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Effects of consumption of fatty fish rich in omega-3 polyunsaturated fatty acids on plasma lipids and prothrombin time in hyperlipidemic subjects

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EFFECTS OF CONSUMPTION OF FATTY FISH RICH IN OMEGA-3 POLYUNSATURATED FATTY ACIDS ON PLASMA LIPIDS AND PROTHROMBIN TIME IN HYPERLIPIDEMIC SUBJECTS

by

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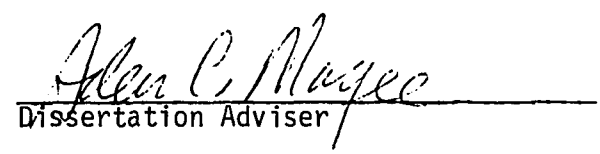

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APPROVAL PAGE

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The effects of consumption of low levels of omega-3 polyunsaturated fatty acids (n-3 PUFAs) on plasma lipids and prothrombin time were examined in hyperlipidemic men (n = 18) attending a cardiac rehabilitation program. Three dietary treatments were evaluated: the men's customary, the American Heart Association Phase I diet, and a food source of n-3 PUFAs fed at two levels. Eight ounces of salmon were fed either two times or four times per week providing approximately 4.5 gm or 9 gm n-3 PUFAs per week, respectively. A Latin-square design which allowed adjustment for any carry-over effect of previous treatment into the succeeding period without separating the three test periods by a washout phase was employed. Each man consumed all three diets but in a different sequency; each dietary treatment lasted four weeks. The salmon was prepared, packed, and distributed to the men after completion of their exercise program at the center. They were instructed to substitute the fish for an entree at lunch or dinner. Adherence to diet instruction was ascertained from a food diary kept for four days during the last week of each treatment phase. Fasting blood samples were collected at baseline and at the end of each dietary treatment phase. Triglycerides, total- and low-density-lipoprotein cholesterol (LDL-cholesterol) were decreased and prothrombin time was increased during the fish consuming dietary phases. The greatest decline was observed in the blood lipid measures when eight ounces of salmon were fed four

times per week as compared to the customary diet. Moderate amounts of fish, eight ounces of salmon fed two times per week, were only observed to reduce triglycerides in these hyperlipidemic men. It would appear that an increase in consumption of fish, rich in n-3 PUFA, would be beneficial in the management of hyperlipidemia.

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CHAPTER I

INTRODUCTION

Epidemiological observations on Greenland Eskimos and subsequent clinical studies have demonstrated that the consumption of omega-3 polyunsaturated fatty acids (n-3 PUFAs), i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) abundant in marine organisms, may reduce the risk of coronary heart disease (CHD). Omega-3 polyunsaturated fatty acids appear to have two important effects in the body which impact on the pathogenesis of heart disease. These fatty acids are hypolipidemic, and they alter platelet adhesiveness.

The hypolipidemic effects of n-3 PUFAs appear to be complex, and consistent results have only been achieved in those studies which used high levels of either fatty fish or fish oil extract. At high dosage normolipidemic individuals generally show a reduction in total cholesterol which is accompanied by a decrease in low density lipoprotein (LDL). The hypolipidemic effect of n-3 PUFAs on triglycerides (TGs) is much more impressive, and the effect is observed at lower levels of n-3 PUFA consumption than required to produce a hypocholesterolemic effect.

There have been few studies which have attempted to determine the impact of consumption of low levels of n-3 PUFAs. Fehily, Burr, Phillips, and Deadman (1983) reported that 5 oz of fatty fish which

provided 3 g n-3 PUFAs per week, consumed twice a week had a beneficial effect on TGs but not on cholesterol levels. A retrospective study conducted by Kromhout, Basschieter, and Coulander (1985) which examined fish consumption in 1960 with CHD mortality data collected in the 1980s suggested that subjects who consumed approximately 30 g of fish per day were less likely to die from CHD. They found no relationship between fish consumption and total cholesterol levels. It has been hypothesized that if similar amount of fish (2-4 servings per week) were consumed by subjects with hyperlipidemia the overall effect would be more dramatic (Leaf & Weber, 1988).

Estimates of fatty fish consumption indicate that the general U.S. population probably accrues little benefit from the ingestion of n-3 PUFAs. The mean daily capita intake of fish is currently about 14 g per day (approximately 3 oz per week) which provides approximately 0.04-0.15 g of n-3 PUFAs per day (Herold & Kinsella, 1986). Estimates of n-3 PUFA content of fatty fish range from 0.3-1.1 g per 100 g dependent on the fish selected. Good fish choices would include salmon, mackerel, herring, sardines and trout. Any increase in fatty fish consumption might prove beneficial in reducing the risk of CHD, particularly in those individuals with elevated serum lipids. At present there is little information on the role of n-3 PUFAs fed at realistic levels in the management of hyperlipidemias.

The present study was designed to determine the effect of supplementing the customary diet with a food source of n-3 PUFAs (salmon) on plasma lipid levels and prothrombin time in hyperlipidemic

men. Two levels of salmon were fed--one providing approximately 9 g and the other 4.5 g n-3 PUFAs per week.

The specific objectives of the study were as follows:

1. To study lipid (TG, total- and LDL-cholesterol) lowering effects of consumption of fish rich in n-3 PUFA.
2. To determine the effect of feeding realistic amounts of fish on high density lipoprotein (HDL)-cholesterol.
3. To examine the increase in prothrombin time of hyperlipidemic subjects on a fish supplemented diet.
4. To compare the two levels of fish consumption with customary diet (American Heart Association [AHA Phase I]) of hyperlipidemics for their desirable effects and their acceptability.

CHAPTER II

REVIEW OF LITERATURE

Accumulating evidence from epidemiologic studies and controlled feeding trials indicate that n-3 PUFAs in the diet might have protective effects against CHD (Leaf & Weber, 1988). Populations consuming diets abundant in seafoods rich in n-3 PUFAs have a lower than expected incidence of CHD (Dyerberg & Bang, 1982). The feeding trials have focused on the actions of the n-3 PUFAs in the body that might diminish the risk of CHD; first, the hypolipidemic effect and second, alteration in platelet function. This review is organized as follows:

I. Epidemiological Studies
II. Composition of n-3 PUFAs
III. Effects of n-3 PUFAs on Plasma Lipids
IV. Effects of n-3 PUFAs on Platelet Function

I. Epidemiological Studies

Several epidemiological-based comparison studies between Eskimos of Greenland and those residing in Denmark were conducted in the 1970s to explore the factors which could possibly explain the differences in CHD incidence (Bang & Dyerberg, 1972; Bang & Dyerberg, 1980; Band, Dyerberg, & Nielsen, 1971; Dyerberg, Bang, & Hjorne, 1975). These studies demonstrated that the Greenland Eskimos had lower serum TGs, lower very low density lipoprotein (VLDL), lower

LDL and higher HDL levels as compared to their Danish controls. All of these parameters have been associated with a decreased risk of cardiac disease.

The diets of the Greenlanders and Danes also differed. The Greenland Eskimos consumed nearly 1 lb (454 g) of fish per day, while the Danish Eskimos consumed less than 20 g of fish per day. The total amount of fat consumed by both groups was similar, approximately 40% of evergy from fat. However, the fat composition of the diets differed; the diet of Greenlanders was low in saturated fatty acids (SFAs) and rich in n-3 PUFAs, whereas the diet of the Danes was higher in SFA and the predominant PUFA was n-6 PUFA from vegetable sources. The lower incidence of CHD in the Greenland Eskimo population may in part be attributed to their abundant consumption of seafoods rich in n-3 PUFAs and lower ingestion of n-6 PUFA and SFAs as compared to the Danish population.

Studies in Japan also support the hypothesis that the intake of fatty acids from marine sources might lower the incidence of CHD (Hirai, Hamazaki, Terano, Nishikawa, Tamura, Kumagai, & Sijiki, 1980). In Japan the lowest mortality rate from CHD is on the island of Okinawa where fish consumption is twice the national average. Hirai et al. (1980) compared a fishing village with a farming village for fish consumption and relative mortality rate from CHD. The average fish consumption in the fishing village was 250 g per day as compared to 90 g per day in the farming village. The mortality rate from CHD was found to be lower in the fishing village.

A study carried out in the Netherlands suggested that even a small intake of fish over a long period might reduce the incidence of coronary disease (Kromhout et al., 1985). Kromhout et al. (1985) investigated the association between fish consumption of 852 middle-aged men in Zutphen, Netherlands and mortality attributed to coronary disease during the ensuing 20 years. The results revealed that subjects who consumed fish each day in 1960 had lower death rates from coronary artery disease. Mortality was more than 50% lower among men who 20 years earlier had said they ate at least 30 g of fish per day compared to those who ate no fish. The diet history indicated that about two thirds of the fish eaten by these men was lean fish (cod and plaice) and one third consisted of fatty fish (herring and mackerel). Although no relation was found between fish consumption and serum total cholesterol levels, Kromhout et al. (1985) concluded that as little as two fish dishes per week might be beneficial in preventing CHD.

II. Composition of n-3 PUFAs

Human diets contain two series of PUFAs: the n-6 and n-3, derived from linoleic acid (18:2 n6) and alphalinoleic acid (18:3, n3), respectively. Omega-3 PUFAs differ from n-6 PUFAs in the position of the first unsaturation from the methyl (omega) end. The first unsaturation in case of n-3 PUFAs occurs three carbon atoms distant from the methyl end of the chain, whereas n-6 PUFAs have the first unsaturation at the sixth carbon atom from the methyl end of the chain.

Both linoleic and alphalinoleic acids are synthesized only by plants, but both can be further desaturated and elongated in animals. In humans linoleic acid is rapidly converted to arachidonic acid, whereas elongation and desaturation of alphalinoleic acid occurs very slowly to yield the n-3 PUFAs--EPA and DHA. Some plant oils such as soybean and linseed oil contain appreciable amounts of alpha-linoleic acid but the conversion to EPA and DHA is very selective and in small quantities (Glomset, 1985). Marine phytoplankton and zoo-plankton, on the other hand, are rich sources of the n-3 PUFAs and are sources of abundant EPA and DHA to other marine animals (Tinoco, 1982). Therefore, a diet containing fish might provide more EPA and DHA than that formed from alphalinoleic acid which is consumed by vegetarians. From a health perspective eating fish rich in EPA and DHA might provide protection against CHD.

III. Effects of n-3 PUFAs on Plasma Lipids

Several controlled feeding trials have been conducted using both normo- and hyperlipidemic subjects. This review is organized according to the form in which n-3 PUFAs were fed. Diets used for most human studies included those supplemented with (1) fish oil, (2) a combination of fatty fish and oil, or (3) fatty fish. The results are summarized with respect to plasma lipid profiles in normolipidemics (Tables 1-4) and hyperlipidemics (Tables 5-7).

