and every three weeks for the remainder of the study. The plasma samples were analyzed for plasma TC, TG and lipoprotein fractions of HDL-C, HDL_2-C, LDL-C and VLDL-C.

The swimming treatment consisted of a training regime, beginning with a 5 minute period every other day and working

up to a 60 minute period. The fish oil group received 50 ul (.175 g omega-3 fatty acids/kg body weight) per day of MaxEPA fish oil for the first six weeks, and then the dose was increased to 100 ul (.35 g omega-3 fatty acids/kg body weight) per day for the last six weeks of the study. The swimming/fish oil group combined the swimming and fish oil treatments.

All four groups had a significant increase in feed intake from week 0 to 6. A significant decrease in feed intake was observed from week 6 to 12, although the feed intakes were significantly higher than at week 0. Weight gain was observed in all hamsters probably due to growth over the study period. All four groups significantly increased weight from week 0 to 12. However, a significant loss in body weight was observed in the swimming group from week 6 to 12, 141 to 135 grams, which was not observed in the other three groups.

A significant increase in plasma TC was observed in all four groups from week 0 to 6 ($P<0.001$). This may have been partially due to a continued plasma TC response to the hypercholesterolemic diet with a continued increase (34%) in feed intake. However, no significant correlation was observed between the plasma TC and feed intake.

At week 12, the control group remained at the same level of plasma TC of 230 mg/dl as in week 6. A significant decrease in plasma TC was seen in the three treatment groups

from week 0 to week 12. The fish oil group plasma TC levels were significantly lower than the swimming, swimming/fish oil and control groups at week 12.

A significant decrease in plasma TG was not observed in the control, swimming or fish oil groups from week 0 to week 12. However, a significant decrease in plasma TG was seen in the swimming/fish oil group. The swimming group had a non-significant 44% decrease in plasma TG which may have contributed to the significant decrease seen in plasma TG in the swimming/fish oil group.

The hamster may have the ability to adapt to dietary or environmental changes over time. In the present study, increases at week 6 and decreases at week 12 were observed for plasma TC, TG, and feed intake in all three treatment groups and the control. Similar changes were observed over the 12 week period in the pooled lipoprotein fractions of the three treatment groups and the control. By week 12, the plasma lipoprotein fractions were similar between the control, the fish oil and the swimming/fish oil groups. However, the swimming group did have an increase in plasma HDL-C, specifically HDL_2-C, and a decrease in plasma LDL-C.

In conclusion, it appeared that the swimming, fish oil, and swimming/fish oil treatments had beneficial results in reducing CHD risk variables by significantly lowering plasma TC. In addition, the swimming/fish oil group had a significant reduction in plasma TG. The changes in plasma

TC and TG at week 6 and week 12 may have been associated with the changes in the feed intake of the hamsters, although plasma TC did not change in the control group at week 12. An apparent increase in plasma HDL-C, especially HDL_2-C, and a reduction of plasma LDL-C was observed in the swimming group. In the present study it appeared that the fish oil was more effective in lowering plasma TC, and the swimming regime was more effective in reducing plasma TG and raising plasma HDL-C.

The use of the hamster in the present study provided some valuable information on the effects of swimming and fish oil supplements on blood lipids levels. More research is necessary to further explain the metabolic adaptations of the hamster from the effects of the swimming and the fish oil treatments on their plasma lipid levels.

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APPENDICES

APPENDIX A

METHODOLOGY

METHODOLOGY

Analytical Procedures

Blood samples were drawn before the introduction of the hypercholesterolemic diet and then every three weeks throughout the remainder of the study. Blood samples (approximately 1.5 ml) were drawn from the orbital sinus of the 12-hour fasted hamsters after the administration of ketamine (Timm, 1979). Plasma samples were obtained by reduced temperature centrifugation at 3000 rpm for 30 minutes. Approximately 500 ml of each plasma sample was saved for the pooling of two samples of each treatment to do lipoprotein fraction cholesterol analysis.

