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Effects of n-3 PUFA and n-6 PUFA on plasma lipids, bleeding time, prothrombin time, and liver cholesterol in the gerbil

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EFFECTS OF N-3 PUFA AND N-6 PUFA ON PLASMA LIPIDS, BLEEDING TIME, PROTHROMBIN TIME, AND LIVER CHOLESTEROL IN THE GERBIL

by

P. Carolyn Dunn

A Dissertation Submitted to
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APPROVAL PAGE

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This dissertation research assessed the effects of n-3 and n-6 PUFA fed in varying combinations on plasma lipids, bleeding time, prothrombin time, and liver cholesterol in 90 adult male gerbils. Gerbils (15 per group) were randomly assigned to one of six dietary treatments. The dietary treatments differed only in type of fat fed. All diets contained 15% fat by weight (14% as the test fat and 1% as safflower oil). Group 1 consumed 100% of their test fat as lard (SFA), group 2 100% as safflower oil (n-6 PUFA), group 3 100% as MaxEPA oil (n-3 PUFA), group 4 25% n-3 PUFA and 75% n-6 PUFA, group 5 25% n-6 PUFA and 75% n-3 PUFA, and group 6 50% n-6 PUFA and 50% n-3 PUFA. The gerbils remained on the test diets for five weeks.

Plasma lipids measured in the study included alpha (HDL-C), beta (LDL-C and VLDLC), total cholesterol, and triglycerides. The only statistically significant effect of dietary treatment on any plasma lipid measure was for triglycerides. The gerbils consuming the 100% n-6 PUFA containing diet exhibited the highest mean triglyceride level. This was significantly different ($p < .05$) from mean levels exhibited by those animals consuming the 50% n-6 PUFA 50% n-3 PUFA and 25% n-6 PUFA 75% n-3 PUFA containing diets. No significant overall effects of the dietary treatments were observed for bleeding time, prothrombin time, or liver cholesterol.

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CHAPTER I

INTRODUCTION

Modification of risk factors is the major clinical and public health approach to the prevention of coronary heart disease (CHD). Scientific evidence implicates high levels of blood cholesterol as a major risk factor for CHD which has been observed in epidemiological, genetic, clinical, metabolic, experimental, pathological, and intervention studies. Animal, metabolic, and clinical studies have all shown that it is possible to favorably alter plasma cholesterol levels by dietary means. Recently, attention has focused on the possible beneficial effects of omega-3 polyunsaturated fatty acids (n-3 PUFA) in reduction of incidence of CHD.

Several epidemiological based comparison studies between Eskimos of Greenland and those residing in Denmark were conducted in the 1970s (Bang & Dyerberg, 1972; Band & Dyerberg, 1980; Bang, Dyerberg, & Nielson, 1971; Dyerberg, Bang, & Hjorne, 1975). These studies noted that the Greenland Eskimos had lower serum triglycerides, lower very low density lipoprotein cholesterol (VLDL-C), lower low density lipoprotein cholesterol (LDL-C), and higher high density lipoprotein cholesterol (HDL-C). Eskimos living in Greenland also exhibited prolonged bleeding times and a lower incidence of CHD than those residing in Denmark.

The diets of the Greenlanders and Danes also differed. The Greenland Eskomos consumed nearly 454 gm (1 lb) of fish per day, while the Danish Eskimos consumed less than 20 gm (< 1 oz.) of fish per day. Both fat and cholesterol content were higher in the Greenlanders' diet; these increases were contributed by whale blubber and other marine fats.

The amount of fat actually consumed by populations with high fish consumption is similar to the fat content of a more Western diet. However, the fat composition of the high fish diets is low in saturated fatty acids (SFA) and consists primarily of unsaturated fatty acids. These unsaturated fatty acids differ from the PUFAs consumed in the typical Western diet. Those unsaturated fats are derived primarily from plant sources, and the desaturation occurs at the sixth position (n-6 PUFA) from the terminal methyl group, whereas, marine oils are desaturated at the third position (n-3 PUFA). The primary n-3 PUFA found in marine oils are eicosapentaenoic acid (EPA) (C 20:5) and decosahexaenoic acid (DHA) (C 22:6). These two PUFAs, EPA more so than DHA, are found in large amounts in the Eskimos' diet. Linoleic acid (LA) C 18:2), an n-6 PUFA, is the predominant PUFA in the typical Western diet. It is these differences (i.e., abundance of n-3 PUFA and lower SFA consumption) that are apparently the dietary factors related to the Eskimos' relative freedom from CHD.