The main findings of all feeding studies conducted in normo-lipidemics are summarized in Table 1. The most consistent finding

Table 1

Main Findings of n-3 PUFA Feeding Studies Conducted in Normolipidemics

Parameter	Number of Studies That Measured Parameter	Number of Studies That Show a Decrease	Number of Studies That Show an Increase
Total - TG	19	16	
Total Cholesterol (C)	17	7	
HDL-C	17	1	8
LDL-C	9	4	1
VLDL-C	4	3	1

(16 of 19 studies) has been an impressive reduction in plasma TG levels. The impact on total cholesterol and lipoprotein cholesterol fractions has been less consistent and appears to be more dependent upon the amount of n-3 PUFAs consumed and length of the period over which the n-3 PUFA source was fed. The reported effects of the n-3 PUFAs on the lipoprotein cholesterol fractions are to lower those plasma fractions that are associated with high CHD risk, namely, total, LDL and VLDL. Interestingly, n-3 PUFAs fed at high levels appear to increase HDL-cholesterol, one of the few dietary factors that has such an effect. However, one difficulty in comparing results across studies is the ability to translate the food item consumed into a meaningful level of n-3 PUFAs.

Results of the studies that supplemented diets with fish oils are presented in Table 2. Some studies have used fish oils isolated from salmon flesh and cod liver. MaxEPA is a commercially prepared fish oil concentrate that is rich in EPA. It is a popular fish oil supplement and has also been used in many intervention trials (Herold & Kinsella, 1986). A reduction in plasma TG levels as a result of fish oil supplementation was observed in all of these studies. A threshold response effect of n-3 PUFAs on plasma TG was observed by those investigators who fed the fish oils at several levels (Brongeest-Schoute, van Gent, Luten, & Reuter, 1981; Sanders & Roshanai, 1983; von Schacky, Fischer, & Weber, 1985). Brongeest-Schoute et al. (1981) found a significant reduction in plasma TG when 8.2 g of n-3 PUFAs were consumed per day for four weeks. Amounts such as 1.4 g, 2.3 g and 4.1 g of n-3 PUFAs per day were not sufficient to significantly reduce plasma TG. Von Schacky et al. (1985) also found significant reductions in plasma TG level of normolipidemic subjects fed the highest level (40 ml) of cod liver oil.

A more prominent threshold effect can be seen with respect to plasma cholesterol when individuals were fed increasing amounts of MaxEPA. Five healthy male subjects consumed 5, 10 and 20 g of MaxEPA per day in a random order for three-week periods (Sanders & Roshanai, 1983). Each experimental period was separated by a rest period of at least six weeks. The results revealed that a significant hypocholesterolemic effect was observed only when subjects consumed 20 g of MaxEPA per day. Diets supplemented with 5 and 10 g of MaxEPA per day were unable to significantly lower the cholesterol levels.

Table 2

Summary of Effects of Fish Oils on Plasma Lipids in Normolipidemic Subjects

Reference	Diets Compared to	Amount Fed Per Day	Duration of Diet (Weeks)	mg/100 ml TG	Total-C	HDL-C	LDL-C	VLDL-C
Saynor & Verel (1980)	Customary	20 ml MaxEPA oil	5	35%↓	NSD	22%↑	-	-
Brongeest-Schoute, van Gent, Luten, & Reuter (1981)	1 g olive oil oil	1.4 g n-3 PUFA	4	NSD	NSD	NSD	NSD	NSD
		2.3 g n-3 PUFA	4	NSD	NSD	NSD	NSD	NSD
		4.1 g n-3 PUFA	4	NSD	NSD	NSD	NSD	NSD
		8.2 g n-3 PUFA	4	39%	NSD	NSD	NSD	↓
Sanders, Vickers, & Haines (1981)	Customary	20 ml cod liver oil	6	22%↓	NSD	9%↑	-	-
Sanders & Hochland (1983)	10 g olive/ corn oil	10 g MaxEPA oil	2	↓	NSD	↑	-	-
Sanders & Roshanai (1983)	20 ml linseed oil	MaxEPA oil						
		5 g	3	14%↓	NSD	NSD	-	-
		10 g	3	23%↓	NSD	NSD	-	-
		20 g	3	32%↓	9%↓	31%↑	-	-
Illingworth, Harris, & Connor (1984)	Customary 30-40% fat	120 g salmon oil (30-40% fat) (24 g n-3 PUFAs)	4	43%↓	23%↓	NSD	20%↓	-
Nestel, Connor, Reardon, Connor, Wong, & Boston (1984)	30% energy from safflower oil	30% energy as MaxEPA oil	4	↓	-	↓	↑	↓

Reference	Diets Compared to	Amount Fed Per Day	Duration of Diet (Weeks)	mg/100 ml TG	Total-C	HDL-C	LDL-C	VLDL-C
Sanders & Mistry (1984)	Customary	10 gm MaxEPA oil	3	↓	-	↑	-	-
			5	↓	-	↑	-	-
			8	↓	-	↑	-	-
			14	↓	-	↑	-	-
Von Schacky, Fisher, & Weber (1985)	Customary	cod liver oil 10 ml	4	NSD	NSD	NSD	-	-
		20 ml	4	NSD	NSD	NSD	-	-
		40 ml	4	66% ↓	NSD	NSD	-	-
		20 ml	8	NSD	NSD	NSD	-	-
Sullivan, Sanders, Trayner, & Thompson (1986)	Customary	15 g MaxEPA oil	2	↓	NSD	-	-	-
			4	↓	NSD	-	-	-
			8	↓	NSD	-	-	-

NSD = Not statistically different
- = Not measured

Changes in HDL-cholesterol concentration are observed only at the highest doses of fish oil. A significant increase in HDL levels was observed when 20 g of MaxEPA was ingested by 23- to 30-year-old healthy volunteers for three weeks (Sanders & Roshanai, 1983). Saynor and Verel (1980) also reported that 20 ml of MaxEPA oil per day was a sufficient amount to produce an increase in HDL-cholesterol level. The same amount, however, failed to reduce total plasma cholesterol levels.

The effect of fish oil or n-3 PUFAs on VLDL- and LDL-cholesterol has not been extensively investigated in human subjects. The amount of fish oil required to demonstrate an observed difference in LDL and VLDL levels appears to be similar to the level required for total cholesterol. The study by Nestel, Connor, Reardon, Connor, Wong, and Boston (1984) yielded conflicting results--an increase in LDL- and VLDL-cholesterol and a decrease in HDL-cholesterol. The level at which the n-3 PUFAs were provided is much higher than in any of the other studies; approximately 85 g of fish oil were consumed daily assuming that the diet provided 200 Kcal. Intakes at this level might possibly avoid the usual metabolic pathways for lipoprotein metabolism.

In summary, it appears that the minimum amount of MaxEPA oil that is required to significantly impact on the plasma and lipoprotein cholesterol levels is at least 20 g per day. However, a much lower level of n-3 PUFAs (10 g of MaxEPA oil) is sufficient to significantly reduce the plasma TGs.

Those studies that have fed a fatty fish in combination with a fish oil supplement as a source of n-3 PUFAs have generally provided the n-3 PUFAs at much higher doses than in those studies that fed the fish oil alone (Table 3). The results have consistently shown a significant decrease in total TG and total-, LDL- and VLDL-cholesterol (Harris & Connor, 1980; Harris, Connor, & McMurry, 1983). However, these studies have failed to demonstrate any increase in HDL-cholesterol.

Studies in which fatty fish has been used as a source of n-3 PUFAs (Table 4) need to be interpreted in light of both the amount of fish consumed and the duration of the treatment. Consumption of 200 g mackerel per day for three weeks resulted in significant decreases in plasma TG and total cholesterol (Von Lossonczy, Reuter, Brongeest-Schoute, van Gent, & Hermus, 1978). A higher amount (500-800 g mackerel per day) when fed for only one week, however, failed to reduce the plasma TG and total cholesterol (Siess, Scherer, Bohlig, Roth, Kurzmann, & Weber, 1980). These results indicate that consumption of fish even at high levels for one week is too short a period to observe any changes in plasma lipids. It is usually assumed that 3-4 weeks are required to demonstrate an effect of diet on lipoprotein cholesterol metabolism. Feeding levels as low as 28-85 g of fatty fish per day for three months were associated with a significant decrease (6%) in plasma TG but did not cause a decrease in any other plasma lipids (Fehily et al., 1983). These authors suggested that an effect of consumption of such low amounts might be manifested only

Table 3

Summary of Effects of Fatty Fish and Fish Oil on Plasma Lipids in Normolipidemic Subjects

Reference	Diets Compared to	Amount Fed Per Day	Duration of Diet (Weeks)	mg/100 ml TG	Total-C	HDL-C	LDL-C	VLDL-C
Harris & Connor (1980)	Customary diet	salmon flesh + Max EPA oil	4	41%↓	17%↓	NSD	15%↓	40%↓
Goodnight, Harris, & Connor (1981)	Customary 45% fat diet 500 mg cholesterol	1 lb salmon flesh + 60-90 ml MaxEPA oil (10 g n-3 PUFA)	4	-	↓	-	-	-
Harris, Connor, & McMurry (1983)	Saturated fat control diet vegetable oil control diet (40% fat)	1 lb salmon flesh + 3-6 T MaxEPA oil (20-29 g n-3 PUFA) (40% fat)	4	38%↓	14%↓	NSD	16%↓	38%↓

NSD = Not statistically different
- = Not measured

Table 4

Summary of Effects of Fatty Fish on Plasma Lipids in Normolipidemic Subjects

Reference	Diets Compared to	Amount Fed Per Day	Duration of Diet (Weeks)	mg/100 ml TG	Total-C	HDL-C	LDL-C	VLDL-C
VonLossonczy, Reuter, Brongeest-Schoute, van Gent, & Hernus (1978)	150 g cheese	200 g mackerel	3	35%↓	7.5%↓	7%↑ (Females only)	-	-
Siess, Scherer, Bohlig, Roth, Kurtzman, & Weber (1980)	Customary	500-800 g mackerel (7-11 g n-3 PUFA)	1	NSD	NSD	-	-	-
Fehily et al. (1983)	Customary	28-85 g fatty fish	12	6%↓	NSD	NSD	NSD	-
Singer, Jaeger, Wirth, Naumann, Zimontkowski, Hazda, & Groedicke (1983)	Customary before mackerel feeding	280 g mackerel	2	47%↓	7%↓	NSD	NSD	-
	Customary 3 months after mackerel feeding	280 g herring	2	NSD	NSD	NSD	NSD	-
Atkinson, Wheeler, Mendelsohn, Pienaar, & Chetty (1987)	Customary	750 g trout	2	NSD	NSD	↑	-	-
		(meat free diet)	4	NSD	NSD	NSD	-	-
Spiller, Jensen, & Scala (1987)	Customary	100 g fish	4	↓	NSD	↑	NSD	-
		300 g fish	4	↓	NSD	↑	NSD	-
		600 g fish	4	↓	NSD	↑	NSD	-

NSD = Not statistically different
- = Not measured

when consumed for longer periods of time, and that these amounts might be insufficient to impact on plasma cholesterol.

Consumption of fatty fish has also produced variable effects on HDL-cholesterol levels. Consumption of 200 g mackerel (2-3 g n-3 PUFAs) per day has been shown to significantly increase HDL-cholesterol and decrease total cholesterol (Von Lossonczy et al., 1978). Whereas greater amounts of mackerel (280 g per day) when consumed for a shorter period, i.e., two weeks failed to bring about a significant increase in HDL-cholesterol although total cholesterol was significantly reduced (Singer, Jaeger, Wirth, Voigt, Naumann, Zimontkowski, Hajdu, & Goedicke, 1983). In a recent study the supplementation of a typical western diet of 28 normolipidemic men with fish yielding 1, 3 and 6 g of n-3 PUFAs per day for one month significantly increased HDL-cholesterol at all levels of n-3 PUFAs (Spiller, Jensen, & Scala, 1987).