Chemical analysis of individual and pooled plasma total cholesterol (TC) was determined by methods using the Lieberman-Burchard colorimetric reaction (Schoenhemier and Warren, 1934). Individual plasma triglycerides (TG) were determined by a quantitative, fully enzymatic colorimetric method using glycerophosphate oxidase supplied by Stanbio* TG kit #2000-75 (Wahlefeld, 1976). The remaining plasma was pooled up to about 3.5 ml per sample (2 samples per treatment) and was spun and separated by ultracentrifugation for 18 hours (Lipid Research Clinics Program, 1974). The plasma high density lipoprotein cholesterol (HDL-C) was

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*Stanbio Laboratory, Inc., San Antonio, Texas

determined in the supernatant after being separated by precipitation of the plasma LDL and VLDL by a heparin-manganese chloride solution (Albers et al, 1978). After ultracentrifugation, the VLDL was taken off the top and discarded and the combined HDL-C and LDL-C were determined in the natant and the LDL-C and VLDL-C were determined by difference: TC - (HDL-C + LDL-C) = VLDL-C and (HDL-C + LDL-C) - HDL-C = LDL-C. Plasma HDL_3-C was determined in the supernatant obtained after precipitation of HDL_2-C with a dextran sulfate solution (Gidez et al, 1982). Plasma HDL_2-C was determined by difference: HDL-C - HDL_3-C = HDL_2-C.

A pilot study was conducted in order to allow the researcher to become familiar with experimental and analytical procedures and sampling and handling animals. Blood samples were taken throughout the pilot study in order to establish technique and a reasonable level of consistency in plasma lipid analysis. Feed consumption and weight gain were monitored in the pilot study to make sure the experimental diet was acceptable to and would support normal growth in the animals.

Laboratory Procedures

DETERMINATION OF FATTY ACIDS BY GAS CHROMATOGRAPHY

Methylation Procedure

1. Place 300 mg of vegetable oil into a glass test tube.

2. Add 5 ml of absolute methanol and 4 ml of 0.8N sodium methoxide (1.4 g/100 ml methanol).
3. Heat at 68 C for 15 minutes.
4. Allow to cool or place on ice.
5. Transfer to a separatory funnel.
6. Add 10 ml of petroleum ether and 5 ml of distilled water, rinsing tube. Mix by inversion.
7. Allow to separate.
8. Save the top layer and discard the bottom layer.
9. Place top layer into a test tube and evaporate down to approximately 3 ml. Vortex, purge with argon and seal with a teflon lined cap. Freeze until gas chromatographic (GC) analysis.

GC Analysis

1. Shimadzu Model 9A gas chromatograph.
2. Use temperature programming mode.
3. Column SP-2330, 30 meters, 0.32 I.D., 0.20 uM Film
4. Start temperature 180 C; 6 C per minute to final temperature of 200 C.
5. Split injection.
6. Injection amount 0.5 ul.

DETERMINATION OF PEROXIDE VALUES (PV)

1. Prepare acetic acid/chloroform solution by mixing 3 volumes acetic acid with 2 volumes chloroform.

2. Prepare saturated potassium iodide (KI) solution by dissolving excess KI in freshly boiled water. Excess solid must remain.
3. Weight 5 g sample accurately into a 250 ml erlenmeyer flask. Add 30 ml acetic acid/chloroform solution. Swirl to dissolve.
4. Add 0.5 ml saturated KI solution and let stand with occasional shaking for one minute.
5. Add 30 ml water. Add starch indicator solution.
6. Slowly titrate with 0.1N sodium thiosulfate until the blue color just disappears in the top layer of the solution. Bottom layer may remain slightly yellow due to the color of the oil.
7. Calculation of PV:

$$\text{PV (meq perioxide/kg sample)} = \frac{S \times N \times 100}{\text{g sample}}$$

S = ml sodium thiosulfate titrated (blank corrected)
N = normality of sodium thiosulfate solution

ORBITAL SINUS BLEEDING PROCEDURE

1) Allow 8 hour fast and then administer 15cc of ketamine to hamster, allow approximately 10 minutes to take affect.
2) Holding animal on a flat surface, the operator's thumb is used to apply pressure to the external jugular vein. The forefinger of the same hand is used to pull the dorsal eyelid back.