The n-3 PUFA rich diets appear to have two biological effects which impact on the incidence of CHD. They lower plasma lipids and reduce platelet adhesiveness (i.e., increase bleeding time). However,

it appears that unless n-3 PUFAs are fed at high levels, they do not exert much of a hypocholesterolemic effect. Omega-6 PUFAs are known to be powerful hypolipidemic agents, but their effect on platelet aggregation is not as pronounced as that seen with feeding n-3 PUFAs (Keys, Anderson, & Grande, 1957).

The present study was designed to examine the effects of n-3 PUFA when fed in combination with n-6 PUFA on plasma lipids and platelet function as measured by bleeding time and prothrombin time. Several combinations of the two PUFA types were fed, and one objective of the study was to determine combinations that optimize the lipid lowering effect while at the same time did not compromise the beneficial platelet function effect. Measurement of liver cholesterol was measured to provide insight into the mechanism of action of combination of n-3 and n-6 PUFA on synthesis and storage of hepatic cholesterol.

Objectives of the Study

The specific objectives of the study were as follows:

1. To assess the effect of differing levels of n-3 and n-6 PUFAs fed in combination on plasma lipids, bleeding time, prothrombin time, and liver cholesterol.
2. To assess the effect of n-3 PUFA, n-6 PUFA, and SFA on plasma lipids, bleeding time, prothrombin time, and liver cholesterol. Observations from these three groups will serve as reference or comparison values.

3. To determine combinations of the n-3 and n-6 PUFAs that optimize the hypolipidemic effect without compromising the beneficial reduction in platelet adhesiveness.
4. To determine the usefulness of the gerbil as a model in the study of diet and CHD risk factors.

Hypotheses

The hypotheses to be tested are as follows:

H_1: There will be no significant difference in plasma triglycerides across the six dietary treatments.

H_2: There will be no significant difference in total cholesterol across the six dietary treatments.

H_3: There will be no significant difference in HDL cholesterol across the six dietary treatments.

H_4: There will be no significant difference in LDL and VLDL cholesterol across the six dietary treatments.

H_5: There will be no significant difference in bleeding time across the six dietary treatments.

H_6: There will be no significant difference in prothrombin time across the six dietary treatments.

H_7: There will be no significant difference in liver cholesterol across the six dietary treatments.

CHAPTER II

REVIEW OF LITERATURE

Studies presently indicate two important actions of n-3 PUFAs, decrease in plasma lipids and a beneficial change in platelet function. Few studies, however, have seen a lipid lowering effect, with the exception of plasma triglycerides, when n-3 PUFAs were fed in amounts feasible for consumption by the American population. On the other hand, even low levels of n-3 PUFAs were observed to influence platelet function as measured by bleeding time. This review will firstly examine the effects of n-3 PUFA feeding on plasma lipids; secondly, their effect on bleeding time and prothrombin time, indicators of platelet function; and lastly, the effect of differing ratios of n-6 to n-3 dietary PUFA.

Plasma Lipids

Published reports of observations on the Greenland Eskimos prompted many subsequent studies in both humans and various animal models. The results are summarized, with respect to plasma lipid profiles, in Table 1 (animal feeding trials) and Table 2 (human feeding trials). Omega-3 PUFA concentrations of fish oils and fatty fish fed in the studies reviewed are presented in Table 3.

Animal studies. The effects of n-3 PUFA on plasma lipids have been investigated in several animal models. Due in part to differing

Table 1

Animal Feeding Trials: Summary of Effects of Marine Oil or N-3 Polyunsaturated Fatty Acid on Plasma Lipid Parameters

Reference	Diets: Compared to		Duration of Diet	mg/100 ml TG	Cholesterol	HDL-C	LDL-C	VLDL-C
I. Rats								
Kobatake et al. (1983)	5% squid liver oil	5% lard	2 weeks		↓[c] 43%	↑[b] 109%		
Wong et al. (1984)	15 wt % MaxEPA	15 wt % safflower oil	2 weeks	↓40%	NSD[a]			
Morisaki et al. (1983)	100 mg EPA[d]/day							
	100 mg EPA[d]/day	basal diet	2 weeks	NSD	NSD	NSD	NSD	NSD
	100 mg DHA[e]/day	basal diet	2 weeks	NSD	NSD	↑11%	NSD	NSD
Haug et al. (1987)	21% energy as lard			↓sig[f]				↓sig
	21% energy as fish oil	42% energy as lard	4 weeks					
	42% energy as fish oil			↓sig				↓sig
II. Rabbits								
VasDias et al. (1982)	before consumption of 60 gm/Kg MaxEPA oil	after consumption of MaxEPA oil	60 days	NSD	NSD			
III. Pigs								
Ruiter et al. (1978)	10% mackerel oil	10% olive oil	4 weeks	↓44%	NSD			↓35%
Hartog et al. (1987)	9.1 wt % mackerel oil	9.1 wt % lard	8 weeks	↓6.2%	↓41%	↓47%		
Hartog et al. (1987)	4.5 wt % mackerel oil	9 wt % lard	16 weeks	↓sig	↓sig	NSD		
	4.5 wt % lard							
IV. Dogs								
Culp et al. (1980)	25% energy as menhaden oil	Standard chow	36-45 days	NSD				