None of the feeding studies conducted observed that supplements of fatty fish as a source of n-3 PUFAs had any significant impact on LDL-cholesterol levels. The effect of fatty fish on VLDL-cholesterol in healthy subjects has not been extensively studied.

The main findings of all feeding studies conducted in hyperlipidemics are summarized in Table 5. As in normolipidemics the most consistent effect was in a reduction of total TG. However, a decrease in total cholesterol and increase in HDL-cholesterol is evident in the majority of studies. The differences observed between normo- and hyperlipidemics appears to be in the LDL fraction. In normolipidemics

Table 5

Main Findings of n-3 PUFA Feeding Studies Conducted in Hyperlipidemics

Parameters	Number of Studies That Measured Parameter	Number of Studies That Show a Decrease	Number of Studies That Show an Increase
Total TG	10	10	
Total-C	8	7	
HDL-C	5	1	4
LDL-C	3	2	2
VLDL-C	3	3	

if there is an observed effect in the LDL fraction it is a lowering effect and in hyperlipidemics the effect is inconsistent and dependent upon the hyperlipidemia disorder under observation. The reduction in total cholesterol appears to be contributed from VLDL rather than LDL fraction.

Studies conducted with hyperlipidemic subjects have generally used either pure fish oil or fish supplemented with fish oils; results from which are presented in Tables 6 and 7. In general, feeding experiments with hyperlipidemic subjects have resulted in impressive reductions in plasma lipids. The greatest plasma lipid reductions were reported in an interventional trial in 20 subjects with hyperlipidemia (type IIb or type V) (Phillipson, Rothrock, Connor, Harris, & Illingworth, 1985). The study compared the effect of three diets that differed in fatty acid composition and fat content; a standard

Table 6

Summary of Effects of Fish Oils on Plasma Lipids in Hyperlipidemic Subjects

Reference	Diets Compared to	Amount Fed Per Day	Duration of Diet (Weeks)	mg/100 ml TG	Total-C	HDL-C	LDL-C	VLDL-C
Harris, Connor, Illingworth, & Foster (1984)	75% high CHO diet	50 g MaxEPA oil in 75% CHO diet	1	61%↓	↓	-	-	-
Sanders & Mistry (1984)	corn/olive oil	10 g MaxEPA oil	3	↓	-	↑	-	-
			5	↓	-	↑	-	-
			8	↓	-	↑	-	-
			14	↓	-	↑	-	-
Saynor (1984)	corn/olive oil	10 g MaxEPA oil	8	48%↓	8%↓	17%↑	-	-
Saynor, Verel, & Gillott (1984)	Customary	20 ml MaxEPA oil	4	37%↓	NSD	10%↑	-	-
			12	36%↓	2%↓	7%↑	-	-
			26	41%↓	4%↓	10%↑	-	-
			38	38%↓	NSD	NSD	-	-
			52	37%↓	NSD	NSD	-	-
			104	41%↓	5%↓	14%↑	-	-
Woodcock, Smith, Lambers, Morris, Jones, Galloway, Greaves, & Preston (1984)	10 g corn/olive oil mix	10 g MaxEPA oil	7	27%↓	NSD	-	-	-
Phillipson, Rothrock, Connor, Harris, & Illingworth (1985)	150 mg cholesterol 20-30% low fat diet Type IIb	salmon oil diet (20 g n-3 PUFA) (30% fat diet) (325 mg cholesterol)	4	64%↓ Type IIb	27%↓ Type IIb	17%↓ Type IIb	12%↓ Type IIb	73%↓ Type IIb
	5% fat diet <100 mg cholesterol Type V	salmon oil diet (20 g n-3 PUFA) (30% fat) (350 mg cholesterol)	4	79%↓ Type V	48%↓ Type V	NSD	48%↑ Type V	71%↓ Type V

Reference	Diets Compared to	Amount Fed Per Day	Duration of Diet (Weeks)	mg/100 ml TG	Total-C	HDL-C	LDL-C	VLDL-C
Simons, Hickie, & Balsubramaniam	6 g olive oil placebo	6 g MaxEPA oil	12	28%↓ Type IIb 41%↓ Type IV	NSD	-	-	-
	16 g olive oil placebo	16 g MaxEPA oil	12	58%↓ Type V	34%↓ Type V	6%↑	7%↑	42%↓
Sullivan, Sanders, Traynor, & Thompson (1986)	Customary	15 g MaxEPA oil	4	48%↓	-	-	-	-

NSD = Not statistically different
- = Not measured

Table 7

Summary of Effects of Fatty Fish and Fish Oil on Plasma Lipids in Hyperlipidemic Subjects

Reference	Diets Compared to	Amount Fed Per Day	Duration of Diet (Weeks)	mg/100 ml TG	Total-C	HDL-C	LDL-C	VLDL-C
Harris & Connor (1980)	Customary	salmon flesh + Max EPA oil	4	67%↓	20%↓	-	-	-
Phillipson, Harris, & Connor (1981)	100 mg cholesterol 20% low fat diet Type IIb	salmon and salmon oil with fat content of 20-50% of total calories	1 1/3 - 4	65%↓ Type IIb	24%↓ Type IIb	-	15%↓ Type IIb	86%↓ Type IIb
	5% fat eucaloic Type V		1 1/2 - 4	50%↓ Type V	13%↓ Type V	-	104%↓ Type V	50%↓ Type V

NSD = Not statistically different
- = Not measured

low-fat control diet, a fish (salmon) oil diet (20 g n-3 PUFAs per day) and a vegetable oil diet (n-6 PUFA). The control diet was lower in cholesterol than the other two diets. In both groups of subjects (those with type IIb and those with type V hyperlipoproteinemia) the fish oil diet resulted in pronounced reductions of plasma cholesterol, TG and VLDL. It has been postulated that the mechanism for the observed changes was a decreased production of VLDL rather than an increase in catabolism of VLDL. This decreased production of VLDL was consistent with the observed lipid changes of a considerable decrease in TG and greater reduction in VLDL than in LDL- or HDL-cholesterol. Consistent reduction in elevated plasma TG levels by feeding 5, 10, 15 and 20 ml of MaxEPA oil per day for periods ranging from 1 month to 24 months were observed by Saynor (1984), Saynor, Verel, and Gillott, (1984), Woodcock, Smith, Lambert, Morris, Jones, Galloway, Greaves, and Preston (1984), Sullivan, Sanders, Trayner, and Thompson (1986), and Simons, Hickie, and Balasubramaniam (1985) have also observed significant reductions in plasma TG of both type IIb and type V hyperlipidemics when 6-16 g of MaxEPA oil were fed for three months.

Fish oils have also been reported to reduce the elevated plasma TG and VLDL levels accompanying a high carbohydrate diet (Harris, Connor, Illingworth, & Foster, 1984). Type V hyperlipidemic individuals with abnormally low LDL levels have shown increases in their mean LDL-cholesterol levels after consuming fish oil containing diets. This observation was also accompanied by reduction in VLDL-

cholesterol and VLDL-TG levels which suggested an improved conversion of VLDLs (Phillipson, Rothrock, Connor, Harris, & Illingworth, 1985).

The cholesterol lowering effect of fish oils, however, has not been reported by all the studies that have used fish oils to correct hyperlipidemia. Saynor (1984) and Saynor et al. (1984) have observed reductions in cholesterol levels of hyperlipidemics at dosages of 10 and 20 g MaxEPA oil per day, whereas, Woodcock et al. (1984) failed to reduce total cholesterol in hyperlipidemics fed 10 g of MaxEPA per day. Simons et al. (1985) have shown a reduction in plasma cholesterol in type V hyperlipidemics when 16 g of MaxEPA was fed per day for three months.

Studies with hyperlipidemics have produced effective results with respect to HDL-cholesterol. Most studies have found an increase in HDL-cholesterol in those individuals supplemented with fish oil (Sanders & Mistry, 1984; Saynor, 1984; Saynor et al., 1984; Simons et al, 1985). Only Phillipson et al. (1985) found reductions in HDL-cholesterol for type IIb hyperlipidemics but no significant difference in type V hyperlipidemics consuming a salmon oil diet.

The effect of fish oils on LDL- and VLDL-cholesterol in hyperlipidemics has not been extensively studied. Phillipson et al. (1985) have reported a reduction in LDL-cholesterol of type IIb hyperlipidemics but an increase in LDL-cholesterol of type V hyperlipidemics. A reduction in VLDL, however, is consistently seen across studies (Phillipson et al, 1985; Simons et al., 1985). Thus, it appears that the effect of fish oils on total cholesterol and

cholesterol in different lipoprotein fractions is variable. The type of hyperlipidemia might influence the effect of n-3 PUFAs.

The two clinical trials with hyperlipidemics that have used fatty fish and fish oils have reported significant reductions in plasma TG, total cholesterol, LDL- and VLDL-cholesterol (see Table 7) (Harris & Connor, 1980; Phillipson et al., 1981). Phillipson et al. (1981) fed salmon flesh in combination with salmon oil and the subjects appeared to benefit from eating fatty fish usually contraindicated in type IIb and type V hyperlipoproteinemia. Salmon contains approximately 10% total fat by weight and is generally restricted in diets of persons with hypertriglyceridemia. There are no studies that have attempted to study the effect of fish alone in hyperlipidemics.

IV. Effects of n-3 PUFAs on Platelet Function

Dyerbert and Bang (1982) conducted epidemiological studies in Greenland Eskimos and observed that this population group exhibited prolonged bleeding time and reduced platelet aggregation as compared to their Danish counterparts. The role of dietary marine fatty acids on platelet function might explain, in part, their low incidence of coronary heart disease.

There appears to be several mechanisms by which n-3 PUFAs might be involved in altered platelet function. Their role might be explained, in part, by alterations in prostaglandin synthesis. Arachidonic acid (AA) is the major precursor of the prostaglandins

(Figure 1). Most AA is formed from dietary linoleic acid (LA) by sequential desaturation and elongation; it is then acted upon by cycloxygenase to form the endoperoxides (PGG2 and PGH2). These endoperoxides are the intermediate compounds in prostaglandin synthesis. In platelets the endoperoxides are transformed into thromboxane A2 (TXA2) which has strong platelet aggregating and vasoconstrictive effects. In vascular endothelium the endoperoxides are converted to prostacyclin (PGI2) which is a vasodilator and a potent inhibitor of platelet aggregation. The balanced antagonistic effect of TXA2 and PGI2 regulate the initial steps of blood clotting.

It is hypothesized that increased dietary n-3 PUFAs should increase their levels in platelet phospholipids which in turn would allow them to complete effectively with AA for cycloxygenase. Alterations in prostaglandin synthesis should result in a reduction of platelet aggregation and an increase in bleeding time. Indeed, reduced platelet aggregation and increased bleeding time were observed in the study conducted by Fischer and Weber (1984) in the n-3 PUFA supplemented groups as compared to nonsupplemented controls. Others have also reported increased EPA and DHA levels in platelet lipids following the supplementation with n-3 PUFAs (Goodnight, Harris, & Connor, 1981; Sanders, Naismith, Haines, & Vickers, 1980). These increases were accompanied by decreased levels of LA and AA in platelet lipids. Related effects, such as reduced platelet aggregation and prolonged bleeding time, have also been reported and could be attributed to altered prostaglandin synthesis (Herold & Kinsella, 1986).