3) Use a heparin capillary tube (used for hematocrit determination) to penetrate the orbital conjuntiva and rupture the orbital sinus.
4) Blood flow will cease when the tube is released and pressure is removed from the external jugular vein.
5) Animals completely recover within 24 to 48 hours allowing for serial sampling on a routine basis.

DETERMINATION OF PLASMA TOTAL CHOLESTEROL (TC)

1) To blank test tube add 25 ul distilled water.
2) To standard test tubes add 25 ul each of 50, 100, 200 and 400 mg/dl cholesterol calibrator. (These were used for the TC standard curves). Run in duplicate.
3) To test tubes, add 25 ul plasma standard in duplicate.
4) To test tube add 25 ul unknown plasma. Run in duplicate.
5) To each test tube add 1.5 ml of SR Direct Cholesterol Reagent* and mix well (vortex).
6) Place all test tubes in a 37 C water bath for 20 minutes.
7) Remove all test tubes from water bath and vortex thoroughly. Zero the spectrophotometer with the reagent blank at 625 nm. Read specimens and standards within 30 minutes.
8) Determine unknown plasma TC values from the standard curve.

* Cholesterol Reagent was mixed under a hood in an ice bath the day before. Combine in the following order and slowly add to the reagent bottle--DO NOT MIX! USE CAUTION, very strong reaction possible.

1245 mls acetic anhydride
1245 mls glacial acetic
408 mls sulfuric acid
102 mls phosphoric acic

Put bottle in ice overnight in the refrigerator. Gently mix before use after the solution has cooled.

DETERMINATION OF PLASMA TRIGLYCERIDES (TG)

1) To test tube add 1 ml reagent for the blank.

2) To standard test tubes add 1 ml reagent, 10 ul of standard (200 mg/dl) in duplicate.

3) To test tube add 1 ml reagent and 10 ul of unknown samples in duplicate.

4) Incubate all test tubes at room temperature for 10 minutes.

5) Zero spectrophotometer with reagent blank at 500 nm.

5) Read standard and samples at 500 nm within 60 minutes.

6) Plasma TG calculations:

$$\text{Plasma TG(mg/dl)} = \frac{Au}{As} \times 200$$

DETERMINATION OF PLASMA HDL-C

Reagents

1. Manganese chloride solution 1.06M (MnCl 4H O-MW=197.91) Weigh out 209.78g MnCl .H 0 and dissolve in a small amount of distilled water. Dilute to 1 liter with

distilled water. This solution is thought to be stable indefinitely.

2. Sodium chloride 0.15M(saline): Weigh out 8.77 g NaCl and dissolve in about 500 ml distilled water. Bring to 1 liter with distilled water.
3. Heparin solution (40,000 units/ml): Commercial heparin preparations contain different strengths of heparin by weight (units per mg). CHECK LABEL ON HEPARIN VIAL BEFORE PREPARATION: If heparin preparation contains 140 units/mg, weigh out 0.286g heparin and dissolve in 1 ml .15 M saline. Since the volume is so slight, use a very small glass vial to prepare solution. The heparin is hard to dissolve so it will be necessary to vortex the solution vigorously for some time, perhaps even overnight. Before removing an aliquot for the combined reagent, let the heparin solution set awhile and check to make sure all the heparin is in solution. Shoud be stable for about one week if refrigerated.
4. Combined heparin-manganese reagent: Add 0.6 ml 40,000 units heparin/ml to 10 ml 1.06 M MnCl .4H 0. Prepare fresh for each run.

Procedure

1) Warm plasma samples to room temperature.
2) Mix plasma samples well. Using a calibrated micropipet transfer 200 ul plasma into a small plastic

conical centrifuge tubes. Run in duplicate if possible.

3) Vortex combined heparin-manganese reagent well. Transfer 20 ul combined reagent into conical centrifuge tube containing plasma sample.

4) Vortex each tube lightly and allow the tubes to stand at room temperature for 10 minutes.

5) Centrifuge tubes in refrigerated (4C) centrifuge for 10 minutes.

6) Carefully transfer the supernatant with a disposable pipet into a 7 ml or larger vial.