[a] NSD = no significant difference ($p \leq 0.05$) between comparison times

[b] = significant increase between comparison times

[c] = significant decrease between comparison times

[d] EPA = eicosapentaenoic acid

[e] DHA = docosahexaenoic acid

[f] sig = significant difference ($p \leq 0.05$) between comparison times

Table 2

Human Feeding Trials: Summary of Effects of Marine Oil or N-3 Polyunsaturated Fatty Acid on Plasma Lipid Parameters

Reference	Diets: Compared to		Duration of Diet	mg/100 ml TG	Cholesterol	HDL-C	LDL-C	VLDL-C
I. Fatty Fish								↓
von Lossonczy et al. (1978)	150 gm cheese	200 gm mackerel	3 weeks	↓[b] 38%	↓ 9%	↑[c] 7% (females only)		
Singer et al. (1983)	280 gm herring	280 gm mackerel	2 weeks	↓ 47%	↓ 7%	NSD[a]	NSD	
Phillipson et al. (1985)	control diet	salmon and salmon oil	4 weeks	Type IIb hyperlipidemia ↓ 64%	↓ 27%	↓ 17%	↓ 12%	↓ 73%
				Type V hyperlipidemia ↓ 79%	↓ 48%	NSD	↓ 48%	↓ 71%
Goodnight et al. (1981)	control diet	1 lb salmon/day & 60-90 ml MaxEPA oil/day	4 weeks		↓			
Harris et al. (1983)	control diet	1 lb salmon/day & 3-6 % MaxEPA oil/day	4 weeks	↓ 38%	↓ 14%	NSD	↓ 16%	↓ 38%
Fehily et al. (1983)	usual diet	317 gm fatty fish/week (average)	3 months	↓ 6%	NSD	NSD	NSD	
II. Cod Liver Oil (CLO)								
Sanders et al. (1981)	before supplementation	after supplementation of 20 ml CLO	6 weeks	↓ 22%	NSD	↑ 9%		
Bronsgeest et al. (1981)	before supplementation	after supplementation	4 weeks					
		1.4 gm w-3 FA		NSD	NSD	NSD	NSD	NSD
		2.3 gm w-3 FA		NSD	NSD	NSD	NSD	NSD
		4.1 gm w-3 FA		NSD	NSD	NSD	NSD	NSD
		8.2 gm w-3 FA		↓ 39%	NSD	NSD	NSD	↓
III. MaxEPA Oil								
Nestel et al. (1984)	before supplementation	after supplementation with 30% energy as MaxEPA oil	4 weeks	↓		↓		↓
Sanders et al. (1983)	10 gm olive & corn oil (crossover)	10 gm MaxEPA oil	2 weeks	↓	NSD	↑		
Sanders et al. (1983)	20 ml linseed	some MaxEPA oil	2 weeks	↓				
	before supplementation	5 gm/day MaxEPA oil	3 weeks	↓ 14%	NSD	NSD		
		10 gm/day MaxEPA oil		↓ 23%	NSD	NSD		
		20 gm/day Max EPA oil		↓ 32%	↓ 9%	↑ 31%		
Saynor et al. (1984)	before supplementation	20 ml MaxEPA oil	1 month	↓ 37%	NSD	↑ 10%		
			6 months	↓ 41%	↓ 4%	↑ 10%		
			12 months	↓ 37%	NSD	NSD		
			24 months	↓ 41%	↓ 15%	↑ 14%		
Illingworth et al. (1984)	control diet	120 gm salmon oil	4 weeks	↓ 43%	↓ 23%	NSD	↓ 20%	

[a] NSD = no significant difference ($p \leq 0.05$) between comparison times

[b] = significant increase between comparison times

[c] = significant decrease between comparison times

Table 3

Content of Omega-3 Fatty Acids and Other Fat Components of Selected Seafood Products (100 gm edible portion, raw) (Hepburn, Exler, & Weihrauch, 1986)