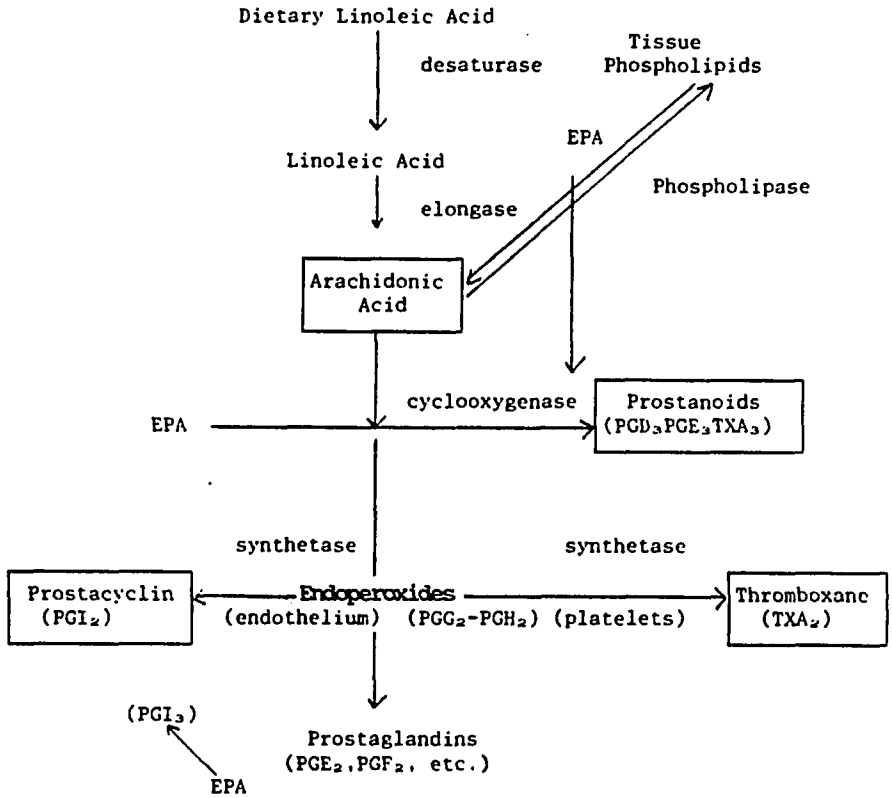

Figure 1: Roles of Linoleic Acid and Arachidonic Acid in Prostaglandin Synthesis.

Evidence also suggests that EPA might be converted to PGI_3 which is an effective antiaggregatory agent (Fischer & Weber, 1984). The major urinary metabolite of endogenous prostaglandin I_3 was present in human subjects consuming diets high in EPA either as a cod liver oil (4 g EPA daily) or as mackerel flesh (10-15 g EPA daily).

Another possible effect of n-3 PUFAs has been reported by Lee, Hoover, Williams, Sperling, RaValese, Spur, Robinson, Corey, Lewis, and Austin (1985). These researchers hypothesized that n-3 PUFAs also effect the archidonic acid metabolic pathway leading to leukotriene formation. Omega-3 PUFAs cause a reduction in leukotriene B_4 generation with loss of functional competence as assessed by endothelial cell adherence. Endothelial cell adherence enhances the adherence of monocytes to the arterial wall and the migration to the internal layer where they are transformed into macrophages which accumulate lipids and stimulate smooth muscle cell profiferation (Ross, Bowen-Pope, Raines, & Faggiotto, 1982). Eicosapentaenoic acid and DHA are known to form leukotriene B_5 which is different from leutotriene B_4 and is known to reduce monocyte adherence. This action might help to reduce the atherosclerotic lesions (Glomset, 1985).

Lower blood viscosity is also another parameter under investigation because it is believed to be a measure of platelet stickiness (Lee, Hoover, Williams, Sperling, RaValese, Spur, Robinson, Corey, Lewis, & Austin, 1985). A study of Japanese fishermen showed that higher concentrations of ADP were required to result in half-maximal aggregation than in a group of farmers (Kabayashi, Hirai, Terano,

Hamazaki, & Kumagai, 1981). The researchers believed that even small amounts (2.5 g) of n-3 PUFAs might lower whole blood viscosity and, consequently, alter platelet aggregation.

CHAPTER III

METHODOLOGY

Study Design

The present study was designed to assess the effects of n-3 PUFA consumption on various plasma lipid levels and prothrombin time in hyperlipidemic men. A Latin square design (Figure 2) which allowed adjustment for any carry-over effect of previous treatment into the succeeding period without separating the three test periods by a wash-out phase was employed. Three dietary treatments were evaluated: the men's customary and a food source of n-3 PUFA fed at two levels. In Figure 2, high (H) and moderate (M) fish diets and customary (C) denote the three dietary treatments; the composition of the three experimental diets was as follows:

H - 8 oz (225 g) of salmon consumed four times per week yielding approximately 900 g of salmon per week or 9 g of n-3 PUFA per week.

M - 8 oz of salmon consumed two times per week yielding approximately 450 g of salmon per week or 4.5 g n-3 PUFAs per week.

C - Customary AHA Phase I diet

The phase I diet restricts total fat intake to 30% of total energy with approximately 10% of total calories from each of the following: monounsaturated fatty acids (MFAs),

SFAs and PUFAs, respectively, and which contains about 100 mg cholesterol per 1000 calories (American Heart Association, 1986).

Week	Period	Sequence					
		1	2	3	4	5	6
1 2 3 4	1	H	M	C	H	M	C
1 2 3 4	2	M	C	H	C	H	M
1 2 3 4	3	C	H	M	M	C	H

Figure 2: Experimental Design: Sequence of the Test Diets

Each participant consumed all three diets but in a different sequence. Subjects were randomly assigned among six sequences. Each dietary treatment lasted for four weeks.

Fasting blood samples were obtained at four points in the study, at the start and at the end of each dietary treatment phase. Each participant was requested to keep a food diary for four days during the last week of each treatment phase. At the end of H and M treatment phases of the study, a questionnaire was administered to obtain information on the acceptability of the fish provided in the

preceding four weeks (Appendix A). Data was also collected on smoking history, medication use, including aspirin, and/or any other anti-coagulant and lipid lowering agents.

Subjects

Eighteen men between 45-65 years of age were selected from a pool of hyperlipidemic patients who were participants of a cardiac rehabilitation program at Wake Forest University in Winston-Salem, NC. Subjects were included if they met the following criteria: hyper-lipidemic, no allergy to fish, did not regularly consume more than 16 oz of fish per week, and were generally complaint to the AHA phase I diet. Dietary adherence to the AHA diet was assessed by asking eligible participants to keep a four-day food record prior to entry into the study.

Only those subjects who had fasting plasma lipids either equal to or greater than the age and sex specific 75th percentile values for total cholesterol and TGs, observed in the prevalence study of the Lipid Research Clinics (LRC), were included (Appendix B) (U.S. Department of Health and Human Services, 1980). Men were excluded if their body mass index (BMI) exceeded the value estimated to represent 40% overweight for men (Metropolitan Life Insurance Co., 1983). The experimental protocol was fully explained to each participant and an informed consent was obtained (Appendix C).

Diets

The three different dietary treatments (Figure 2) were followed by all the participants. The fish was prepared, packed and distributed weekly to the subjects in the morning after the completion of their exercise program at the center. They were instructed to substitute the fish for an entree at lunch or dinner.

Several fish recipes were pretested using the staff of the Cardiac Rehabilitation Program as the evaluators. The testers were asked to rank the recipes for their taste, appearance, portion size, and overall acceptability on a four-point scale. The recipes that scored excellent, good, and average were included in the study. All recipes used in the study are presented in Appendix D.

At the start of a new dietary treatment individualized guidelines were provided to each participant to promote adherence to the new diet. Participants were asked to keep a record of any leftovers of the fish on a weekly basis. They were also requested to keep a diary of all foods consumed during four days (2 week days and the weekend) of the last week of each treatment phase. A diet record designed by the Cardiac Rehabilitation Program was used because it was familiar to the participants.

Analytical Methods

Dietary Analysis

A diet analysis software program, the Nutritionist II, was employed to analyze the four-day food record for cholesterol, total

calories, total and percent of calories from protein, carbohydrate, fat, saturated and polyunsaturated fatty acids. To ensure that the investigator was assigning codes to foods in a manner similar to dietitians at the Cardiac Rehabilitation Center, six food records were coded by each individual and the codings reviewed. Adherence to diet and consistency of dietary behavior throughout the study period were ascertained from the dietary analysis.

A Keys' score was used as a means of describing the effect of dietary pattern on serum cholesterol. The score is based on saturated (sat) and polyunsaturated (polyunsat) fat, cholesterol and calories in the daily diet (Anderson, Jacobs, Foster, Hall, Moss, Majonnier, & Blackburn, 1979).

Keys' formula:

$$\text{dietary index} = 2.70\ (\text{sat fat}) - 1.35\ (\text{polyunsat fat}) + 1.52\ \sqrt{1000\ \text{dietary C/total energy}}$$

where both saturated fat and polyunsaturated fat are calculated as a percent of total calories.

Blood Collection

Fasting blood samples (minimum of a 12-hour fast) were drawn at baseline and weeks 4, 8 and 12 into vacutainers containing either ethylenediaminetetracetic acid (EDTA) or sodium citrate as anticoagulants. The whole blood was centrifuged for 15 minutes at 2500 rpm in order to separate the plasma from the red blood cells. An aliquot of fresh citrated plasma was used for prothrombin time. The remaining

citrated plasma and plasma collected in EDTA were stored in polyethylene vials and frozen at -20°C.

Plasma Lipid Analysis

Lipid profiles including total TGs, total-, HDL- and LDL-cholesterol were determined for each sample. Total TG levels were assessed by the colorimetric enzymatic assay based on the method of Bucolo and David (1973). A Sigma diagnostics kit obtained from Sigma Inc., St. Louis, MO was used for this analysis. Total cholesterol and HDL-cholesterol were determined by the enzymatic method described by Allain, Poon, Chan, Richmond, and Fu (1974). The HDL fraction was isolated by using an HDL-precipitating agent obtained from Sigma Inc., St. Louis, MO. The LDL fraction was calculated using the following formula:

$$\text{LDL-C} = \text{total-C} - \left(\frac{\text{total-TG}}{5} + \text{HLD-C}\right)$$

The analytical methods were standardized by using a lipid control containing normal levels of TGs and cholesterol; the normal lipid control was obtained from Sigma Inc. To monitor reliability of test results, frozen aliquots of blood drawn from the investigator at the beginning of the study were assayed for lipids with each group of samples.

Prothrombin Time

Prothrombin time was measured using fresh citrated palsma with an assay kit purchased from Sigma Inc. The kit was based on the method described by Quick (1966). The method was standardized by

using the normal plasma control obtained from Sigma Inc. The normal plasma control was also included with each group of samples to monitor reliability of test results.