7) HDL are suspended in the supernatant and are determined the same as the plasma TC is determined as unfractionated plasma as described earlier. Prepare standard solutions of 25, 50, 100 and 200 mg/dl with appropriate dilutions of the test standards for making the standard curve. Analyze in duplicate.

DETERMINATION OF PLASMA HDL_2-C*

Reagent

1) Dextran sulfate reagent: Add 143 mg to a 10 ml dextran sulfate to a 10 ml volumetric flask. Bring to volume with 10 ml of 0.15M sodium chloride.

Procedure

1) Follow procedure for determining HDL-C.

2) Remove 100 ul from the HDL supernatant and transfer to

plastic conical centrifuge tubes.

3) Add 10 ul dextran sulfate solution. Vortex well.

4) Allow tubes to sit at room temperature for 20 minutes.

5) Centrifuge tubes in refrigerated (4C) centrifuge for 15 minutes. A hard pellet will form at the bottom of the tube.

6) Transfer supernatant and analyze as discussed in the determination for plasma TC. Prepare 25, 50 and 100 mg/dl standard solutions for standard curve.

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* VPI & SU, Blacksburg, VA. Procedure was modified by Leslie Reynolds.

APPENDIX B

GAS CHROMATOGRAPHY AND LIPID PEROXIDE VALUES

TABLE VII. Gas chromatography (GC) and lipid peroxide values (PV) at Week 0 and Week 8.

	Week 0+	Week 8*
GC	30.8% DHA & EPA (12.8% DHA, 18% EPA)	32.4% DHA & EPA (14.4% DHA, 17.9% EPA)
PV	4.27 mEq/kg	10.25 mEq/kg

+ Laboratory analysis done by R. P. Scherer Company.
* Laboratory analysis done by laboratory at VPI & SU.

APPENDIX C

AVERAGE BODY WEIGHTS

Table VIII. Average body weight of hamsters.

	Week 0	Week 6	Week 12
GROUP	----------grams----------		
CONTROL	130.1[a] (2.37)	145.9[b] (2.79)	146.7[b2] (3.71)
SWIMMING	128.7[a] (2.81)	140.6[b] (3.31)	134.9[c1] (4.39)
FISH OIL	128.8[a] (2.46)	142.8[b] (2.90)	154.5[c2] (3.85)
SWIMMING/ FISH OIL	129.8[a] (2.96)	143.1[b] (3.49)	144.7[c12] (4.63)

Values expressed in parenthesis as SEM.
[a-c]mean values with different letters are significantly different in rows ($P<0.05$).
[1-2]mean values with different numbers are significantly different in columns ($P<0.05$).

APPENDIX D

INDIVIDUAL PLASMA TOTAL CHOLESTEROL AND TRIGLYCERIDE VALUES

Table of plasma TC levels (mg/dl) every three weeks.

SUBJECT	GROUP	-3	0	3	6	9	12
34	C	74	257	185	260	221	220
67	C	62	239	201	244	227	248
31	C	72	228	154	222	218	230
27	C	79	225	182	265	197	222
68	C	51	219	226	250	178	231
56	C	54	210	165	178	160	174
18	C	76	203	155	174	268	308
5	C	71	204	203	236	178	217
29	C	75	200	183	224	144	192
3	C	49	198	210	236	178	196
60	C	68	219	179	250	194	216
54	C	72	190	182	224	156	331
39	C	70	185	208	258	214	218
48	C	64	177	180	192	136	225
70	S	76	253	155	242	212	206
55	S	82	240	230	259	182	212
45	S	77	225	202	267	199	222
47	S	77	222	250	302	178	158
25	S	69	212	199	222	170	160
7	S	70	211	168	206	-	-
52	S	76	204	206	254	164	246
6	S	69	202	194	249	147	179
75	S	44	198	217	222	194	220
50	S	60	195	213	264	203	141
61	S	76	191	164	-	-	-
13	S	81	186	-	-	-	-
9	S	78	185	201	238	184	208
12	S	62	177	122	-	-	-
46	F	69	248	226	234	222	209
35	F	73	236	191	250	188	153
44	F	78	240	192	352	166	194
64	F	68	224	228	258	182	179
73	F	45	222	165	181	239	205
59	F	77	209	195	200	142	145
10	F	54	205	183	-	-	-
63	F	72	210	188	221	208	162
58	F	70	198	163	212	109	164
8	F	72	195	200	286	244	173
26	F	63	191	168	184	156	142
17	F	92	185	198	205	196	176
36	F	57	182	176	218	194	172
23	F	49	170	124	160	128	126

(continued)

Table of plasma TC levels (mg/dl) every three weeks.