Statistical Analysis

The Latin square design that was employed allowed for the assessment of residual (carry-over) effects of each of the three dietary treatments (Cochran & Cox, 1968). An analysis of variance--general linear models (GLM) procedures of the Statistical Analysis Systems (SAS, 1985)--was employed to examine the adjusted direct effects and residual effects of each of the three dietary treatments. There was a significant residual effect for only one variable, total TG. The direct effect mean adjusted for the residual effect was calculated according to the method developed by Williams (1949).

A simpler design was employed to further examine the dietary treatment effect on the other variables for which there existed no residual effect, namely, total-, HDL- and LDL-cholesterol, and prothrombin time. The design selected blocked on individuals and period. This design was chosen in preference to a repeated measures design since it more closely resembled the original design by taking into account an ordering effect. If the dietary treatment effect was deemed significant, then differences between individual means were examined. The a priori comparisons of interest were those that involved comparing the two levels of fish consumption to the control mean.

A one-way analysis of variance (ANOVA) was performed to assess the effects of dietary treatment on composition of the diets consumed. If the dietary treatment effect was deemed significant, then differences between individual means were examined. Again, the a priori comparisons of interest were those that involved comparing the two levels of fish consumption to the control mean.

Analysis of covariance was performed to determine if the effect of dietary treatment remained after adjusting for potential confounders. Potential confounders that were examined included those variables for which there was a significant treatment effect and any additional covariates which included those dietary variables for which there was a significant correlation between the nutrient and blood outcome measure. Some dietary data was missing for two individuals and values were assigned to replace the missing values; the value used was the estimated mean dietary value for the treatment within the same period. The potential confounders were assessed as potential effect modifiers by including their interaction with treatment in the full model. All such two-way interactions were assessed together using a chunk test. Only covariables that were determined to add significantly to the model were reassessed individually as effect modifiers by adding a two-way interaction term. None of the covariables were found to be effect modifiers.

Differences in responses to items on the acceptability questionnaire between the two frequencies of salmon consumption were compared using the chi-square statistic.

CHAPTER IV

RESULTS

Baseline characteristics (means and standard error [SE]) of the six groups prior to randomization to treatment groups are presented in Table 8. There were no statistically significant differences noted in any of the measured characteristics among the six groups. Mean age of men in each group ranged from 51 to 61. The baseline values for plasma TGs and total cholesterol indicated that the subjects met the eligibility criteria of being hyperlipidemic. Triglyceride values across six groups were more variable than for total cholesterol levels; the mean TG level for Group 2 was the highest among all groups (433.6 mg per dl). This elevated TG level could be attributed to a single individual in Group 2. This particular individual had a baseline TG level of 786 mg per dl. The baseline BMI values of all subjects were under the estimated value representing 40% overweight value for men.

The subjects were also assessed for their compliance to the AHA Phase I diet which recommended 30% of total calories from fat. The percent of calories from total fat as shown in Table 8 indicated that subjects were generally compliant to 30% calorie AHA diet. A Keys' score was computed as a means of describing the effect of differences in diet on serum cholesterol.

Table 8

Baseline Characteristics of the Six Groups Prior to Randomization to Treatment

Characteristics	1 $\bar{X}$	1 SE	2 $\bar{X}$	2 SE	3 $\bar{X}$	3 SE	4 $\bar{X}$	4 SE	5 $\bar{X}$	5 SE	6 $\bar{X}$	6 SE
Age (years)	55.0	4.1	56.7	39.0	55.0	3.5	61.3	0.9	51.0	4.5	55.0	4.0
Triglyceride (mg/dl)	273.2	59.8	433.6	179.8	242.3	13.7	227.1	30.6	194.8	10.1	313.3	34.5
Total-C (mg/dl)	244.2	9.2	291.7	24.3	247.0	15.5	248.0	10.8	256.1	12.5	265.6	12.8
HDL-C (mg/dl)	27.7	4.5	36.6	3.6	34.9	4.0	30.8	6.6	46.5	1.8	30.6	3.3
LDL-C (mg/dl)	161.9	10.3	168.4	15.4	163.6	14.0	171.7	10.7	170.6	11.7	172.4	9.7
Prothromb Time (PT) (seconds)	20.0	2.8	20.7	2.0	20.7	3.7	19.6	1.9	21.2	0.2	22.5	2.8
BMI	26.7	1.8	29.3	0.9	25.0	2.4	26.1	2.3	25.0	1.9	26.3	1.1
Total Calories (Cal)	1328.0	229.0	1318.0	68.0	1424.0	18.5	1315.3	259.3	1233.7	16.3	1847.3	250.0
Keys' Score	25.6	.4.7	36.8	5.1	33.5	6.6	38.8	5.0	33.3	3.3	32.3	4.6
Total Fat (% Cal)	23.7	5.4	30.7	2.0	29.7	1.2	35.0	0.6	25.3	1.3	34.3	2.0
Cholesterol (mg)	146.0	37.2	202.3	43.4	164.0	11.8	171.3	7.7	118.7	20.9	196.0	43.1

$\bar{X}$ = mean

Individuals were randomly assigned to groups and then these groups were randomly assigned to treatment sequence within each Latin square. The Latin square design employed allowed for the assessment of carry-over effects of each of the three dietary treatments. There was only one variable, total TG, for which there was a significant residual (carry-over) effect. The original design allowed the estimation of the treatment effect adjusted for the residual effect (written as the direct effect in the ANOVA table) (see Appendix E for ANOVA table). Total TG mean values for each treatment were adjusted for the residual effect according to the method by Williams (1949) and are presented in Table 9. There was no significant residual effect for the other parameters--total-, HDL- and LDL-cholesterol and prothrombin time (see Appendix E). In addition to the ANOVA performed above, which included all individuals, an analysis was performed with the removal of the individual in Group 2 who consistently had high TG values. Removal of the individual did not alter the ANOVA results.

For those outcome variables for which there was no residual effect a simpler ANOVA design was used to assess treatment effect. Statistical analyses clearly demonstrated that there was a significant impact of dietary treatment on the outcome variables. Means and standard error of the variables of interest by treatment are presented in Table 10 (see Appendix F for ANOVA tables). The effect of treatment, i.e., feeding of supplementary salmon, appeared to be limited to the higher levels of feeding of salmon (4 time per week).

Table 9

Means and Standard Errors of Outcome Variables by Treatment

Parameters	Dietary Treatment[1]					
	Control		Moderate		High	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Triglycerides[2,3] (mg/dl)	254.8	± 6.7	227.6[4]	± 6.7	229.8[4]	± 6.7
Total-C[2] (mg/dl)	250.6	± 7.0	241.4[4]	± 7.3	236.6[4]	± 7.5
HDL-C[2] (mg/dl)	39.4	± 2.1	40.1	± 2.0	41.4[4]	± 2.0
LDL-C[2] (mg/dl)	160.8	± 5.8	155.9	± 5.3	149.9[4]	± 5.7
PT[2] (seconds)	22.8	± .8	23.6	± .9	24.2[4]	± .7

[1]Each value is the mean ± standard error (SE)

[2]Significant treatment effect $p < .05$

[3]Mean adjusted for residual effect according to method by Williams (1949)

[4]Significantly different from control mean; individual p values set at < 0.025 to maintain an overall alpha of 0.05

Table 10

Dietary Variables-Means and Standard Errors by Treatment

Parameters	Dietary Treatment[1]					
	Control		Moderate		High	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Energy (Kcal)	1476	± 72.5	1338	± 65.0	1360[2]	± 68.9
Total Fat[3] (g)	55.8	± 3.8	43.1[4]	± 4.9	45.5	± 3.7
SFA (g)	16.9	± 1.4	12.8	± 1.2	13.1	± 1.3
Linoleic Acid (g)	8.7	± 0.8	7.7	± 0.8	8.3	± 0.8
Cholesterol[3] (mg)	208.4	± 16.9	174.2	± 15.1	151.4[4]	± 15.1
Keys' Score[3]	38.2	± 1.9	32.7[4]	± 1.7	31.9[4]	± 1.8
Weight (lb)[3]	182.7	± 0.2	181.6[4]	± 0.2	181.4[4]	± 0.2

[1]Each value is the mean ± SE

[2]Adjusted for unequal cell size

[3]Statistically significant treatment effect $p < .06$

[4]Significantly different from control mean; individual p values set at <0.025 to maintain an overall alpha of 0.05

Feeding salmon two times per week showed statistically significant beneficial effecgs only for total TG and total cholesterol. The maximum reduction in total cholesterol was seen at the highest level of salmon feeding (4 times per week), whereas TG levels showed maximum reduction at lower levels of salmon feeding (2 times per week). Beneficial effects of feeding salmon on HDL--an increase--and LDL-cholesterol--a decrease--and prothrombin time--an increase--were seen only at the higher level feeding (4 times per week). It therefore appeared that feeding salmon four times per week was beneficial to all parameters under investigation, whereas reduced amounts of salmon (2 times per week) were only beneficial to total TG and total cholesterol.

The question that remained to be answered was "Cound the effect be attributed to the increase in n-3 PUFA consumption or did the supplementation of salmon to the customary diet result in changes in other dietary factors?" Analysis of the dietary variables indicated that the composition of the diets consumed differed among the three dietary treatment groups (Table 10). Different nutrient consumption patterns were observed between the two frequencies of salmon feeding and the control diet. When salmon was fed twice a week, total fat content of the diet was significantly less than when a control diet was consumed. However, even though the feeding of salmon four times per week tended to increase fat consumption slightly above that observed during the moderate salmon feeding phase, dietary cholesterol intake was significantly reduced and the computed Keys' score was

lower than observed on the control diet. Body weights of men on the different treatment groups also differed.

Results of the analysis of covariance, an attempt to control for differences in diet composition, are presented in Table 11. The analysis of covariance (ACOVA) tables for total-, HDL- and LDL-cholesterol are presented in Appendix G. There remained a statistically significant effect of treatment for four variables--TGs, total- and LDL-cholesterol and prothrombin time--even after adjustment for other dietary factors. The covariates listed added significantly to the model. None of the potential covariates made a significant contribution to the total variance of models containing total TGs and prothrombin time. The other lipid parameters, i.e., HDL- and LDL-cholesterol, were analyzed for treatment effect after adjustments were made for the contribution of total and saturated fat as covariates. Effect on total cholesterol was also analyzed after adjustment for total and saturated fat and weight. After covariate adjustment, the treatment effect on total cholesterol became limited to the higher level of fish consumption (Table 11). The significant treatment effect on HDL-cholesterol disappeared completely after adjusting for covariates (total and saturated fat). The effect of treatment on LDL-cholesterol remained even after adjusting for covariates (total and saturated fat).

Detailed results of the acceptability questionnaire are presented in Appendix H. In general the results indicated that there was little difference between the acceptability scores for smell, taste,

Table 11

Adjusted Means by Treatment

Parameters	Dietary Treatment			Variables Adjusted For
	Control	Moderate	High	
Triglycerides[1] (mg/dl)	254.8	227.6[2]	229.8[2]	none
Total-C[1] (mg/dl)	249.2	245.4	236.6[2]	fat, saturated fat, weight
HDL-C (mg/dl)	40.0	39.8	41.1	fat, saturated fat
LDL-C[1] (mg/dl)	159.4	157.2	149.5[2]	fat, saturated fat
PT[1]	22.8	23.6	24.2[2]	none

[1]Statistically significant treatment effect $p < .05$

[2]Significantly different from control mean, individual p values set at < 0.025 to maintain an overall alpha of 0.05

appearance, and portion size. However, significantly different responses were observed between the two feeding frequencies of salmon consumption. Sixty-seven percent of the subjects consuming salmon four times per week considered this level of consumption as too much, whereas those consuming the fish two times per week considered this level of consumption as just right (72%). The results also indicated that most subjects (67%) would prefer to consume salmon two times per week but consumption of salmon three times per week also appeared to

be well accepted (46%). The percent of subjects who indicated that they enjoyed consuming the salmon was similar between two feeding frequencies (78%) for two times versus 72% for four times. However, the participants indicated that the major problems associated with adherence to the salmon regime were those that involved incorporating the fish into the menu and family routine. The majority of participants expressed willingness to continue consuming as much fish as they did during the study.

CHAPTER V

DISCUSSION

The results of the present study support the well known hypotriglyceridemic effect of n-3 PUFAs. A reduction in serum TG was observed at both levels of salmon feeding. Studies conducted in patients with hyperlipidemia who were fed diets containing marine fish oils have consistently shown marked decreases in plasma TGs. Phillipson et al. (1985) fed 20 g of n-3 PUFAs as salmon oil to 20 type IIb hypertriglyceridemic patients and observed a 64% decrease in plasma TG. Other studies conducted in hyperlipidemic subjects have also reported reductions in plasma TG ranging from 25% to 70% (Harris, et al., 1984; Saynor et al., 1984; Simons et al., 1985; Sullivan, et al., 1986).

All these trials conducted in hyperlipidemic subjects have used fish oils or combinations of fish oils and fatty fish in large quantities; levels far in excess of that which would be expected to be included in the diet. The amounts of n-3 PUFAs fed in the present study were only 3-6% of the amount used by Phillipson et al. (1985), and these small amounts resulted in a 10% decrease in plasma TG.

The only other study that used amounts of n-3 PUFAs similar to the dose in the present study was conducted in normolipidemic individuals (Fehily et al., 1983). These researchers reported a 6% decrease in plasma TG which was approximately half of that which was

seen in the present study. Leaf and Weber (1988) have hypothesized that if similar amounts of n-3 PUFAs as fed to normals were consumed by hyperlipidemic individuals, the results in hyperlipidemic individuals would be much more dramatic. Comparison of the data collected in this study with data of Fehily et al. (1983) tends to support the hypothesis of Leaf and Weber (1988).

Whether a 10% decrease in plasma TG of hyperlipidemic men reduces their risk for CHD remains to be determined. Ahrens (1985) and Austin (1989) have assessed hypertriglyceridemia as an independent risk factor for CHD. Univariate analytic techniques suggest that high TG levels are associated with higher risk for CHD. However, when all other known risk factors are entered into multivariate analysis, plasma TG levels are only slightly indicative of increased risk for CHD. Hulley, Rosenman, Bawol, and Brand (1980) reviewed 27 epidemiologic-based studies and concluded that hypertriglyceridemia was not a reliable risk factor for CHD.

Multiple etiological factors make patients with hypertriglyceridemia an extremely heterogeneous group (Ahrens, 1985). Large intraindividual and interindividual variations lead to lower precision in TG determination as compared to other lipid measurements (Ahrens, 1985; Austin, 1989). Factors such as those listed above may partly explain why TGs do not generally emerge as an independent risk factor in multivariate statistical models. However, the role of TGs in the development of atherosclerosis can not be excluded. Very low density lipoprotein of hypertriglyceridemic subjects is shown to be involved

in the atherogenesis process; they are implicated in the conversion of monocytes to foam cells which are toxic to endothelial cells (Gianturco & Bradley, 1988). Therefore, Austin (1989) has suggested that it is premature to dismiss TG as a risk factor for CHD. Ahrens (1985) reported that despite the heterogeneity seen in hypertriglyceridemic individuals, they are homogeneous in at least one respect; they all show marked decreases in plasma TG levels when fed diets containing fish oils.

To summarize, a recommendation to increase consumption of fish rich in n-3 PUFAs in the management of hypertriglyceridemia is consistent with the TG lowering effect of n-3 PUFAs.

The impact of feeding a diet rich in n-3 PUFAs on total cholesterol was limited, after covariate adjustment, to the higher level of fish consumption. Other studies conducted in hyperlipidemics have always used large amounts of n-3 PUFAs and have generally reported hypocholesterolemic effects (Harris & Connor, 1980; Phillipson et al., 1981; Phillipson et al., 1985; Simons et al., 1985; Sullivan et al., 1986). When n-3 PUFAs were fed in doses of up to 20 g per day, an 8 to 48% reduction in total cholesterol has been reported by these investigators. Since no data are available on hyperlipidemics consuming fatty fish in realistic amounts, it is difficult to compare present results with other feeding trials. The results of the present study suggest that ingestion of as little as 1.3 g of n-3 PUFAs per day from a food source was sufficient to significantly reduce the elevated serum cholesterol levels. However,

at lower levels of salmon feeding (approximately 0.65 g of n-3 PUFAs per day) confounders such as total and saturated fat intake and body weight were partly responsible for the observed reduction in levels of total cholesterol.

A positive relationship between body weight and serum cholesterol has been shown by many investigators Kannel, Gordon, & Castelli, 1979; Kromhout et al., 1983; Montoye, Epstein, & Kjelsberg, 1966; Nichols, Ravenscroft, Lamphiear, & Ostrander, 1976). In the present study average body weight was lower for the groups consuming moderate and high levels of salmon as compared to individuals consuming their customary diet. Analysis of covariate which included weight suggested that in the group where individuals received two servings of salmon per day, the reduction in cholesterol levels could in part be attributed to reduced body weight.

Those studies which have fed comparable amounts of fatty fish, i.e., .65-1.3 g per day, to healthy subjects have not shown a hypocholesterolemic effect (Fehily et al., 1983; Spiller et al., 1987). It has been hypothesized by other researchers who fed similar amounts of n-3 PUFAs that one should expect more dramatic lipid lowering effect in hyperlipidemics than in normolipidemics (Leaf & Weber, 1988).

The average reduction in total cholesterol after covariate adjustment was 10.6 mg which represents a 4% decrease in the cholesterol level from the level of the control group (249 mg per dl). According to the results from the Lipid Research Clinics (LRC)

primary prevention trials conducted in asymptomatic hyperlipidemic men, a 4% reduction in cholesterol may represent an 8% reduction in the CHD events (Lipid Research Clinics Program, 1984). The National Cholesterol Education Program categorized individuals with blood cholesterol levels of 240 mg per dl as high risk and those with blood cholesterol of 200-239 mg per dl are considered borderline risk (Ernst, Cleeman, Mullis, Sooter-Bochenek, & Van Horn, 1988). Results of the present study indicate that consumption of salmon reduced the average cholesterol levels by 10 mg which resulted in the change of status of subjects from high to borderline risk for CHD.

In conclusion, two servings of salmon (8 oz each) per week (0.65 n-3 PUFAs per day) were not sufficient to significantly lower the elevated cholesterol levels, whereas four servings of salmon (8 oz each) per week (1.3 g n-3 PUFAs per day) did have a significant hypocholesterolemic effect in hyperlipidemic individuals.

Beneficial effects of feeding salmon have also been seen with LDL-cholesterol in the present study. The difference, a decrease in LDL-cholesterol, was observed between the higher level of feeding salmon and the customary diet. Consistent results have been reported when type IIb hyperlipidemics have been fed n-3 PUFAs. Some investigators have shown a decrease in LDL-cholesterol levels as a result of n-3 PUFA feeding. A mechanism has been hypothesized by which n-3 PUFAs might affect LDL-cholesterol. Saynor et al. (1984) suggest that reduction in LDL-cholesterol may result from the reduced synthesis of VLDL. Although VLDL concentration was not measured in the present study, the reduced LDL levels which were a result of salmon feeding

tend to support the latter hypothesis. Analysis of covariance indicated that the beneficial effect of n-3 PUFAs on LDL-cholesterol continued to persist even after adjusting for changes in dietary variables such as total and saturated fat.

A significant increase in HDL-cholesterol was observed in unadjusted data as a result of salmon feeding. However, analysis of covariance indicated that the effect of n-3 PUFAs on HDL-cholesterol after adjusting for saturated and total fat in the diet disappeared. Studies with hyperlipidemic men have generally shown increased HDL-cholesterol levels (Saynor, 1984; Saynor et al., 1984; Simons et al, 1985). These researchers have reported increases in HDL-cholesterol in subjects who consumed at least 10 g of MaxEPA oil for a minimum of eight weeks. A higher dosage of fish oil (20 ml MaxEPA oil) has been demonstrated to shorten the response time (4 weeks) in the HDL fraction. Of those studies which fed realistic amounts of fatty fish to healthy individuals, only the study conducted by Spiller et al. (1987) has shown increased HDL-cholesterol at levels as low as 100 g fish per day (1 g n-3 PUFAs). However, the studies that have shown an increase in HDL levels have not included an analysis of the effect in the presence of potential confounders as was done in the present study. Probably larger doses of n-3 PUFAs and/or their long time consumption are required before beneficial effects on HDL-cholesterol levels are observed in hyperlipidemic individuals.

An increase in prothrombin time was noted only at the higher level of salmon feeding which provided 1.3 g n-3 PUFAs per day. Prothrombin time is a test of the extrinsic pathway involved in blood

clotting and is the final common pathway of clotting. The final pathway of clotting involves the activity of thrombin. Thrombin is actively controlled via two mechanisms; one involves the existence of a thrombin antagonist antithrombin III, the other involves the inactive thrombin zymogen prothrombin. Since plasma levels of antithrombin III have been demonstrated to increase in subjects fed supplements of 10 g of fish oil (Mortensen, Schmidt, Nielsen, & Dyerberg, 1983), there is the possibility that fibrin clot formation may be altered by n-3 PUFA feeding. A study where 2 g EPA supplements per day were fed to human subjects, however, failed to demonstrate an increase in the prothrombin time (Nagakawa, Orimo, Harasawa, Morita, Kashirok, & Murota, 1983). The results of the present study do not support the findings of Nagakawa et al. (1983). There have not yet been sufficient studies conducted to determine, with confidence, the degree to which n-3 PUFAs alter prothrombin time.

Many scientists believe that the beneficial antithrombotic effects of fish oils on CHD are best explained by their effects on platelets and endothelial cells (National Dairy Council, 1988). Measurement of parameters such as platelet aggregation, amounts of EPA and DHA in the endothelial cells, and measurement of key compound in prostaglandin synthesis would have added to this body of knowledge, but due to financial constraints, these tests were not performed in the present study.

There are several features regarding the design and analysis which strengthen the interpretation of the results obtained in the

present study. First, in the Latin square design employed, subjects served as their own controls thereby reducing the error variance. Second, the analysis design permitted the treatment effect be adjusted for any residual effects of the previous treatment phase. Third, removal of the influence of other dietary factors did not in most part effect the lipid results. It should be noted that most other studies in the literature have not accounted for other confounding dietary and environmental factors. It is possible that if other factors had been accounted for in these studies the effect of n-3 PUFAs on blood lipids might have been somewhat diminished.