SUBJECT	GROUP	-3	0	3	6	9	12
42	SF	70	250	207	230	173	163
40	SF	78	243	199	236	194	164
72	SF	69	234	170	242	201	231
43	SF	74	222	196	249	221	-
16	SF	68	220	202	230	228	244
33	SF	115	205	199	248	242	208
41	SF	73	204	164	252	202	141
51	SF	75	202	226	281	196	192
65	SF	88	199	181	232	188	159
49	SF	65	196	180	230	254	-
1	SF	81	194	191	284	214	199
4	SF	65	189	188	230	147	-
74	SF	68	180	168	218	197	-
37	SF	71	-	-	-	-	-

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group
- = expired hamster

Table of plasma TG levels (mg/dl) every three weeks.

SUBJECT	GROUP	-3	0	3	6	9	12
34	C	120	294	121	406	110	150
56	C	104	102	22	264	56	171
60	C	93	38	48	158	168	106
67	C	136	104	48	232	122	101
18	C	73	232	75	244	177	48
54	C	132	123	65	215	82	52
31	C	66	82	24	113	116	186
5	C	88	45	70	172	74	87
39	C	65	95	105	204	180	98
27	C	43	173	79	216	112	90
29	C	88	100	79	68	68	70
48	C	79	103	108	150	66	94
68	C	93	283	66	174	59	46
3	C	41	144	186	174	59	78
70	S	84	150	30	234	218	96
55	S	104	70	68	212	44	74
45	S	75	144	114	228	80	78
47	S	113	68	65	297	96	30
25	S	45	123	106	192	78	32
7	S	96	193	164	248	-	-
52	S	100	73	50	250	147	68
6	S	93	151	86	226	58	55
75	S	83	139	197	244	102	80
50	S	122	437	173	326	158	88
61	S	67	224	92	-	-	-
13	S	109	-	-	-	-	-
9	S	79	31	37	148	192	181
12	S	100	67	39	-	-	-
46	F	110	62	111	408	271	163
35	F	61	94	80	164	142	95
44	F	188	376	249	748	107	114
64	F	78	85	109	147	74	124
73	F	88	123	39	180	166	128
59	F	45	106	76	184	162	105
10	F	63	52	172	-	-	-
63	F	118	64	31	126	88	94
58	F	51	54	30	240	142	116
8	F	92	218	152	498	309	124
26	F	83	106	103	142	68	107
17	F	113	118	100	222	176	114
36	F	116	81	71	187	111	109
23	F	41	144	94	74	48	42

(continued)

Table of plasma TG levels (mg/dl) every three weeks.

SUBJECT	GROUP	-3	0	3	6	9	12
42	SF	77	224	50	524	195	106
40	SF	72	362	78	375	192	81
72	SF	75	271	43	221	233	208
43	SF	95	139	197	284	193	-
16	SF	92	216	200	350	176	190
33	SF	67	172	101	273	134	63
41	SF	85	256	58	276	41	86
51	SF	134	170	50	498	136	70
65	SF	114	110	388	350	218	159
49	SF	81	127	295	318	400	-
4	SF	86	204	242	346	172	-
74	SF	133	90	128	264	200	-
1	SF	129	196	310	314	95	95
37	SF	92	-	-	-	-	-

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group
- = expired hamster

APPENDIX E

POOLED PLASMA LIPID AND LIPOPROTEIN VALUES

Table of pooled plasma TC levels (mg/dl) taken every three weeks.

GROUP	-3	0	3	6	9	12
C	71	209	191	236	184	194
S		202	224	268	179	176
F		193	202	250	175	160
SF		206	222	266	180	188

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group

Table of pooled plasma TG levels (mg/dl) taken every three weeks.