One limitation of the present study appears to be in the estimation of dietary intake. The total caloric estimate seems to be very low (approximately 1400 Kcal) as compared to values generally reported for men of similar age range (approximately 2500 Kcal). Underreporting of actual intake was suspected, but personal communication with the dietitian at the Cardiac Rehabilitation Center did not support this suspicion. If one accepts that the actual description of foods accurately reflects intake, then the error could have been introduced at the level of the calculation of nutrient intake and may be inherent to the food composition data base. Several recent studies have demonstrated that different nutrient values are obtained depending upon the nutrient data base system utilized in the calculation (Medlin & Skinner, 1988). Nutritionist II was used to analyze food intakes in the present study which has a data base of 900 foods. Medlin and Skinner (1988) have addressed problems which are inherent

in smaller data bases; the availability of a limited number of foods from which to select may lead to an underestimation of actual nutrient intake. In the present study even though actual nutrient intake may have been underestimated, it was a systematic bias across all nutrients, subjects and treatment periods. Thus, it should have had little impact on influencing the results from this study. In addition, the investigative staff had great confidence that the subjects consumed the fish provided, and communication with the subjects, spouses and staff of the Cardiac Rehabilitation Center supported this assertion.

Encouraging results, however subjective in nature, were obtained from the assessment of the acceptability of the fish by the subjects. Results indicated that the participants accepted salmon for its organoleptic characteristics (smell, taste, appearance, and portion size) equally across the two feeding frequencies. As anticipated, most participants indicated that consuming the same fish four times per week was somewhat burdensome and that consuming fish three times per week was more acceptable.

Comparing these results with any other data is not possible, because no one to date has assessed the acceptability of fish in amounts beneficial to reduce the risk for CHD either in healthy or hyperlipidemic individuals. The problems encountered by participants in adhering to the fish diet were those that involved incorporating the fish into the menu and family routine. It is anticipated that these difficulties would be overcome if the salmon was integrated into the entire family's eating pattern.

Despite all the inconveniences the participants responded affirmatively when asked if they would consume similar amounts of fish in the future. However, such affirmative responses need to be interpreted with care because of the way the question was worded. The current question asked was, "Would you consume as much fish again?" and could have been interpreted to mean that someone else was providing the fish. If the question had been "Would you consume as much fish again at your own expense?", the response might have been different.

To summarize, the consumption of fish at levels which provide 1.3 g of n-3 PUFAs does benefit hyperlipidemic individuals by decreasing lipid levels. However, any claim regarding antithrombotic properties of n-3 PUFAs cannot be made based on the present data. It is encouraging, though, that the observed effect on prothrombin time is in a direction similar to that which has been suggested in the literature. The acceptability data provides a bridge between the heart-healthy claims and the acceptance of fish by people and indicates that people may be willing to consume fish more frequently if they perceive that fish can reduce their risk for CHD.

Based on the results of the present study, it seems prudent to recommend that individuals consume more fish rich in n-3 PUFAs, and substituting fish for foods rich in SFAs may also be an effective strategy for reducing the SFA content of the diet. Fish oil capsules are not recommended as a therapeutic measure because their benefits and safety have not been proven. The more prudent procedure, therefore, appears to be for a person to include more fish in the diet.

CHAPTER VI

RECOMMENDATIONS FOR FUTURE RESEARCH

The potential role of dietary augmentation with n-3 PUFAs is promising but has yet to be resolved. Review of the literature and results of the present study have presented many potentials for n-3 PUFAs. Experimental trials in human subjects have generally involved large doses of n-3 PUFAs either in the form of fish oil or combinations of fish oils and fatty fish. Few studies have examined the effects of fatty fish consumption in realistic amounts, and such studies are generally conducted with healthy individuals. The present study was the first to study the effects of n-3 PUFAs when consumed in moderate amounts by hyperlipidemic individuals, and the results have been encouraging. The study was, however, short term, and the sample size of the study was small. Long-term intervention studies of moderate amounts of n-3 PUFA, using a larger sample, are recommended for stronger evidence of the beneficial effects of n-3 PUFAs. Intensive long-term research efforts are also needed to determine the minimum dosage of n-3 PUFAs for optimum benefits before dietary recommendations can be made to the general public.

Subjects in the present study were chosen because they liked fish. One can, therefore, assume that these subjects were receiving some amount of n-3 PUFAs in their diet before the study. It would be worthwhile to study the effects of adding realistic amounts of fish to a totally fish-free diet.

The results of the present study also indicated that the covariates, such as total and saturated fat, had an effect on lipid levels. The effectiveness of n-3 PUFAs in moderate amounts, at different levels of total and saturated fat consumption in humans, needs further assessment. Studies that monitor effects of various dietary levels of n-6 PUFAs on the response to n-3 PUFAs feedings in humans are also required. Research in this area will aid in understanding whether the effect of n-3 PUFAs is more pronounced when diet is limited in other fats. During the course of the present study, it was often asked if salmon was the only fish that was good to reduce the risk of CHD. Since only salmon was used as a source of n-3 PUFAs in the present study, it is recommended for future studies that various fish species be used and comparisons be made of their effects in the same subjects because people generally consume a variety of foods. Success in recommending increased fish consumption can be achieved only if people would consume a variety of different fish to receive the benefits of n-3 PUFAs.

Evidence indicates that n-3 PUFAs may decrease the risk of CHD because of the antiaggregatory effect which results from competitive inhibition of formation of proaggregatory metabolites of AA (Siess et al., 1980). To date parameters such as bleeding time and platelet aggregation have generally been used to study the antiaggregatory effects of n-3 PUFAs. Assessment of additional parameters such as concentration of n-3 PUFAs in platelets and measurement of urinary metabolites of antiaggregatory prostanoids need to be made when

varying amounts of n-3 PUFAs and n-6 PUFAs are fed. This will allow a researcher to determine an optimal ratio of n-3 and n-6 PUFAs in the diet, and the levels of n-3 PUFAs required to favorably alter prostaglandin synthesis.

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APPENDIX A

ACCEPTABILITY QUESTIONNAIRE

Acceptability Questionnaire

Diet _____

Please answer all the following questions which will help us learn how the fish diet over the past four weeks is fitting into your routine.

1. Rate the following characteristics of the fish diet you consumed for the last four weeks.

	Good	Average	Poor	Other
Smell	____	_____	____	_____
Taste	____	_____	____	_____
Appearance	____	_____	____	_____
Portion Size	____	_____	____	_____

2. Rate the frequency of consumption.

   Too much _____

   Just right _____

   Too little _____

3. How many times per week would you like to consume fish?

   Once _____

   Twice _____

   Three times _____

   Other _____

4. Did you enjoy consuming salmon for the past four weeks?

   Yes _____

   No _____

   Other _____

5. If your answer to question no. 4 is no, please check the other kind(s) of fish you would like to consume.

   Mackerel _____

   Herring _____

   Trout _____

   Flounder _____

   Tuna _____

   Other _____

6. Check all the problems you experienced in adhering to the fish diet for the last four weeks.

   Portion size _____
   Smell of fish _____
   Taste of fish _____
   Physical discomfort _____
   Fitting in the menu _____
   Fitting in the family routine _____
   Inflexibility to go out to eat _____
   Other _____

7. Would you consume as much fish as you did for the last four weeks again?

   Yes _____

   No _____

APPENDIX B

PLASMA LIPID VALUES FOR ELIGIBLE PARTICIPANTS

Plasma Lipid Values for Eligible Participants

Age	Total Cholesterol 75th Percentile and Above (mg/dl)	Age	Total Triglycerides 75th Percentile and Above (mg/dl)
40-49	231	45-49	165
50	230	50-54	178
		55-59	167
		60-65	150

Note. Taken from The Lipid Research Clinics Population Studies Data-book (Vol. 2). (NIH Publication No. 80-1527)

APPENDIX C

GENERAL INFORMATION ABOUT THE STUDY AND CONSENT FORM

General Information About the Study

Fish and Heart Disease
Department of Food, Nutrition and Food Service Mangement
School of Home Economics
The University of North Carolina at Greensboro

There is a growing evidence that consumption of dietary omega-3 polyunsaturated fatty acids (n-3 PUFAs), abundant in marin organisma, may reduce the development of cardiovascular disease. Omega-3 PUFAs have two important effects in the body which may impact on the development of heart disease. These fatty acids are known to lower the elevated serum lipids and also alter platelet function (bleeding factors). The hypolipidemic effects of n-3 PUFAs appear to be consistent only when high levels of either fatty fish or a fish oil extract have been consumed by people. Similar observations have been made when dietary habits of the Eskimos were studied.

At lower intakes of n-3 PUFAs, the effects are less discernible and to date there have been fewer studies which have attempted to determine the impact of consumption of low levels of n-3 PUFAs. The studies that have studied the effects of fish consumption in moderate amounts have suggested that the mean capital intake of fish is currently about 3 ozs per week and any increase in fatty fish consumption may prove beneficial in reducing the risk of cardiovascular disease, particularly in those individuals with elevated serum lipids. It is also worth mentioning here that Eskimos are known to have prolonged bleeding time which is associated with their excess consumption of fatty fish. However, the amount of fish consumed by the

United States population at large, and in the proposed study, does not pose a danger of bleeding disorder, and consumption in moderate amounts may not be harmful.

The present study is, therefore, designed to study the lipid lowering effects of fish consumption. An informed consent is sought from the people who would like to volunteer for the participation in the proposed study.

Consent Form

Fish and Heart Disease
Department of Food, Nutrition and Food Service Management
School of Home Economics
The University of North Carolina at Greensboro

General

I understand that the purpose of this research study is to evaluate the effects of fish consumption on blood lipid (fats) levels--total cholesterol, HDL-cholesterol, total triglycerides and platelet function. Participation in the study requires me to follow three different dietary regimes over a period of twelve (12) weeks. I understand that I will be randomly assigned to each dietary regime for a four-week period (random assignment is similar to a flip of a coin where everyone has an equal chance of being selected).

Dietary Regimens

Following are the three dietary regimens:

1. I understand that I will consume eight ounces (ozs) of fish (salmon) four times a week for four weeks. The fish will provide a certain amount of polyunsaturated fatty acids (PUFAs) that I need. The remainder of the PUFAs will be provided in the form of safflower oil which will also be provided by the research investigator.
2. During the other four weeks, I will consume eight ounces of fish (salmon) two times a week for four weeks. Safflower oil will be provided as needed.

3. During another period of four weeks, I will consume only safflower oil to get all the PUFAs I need.

I understand that fish will be provided to me in cooked form, and safflower oil can be used for cooking purposes.

I understand that I will be required to follow certain dietary guidelines during the entire study. A draft of the dietary guidelines is as follows:

1. Do not eat an egg on the fish day.
2. Restrict visible fat (butter, cream, margarine, salad dressing) to three teaspoons a day. Do not prepare foods with added fat from animal sources. Restrict the use of butter and animal fats.
3. Avoid the use of other meats on the fish day.
4. Try to avoid consumption of any vegetable oil during fish meal and restrict it on fish day.

I understand that I will be asked to keep a four-day diet record at the beginning of the study and at the end of each four-week period. This is similar to the seven-day diet record which I provide to the cardiac rehabilitation program.

I will also be required to fill out a simple questionnaire at three different times during the entire study period of twelve (12) weeks. The questionnaire will only assess my acceptability of the diet consumed during the previous four weeks.