GROUP	-3	0	3	6	9	12
C	76	152	73	159	112	90
S		162	101	234	121	70
F		130	112	262	150	107
SF		203	146	322	178	112

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group

Table of pooled plasma HDL-C levels (mg/dl) taken every three weeks.

GROUP	-3	0	3	6	9	12
C	37	99	63	163	130	122
S		96	87	166	130	130
F		92	73	123	98	106
SF		80	96	140	108	118

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group

Table of pooled plasma HDL_2-C levels (mg/dl) taken every three weeks.

GROUP	-3	0	3	6	9	12
C	26	94	53	113	115	78
S		93	72	138	114	106
F		87	62	88	85	72
SF		77	84	111	88	75

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group

Table of pooled plasma LDL-C levels (mg/dl) taken every three weeks.

GROUP	-3	0	3	6	9	12
C	29	99	118	57	48	58
S		83	106	43	39	32
F		81	112	70	63	46
SF		90	92	37	50	48

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group

Table of pooled plasma VLDL-C levels (mg/dl) taken every three weeks.

GROUP	-3	0	3	6	9	12
C	5	36	10	26	6	12
S		24	25	58	10	14
F		20	20	56	16	8
SF		36	32	88	23	16

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group

APPENDIX F

INDIVIDUAL BODY WEIGHT VALUES

Individual hamster body weights (grams) every three weeks.

SUBJECT	GROUP	-3	0	3	6	9	12
34	C	114	137	131	137	138	134
67	C	109	126	138	153	167	178
31	C	111	130	124	146	156	159
27	C	111	139	140	158	166	162
68	C	107	132	127	142	144	144
56	C	115	132	142	161	164	168
18	C	110	136	138	149	137	133
5	C	114	125	133	150	122	131
29	C	103	116	112	124	123	119
3	C	116	136	136	136	139	142
60	C	111	130	128	152	154	151
54	C	120	136	133	154	148	141
39	C	106	125	130	149	152	160
48	C	105	120	124	134	130	131
70	S	115	128	127	136	134	130
55	S	107	132	134	140	126	131
45	S	113	126	123	131	126	119
47	S	108	123	129	141	136	138
25	S	121	136	142	146	144	141
7	S	102	135	122	115	-	-
52	S	112	122	124	142	140	128
6	S	121	142	138	152	148	153
75	S	109	121	118	127	127	116
50	S	116	142	146	157	158	155
61	S	119	106	146	157	158	155
13	S	112	-	-	-	-	-
9	S	107	116	119	133	132	138
12	S	116	130	95	-	-	-
46	F	117	132	140	154	157	165
35	F	113	131	140	156	157	158
44	F	115	147	154	136	166	167
64	F	117	128	132	146	154	155
73	F	110	131	137	137	154	168
59	F	106	127	133	145	149	158
10	F	108	123	124	-	-	-
63	F	103	125	125	131	142	136
58	F	110	118	121	141	150	149
8	F	128	151	158	173	188	182
26	F	105	114	123	130	143	143
17	F	105	120	126	143	145	143
36	F	104	118	121	142	138	145
23	F	116	136	136	136	139	142

(Continued)

Individual hamster body weights (grams) every three weeks.

SUBJECT	GROUP	-3	0	3	6	9	12
42	SF	117	139	140	153	153	15
40	SF	121	135	137	144	145	140
72	SF	112	129	136	146	144	142
43	SF	110	122	130	139	142	-
16	SF	117	129	138	152	159	163
33	SF	110	120	123	130	132	136
41	SF	108	124	127	134	136	144
51	SF	115	130	136	144	148	150
65	SF	112	122	126	134	138	127
49	SF	111	130	134	143	156	-
1	SF	111	139	145	151	149	148
4	SF	109	143	146	153	151	-
74	SF	110	113	123	132	149	-
37	SF	113	121	-	-	-	-

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group
- = expired hamster

APPENDIX G

INDIVIDUAL FEED INTAKE VALUES

Individual feed intake (grams) every three weeks.