I understand that all the aforementioned procedures will be performed at the cardiac rehabilitation program after my exercise.

Explanation of Risks

I understand that 30 cc of blood will be drawn at the beginning of the study and at the end of each study period.

I understand that I may experience some discomfort such as bruising or lightheadedness associated with blood drawing procedures. I realize that all possible precautions will be taken in order to reduce the risks associated with drawing blood.

Explanation of Benefits

The benefits I may gain from participating in this research study include: an assessment of blood lipid profile and an evaluation of my dietary adequacy. In addition, I will be able to consume cooked fish free of cost which is recommended for heart disease patients.

I understand that all data collected will be coded to maintain confidentiality. I understand that I can withdraw from the study at any time.

Dr. Karen Graves or another research staff member will be available to answer any questions about the study. They may be reached at the University of North Carolina at Greensboro Nutrition Department on weekdays at (919) 334-5313. Dr. Henry Miller will also be available to answer questions about the research. Questions regarding the research subjects' rights will be answered by the Chairman, Clinical Research Practices Committee through the Office of Research Development (919-748-4542).

_____
Signature of Subject

_____
Date

_____
Signature of Witness

_____
Date

APPENDIX D

RECIPES

Recipes

Lemon Poached Salmon

4 6 oz fresh or frozen salmon steaks
1/3 cup lemon juice
1/4 cup chopped celery
1/8 teaspoon freshly ground pepper
tartar sauce (see recipe below)
4 cups water
1 small onion, sliced
1/2 teaspoon salt
lemon wedges

Thaw salmon if frozen. In large skillet combine water, lemon juice, onion, celery, salt, and pepper. Bring to boil; simmer five minutes. Add salmon; simmer, covered, 7 to 10 minutes or until fish flakes easily with a fork. Remove salmon from liquid with spatula. Chill or serve hot. Serve salmon with lemon wedges. If desired, top salmon with tartar sauce and garnish with fresh dill weed. Makes 4 servings.

Tartar Sauce

1/2 cup whipped mayonnaise-type salad substitute (no more than 20 calories per tablespoon)
1 teaspoon finely chopped onion
1 teaspoon finely chopped pimento
1/4 cup finely shredded carrot
1 tablespoon finely chopped pickle
1 teaspoon snipped parsley
1 teaspoon lemon juice

Combine salad dressing, carrot, dill pickle, onion, parsley, pimento, and lemon juice. Cover and chill thoroughly. Serve with fish. Makes 3/4 cup sauce. One serving (2 tablespoons)

Lemon-Parsley Salmon

1 quart water
2 teaspoons chicken-flavored bullion granules
1/2 teaspoon whole peppercorns
1/4 teaspoon garlic powder
1 one pound salmon fillet
fresh tarragon leaves (optional)
1 cup fresh parsley sprigs
1 tablespoon fresh tarragon leaves or 1 teaspoon dried whole tarragon
4 1/4 in. thick lemon slices
lemon wedges (optional)

Combine first 7 ingredients in a large skillet. Bring mixture to a boil; reduce heat, and simmer, uncovered, 10 minutes. Rinse salmon under cold, running water; pat dry. Add salmon, if necessary. Return to a simmer; cover and cook over low heat 10 to 12 minutes or until salmon flakes easily when tested with a fork. Remove salmon from skillet, using a slotted spoon; discard liquid. Cut fillet into 4 equal portions; garnish with lemon wedges and tarragon, if desired. Yield: 4 servings.

Baked Salmon Steaks with Caper Sauce

2 tablespoons plain low-fat yogurt
2 tablespoons reduced calorie mayonnaise
1 teaspoon capers, drained
1/2 teaspoon low-sodium lemon-pepper seasoning
2 4-oz. salmon steaks
fresh dill sprigs (optional)
1 small onion, thinly sliced
1 tablespoon dry white wine
1 tablespoon chopped fresh dill or 1 teaspoon dried whole dill weed
lemon slices (optional)
vegetable cooking spray

Combine first 5 ingredients, mixing well. Cover and chill. Rinse salmon under cold, running water; pat dry. Place in a 1-quart baking dish coated with cooking spray. Arrange onion slices over salmon. Pour wine over salmon and sprinkle with dill. Cover and bake at 350° for 15-20 minutes or until salmon flakes easily when tested with a fork. Discard onion and dill and transfer salmon to individual serving plates. Spoon 2 tablespoons caper mixture over each salmon steak and garnish with lemon slices and fresh dill, if desired. Yield: 2 servings

Broiled Cilantro Salmon

1/4 cup chopped onion
1/4 cup chopped tomato
1/8 teaspoon white pepper
2 tablespoons lime juice
1 tablespoon low sodium soy sauce
vegetable cooking spray
1/4 cup chopped red bell pepper
3 tablespoons chopped fresh cilantro
1 tablespoon vegetable oil
1 clove garlic, crushed
4 4-oz. salmon steaks
fresh cilantro sprigs (optional)

Position knife blade in food processor bowl; add first 9 ingredients; top with cover and process until mixture is smooth. Rinse salmon under cold, running water, pat dry. Place salmon in a 12 x 8 x 2 inch baking dish. Pour 1/4 cup cilantro mixture over salmon, turning to coat well. Set aside remaining cilantro mixture. Cover and chill at least 4 hours, turning occasionally. Remove salmon from marinade, discarding marinade. Place on rack of a broiler pan coated with cooking spray. Broil 4-5 inches from heat for 5 minutes or until fish flakes easily when tested with a fork. Transfer to a serving platter; serve with reserved cilantro mixture. Garnish with cilantro sprigs, if desired. Yield: 4 servings

Poached Fish with Dill Sauce

1 3-lb. fresh or frozen dressed fish
1/4 teaspoon dried tarragon, crushed
2 tablespoons butter or margarine
4 teaspoons all-purpose flour
1/2 teaspoon sugar
1 beaten egg yolk
2 cups water
3 lemon slices
1 bay leaf
2 teaspoons lemon juice
1/2 teaspoon dried dill weed

Thaw fish, if frozen. Place on large piece of cheesecloth; fold cloth over fish. Place on rack in poaching pan. Add water, lemon slices, bay leaf, tarragon, and 1 teaspoon salt. Cover and simmer 25-30 minutes or until fish flakes easily when tested with fork. Remove from pan; keep warm. Strain and reserve 1 cup cooking liquid. For sauce, in saucepan melt butter; stir in flour. Add reserved liquid, lemon juice, sugar, dill week and dash of salt. Cook and stir until thickened and bubbly. Gradually stir 1/2 cup of hot mixture into egg yolk; return to hot mixture. Cook and stir 1-2 minutes more. Pull cloth away from fish; remove and discard skin. Transfer fish to platter using 2 spatulas. Top with some sauce. Pass remaining. Makes 6 servings

APPENDIX E

ANALYSIS OF VARIANCE RESULTS: ANALYSIS TO ESTIMATE RESIDUAL EFFECT

Analysis of Variance Results: Analysis to Estimate Residual Effect

Triglyceride

Source	df	SS	F	P
ID	17	937403		
Period (sq)	4	3481		
Direct Effect (adj)	2	6540	15.40	.0001
Residual Effects (adj)	2	2467	5.81	.0077
Error	28	5946		
Total Cholesterol				
ID	17	47173		
Period (sq)	4	276		
Direct Effect (adj)	2	1835	20.52	.0001
Residual Effects (adj)	2	134	1.51	.2387
Error	28	1252		
HDL-C				
ID	17	3543		
Period (sq)	4	64		
Direct Effect (adj)	2	46	4.37	.0224
Residual Effects (adj)	2	9	9	.4046
Error	28	147		
LDL-C				
ID	17	26964		
Period (sq)	4	276		
Direct Effect (adj)	2	1347	1308	.0001
Residual Effects (adj)	2	340	330	.0515
Error	28	1442		

Prothrombin Time

Source	df	SS	F	P
ID	17	463		
Period (sq)	4	34		
Direct Effect (adj)	2	18	6.64	.0044
Residual Effects (adj)	2	1	.36	.6975
Error	28	37		

APPENDIX F

ANALYSIS OF VARIANCE RESULTS: ANALYSIS OF BLOCKING ON INDIVIDUAL AND PERIOD

Analysis of Variance Results: Analysis of Blocking on Individual and Period

Source	df	SS	F	P
Total Cholesterol				
ID	17	3543		
Period	2	63		
Treatment	2	37	3.7	.0349
Error	32	159		
HDL-C				
ID	17	3543		
Period	2	63		
Treatment	2	37	3.7	.0349
Error	32	159		
LDL-C				
ID	17	26965		
Period	2	214		
Treatment	2	1129	9.8	.0005
Error	32	1846		
Prothrombin Time				
ID	17	463		
Period	2	32		
Treatment	2	18	12.8	.0001
Error	32	40		

APPENDIX G

ANALYSIS OF COVARIANCE: ANALYSIS TO ADJUST FOR POTENTIAL CONFOUNDERS

Analysis of Covariance: Analysis to Adjust for Potential Confounders

Total Cholesterol

Source	df	SS	F	P
ID	17	25626		
Period	2	145		
Treatment	2	632	10.08	.0006
Fat	1	441	16.07	.0009
Saturated Fat	1	337	10.74	.0030
Weight	1	119	3.79	.0623
Error	26	816		
HDL-C				
ID	17	2953		
Period	2	61		
Treatment	2	16	1.70	.2016
Fat[1]	1	4	.90	.3503
Saturated Fat	1	14	2.98	.0960
Error	27	129		
LDL-C				
ID	17	25005		
Period	2	180		
Treatment	2	836	8.93	.0011
Fat	1	491	10.58	.0031
Saturated Fat	1	301	6.48	.0169
Error	27	1254		

[1]Type 1 SS showed that fat made a significant difference.

APPENDIX H

RESULTS OF ACCEPTABILITY QUESTIONNAIRE

Results of Acceptability Questionnaire

Question 1: Overall Characteristics (average score)[1]

	2 Times Per Week	4 Times Per Week
Smell	2.27	2.17
Taste	2.56	2.50
Appearance	2.61	2.83
Portion Size	2.61	2.61
Average	2.51	2.53

[1]the higher the score the more positive the response.

Question 2: Frequency of Consumption (% of participants)[1]

	2 Times Per Week	4 Times Per Week
Too much	17	67
Just right	72	33
Too little	11	0

[1]responses were significantly different between the two frequency categories.

Question 3: How many times per week (% of participants)

	2 Times Per Week	4 Times Per Week
Once	6	11
Twice	67	39
Three times	17	44
Other	11	6

Question 4: Enjoyment (% responding positively)

2 Times Per Week	4 Times Per Week
78	72

Question 5: (not analyzed (subject did not follow instructions)

Question 6: Problems with adherence (% responding affirmatively)

	2 times per week	4 Times Per Week
Portion size	5	5
Smell	11	33
Taste	33	

Question 6: Problems with adherence (% responding affirmatively)

	2 Times Per Week	4 Times Per Week
Portion size	5	5
Smell	11	33
Taste	33	11
Physical discomfort	5	5
Fitting in routine	33	44
Fitting into family routine	50	44
Inflexibility	22	17

Question 7: Would you consume as much again (% responding affirmatively)

2 Times Per Week	4 Times Per Week
83	72