SUBJECT	GROUP	-3	0	3	6	9	12
34	C	0	17.7	16.8	25.8	19.5	17.6
67	C	0	19.6	19.2	28.5	27.0	23.9
31	C	0	20.9	17.2	23.1	23.6	22.7
27	C	0	20.1	22.7	31.0	27.5	25.1
68	C	0	18.7	18.6	32.2	24.0	22.0
56	C	0	20.5	36.4	43.4	26.9	25.9
18	C	0	16.4	16.1	22.6	17.5	19.6
5	C	0	14.2	18.1	25.0	14.3	19.5
29	C	0	16.1	13.6	21.2	16.7	18.9
3	C	0	16.2	15.5	20.7	18.7	17.9
60	C	0	20.0	20.7	32.8	28.8	25.7
54	C	0	24.1	16.8	27.9	21.9	16.6
39	C	0	15.8	21.8	28.7	14.5	20.3
48	C	0	17.3	21.4	22.5	18.8	19.6
70	S	0	16.7	17.0	27.0	25.7	22.0
55	S	0	16.4	18.8	26.6	17.5	19.6
45	S	0	15.0	16.4	23.8	19.3	16.2
47	S	0	18.5	20.1	27.0	20.9	18.8
25	S	0	17.0	18.0	26.3	21.7	20.2
7	S	0	15.3	11.3	-	-	-
52	S	0	15.0	17.0	27.7	23.8	16.3
6	S	0	18.4	17.1	26.8	22.8	23.7
75	S	0	16.0	14.3	21.4	20.7	19.1
50	S	0	18.2	16.8	23.3	20.3	19.5
61	S	0	9.5	14.0	-	-	-
13	S	0	20.8	-	-	-	-
9	S	0	19.0	19.0	29.4	24.6	23.0
12	S	0	14.1	8.5	-	-	-
46	F	0	20.8	23.2	30.9	27.8	23.7
35	F	0	17.3	19.8	26.7	21.2	19.2
44	F	0	18.3	19.0	21.2	24.6	21.0
64	F	0	14.7	15.5	22.4	18.6	16.7
73	F	0	21.6	23.0	23.8	27.6	24.3
59	F	0	19.3	21.6	30.7	20.3	21.2
10	F	0	22.0	21.7	-	-	-
63	F	0	18.5	22.4	28.7	26.7	21.6
58	F	0	19.6	24.5	33.1	30.1	21.9
8	F	0	19.0	20.3	32.2	27.8	22.3
26	F	0	13.9	16.2	22.0	20.8	15.4
17	F	0	15.6	18.3	30.5	23.4	20.1
36	F	0	18.0	18.2	26.8	18.6	18.8
23	F	0	16.2	15.5	20.7	18.7	17.9

(Continued)

Individual Feed Intake (grams) every three weeks.

SUBJECT	GROUP	-3	0	3	6	9	12
42	SF	0	18.7	21.4	33.1	25.0	20.3
40	SF	0	18.4	16.4	27.4	22.8	18.1
72	SF	0	20.5	25.5	29.8	19.5	20.1
43	SF	0	15.4	12.4	20.8	18.6	-
16	SF	0	20.9	21.0	28.2	25.0	21.0
33	SF	0	15.2	13.7	17.2	19.5	16.7
41	SF	0	15.1	14.7	26.0	28.3	18.0
51	SF	0	16.5	25.0	33.9	28.3	21.5
65	SF	0	18.9	19.3	25.6	20.5	16.7
49	SF	0	18.7	22.0	23.6	26.6	-
1	SF	0	17.2	20.6	26.5	21.9	17.5
4	SF	0	16.6	15.0	19.9	17.1	-
74	SF	0	20.2	24.1	25.7	22.6	-
37	SF	0	20.4	-	-	-	-

C = Control group
S = Swimming group
F = Fish oil group
SF = Swimming/Fish oil group
- = expired hamster

APPENDIX H

DATA COLLECTION FORMS

RAT SEX: ______________________ EXPERIMENT: ______________________

ANIMAL NO.: __________ AGE: __________ TREATMENT: ______________

BREED: ______________________ EMPTY FEEDER WT.: ______________

DATE	BODY WT. (g)	FEED & FEEDER (g)	FEEDER & REF. FEED (g)	FEED CONSUMED (g)	REMARKS

Study_____ Dates_____

Hamster #_____

DATE	SWIMMING TIMES	COMMENTS

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