Food Item	Total Fat	Total SFA	Total MUFA	Total PUFA	18:3[a]	20:5[b]	22:6[c]
	gm						
Mackerel, king	13.0	2.5	5.9	3.2	-	1.0	1.2
Salmon, chinook	10.4	2.5	4.5	2.1	0.1	0.8	0.6
Cod lever oil	100.0	17.6	51.2	25.8	0.7	9.0	9.5
Menhaden oil	100.0	33.6	32.5	29.5	1.1	12.7	7.9
MaxEPA oil	100.0	25.4	28.3	41.1	-	17.8	11.6
Salmon oil	100.0	23.8	39.7	29.9	1.0	8.8	11.1

[a] ∝ Linolenic Acid

[b] Eicosapentaenoic Acid

[c] Docosahexaenoic Acid

species, variability in diet composition, and duration of the studies, there are some contradictions in results.

Rats have been the most frequently employed model for the study of the effect of n-3 PUFA (see Table 1) (Huag & Hostmark, 1987; Kobatake, Hirahara, Innami, & Nishiole, 1983; Morisaki, Shinomiya, Matsuoka, Saito, & Kumagai, 1983; Wong, Nestel, Trimble, Storere, Illman, & Topping, 1984). Wong et al. (1984) observed a significant

decrease in triglycerides of those rats fed MaxEPA oil (15% by wt.). Triglycerides and VLDL-C were significantly decreased in rats fed 20% by wt. fish oil and in rats fed a combination of 20% by wt. fish oil and 20% by wt. lard as compared to rats fed a diet containing 20% by wt. lard (Haug, 1987). Kobatake et al. (1983) observed a decrease in total cholesterol only in those rats fed 5% by wt. squid liver oil as compared to 5% by wt. lard. High density lipoprotein cholesterol was also altered (109% increase) in those animals consuming the 5% squid oil. Morisake et al. (1983) also saw an increase in HDL-C but only when pure DHA was fed (100 mg/day), suggesting that HDL-C levels may be more sensitive to DHA than EPA intake. Morisake et al. observed no significant change in triglycerides, total cholesterol, LDL-C, or VLDL-C in those rats fed either DHA or EPA. In those rat studies that measured LDL no significant results of feeding n-3 PUFAs was observed.

Several studies have been conducted using other mammalian species and have observed no notable effects of marine oils. New Zealand rabbits were fed 60 gm/Kg (6% by wts.) MaxEPA pil and comparisons made before and after supplementation had no effect on plasma lipids (VasDias, Gibney, & Taylor, 1982). A study employing the dog also observed no effect on plasma lipids after feeding n-3 PUFA (12% by wt.) (Culp, Lands, Luches, Pitt, & Romson, 1980).

The pig has been used to model CHD pathogenecity; physiologically, it closely resembles humans. A significant decrease in triglycerides was observed by Ruiter, Jongblood, van Gent, Danse, &

Metz (1978) when pigs were fed 10% by wt. mackerel oil as compared to 10% by wt. olive oil. However, no significant change in total cholesterol was observed. The fatty acids in olive oil were predominantly monounsaturated and these were demonstrated to be hypocholestrolemic in nature (Keys et al., 1957).

A significant decrease in triglycerides, total cholesterol, and HDL-C occurred when pigs were fed 9.1% by wt. mackerel oil as compared to 9.1% by wt. lard (Hartog, Lamers, Montfoort, Becker, Klompe, Morse, ten Cate, van der Werf, Hulsmann, Hugenholtz, & Verdouw, 1987). When pigs were fed a more moderate amount of mackerel oil (combination of 4.5% by wt. mackerel oil and 4.5% by wt. lard compared to 9% by wt. lard), triglycerides and total cholesterol were still significantly decreased while there was no change in HDL-C (Hartog, Verdouw, Klompe, & Lamers, 1987). These studies by Hartog et al. (1987) suggest that feeding moderate amount of mackerel oil may have beneficial effects on total cholesterol and triglycerides while maintaining HDL-C levels.

The present research employed the gerbil as the model. To date there are no published research articles on the effects of n-3 PUFA on plasma lipids in the gerbil. Gerbils have been demonstrated to be appropriate models in the study of diet-induced hyperlipidemia (Otken & Scott, 1984). Gerbils, like rats, carry the majority of their cholesterol in the HDL-C fraction. However, when challenged with a small amount of cholesterol (i.e., 0.1%) the majority of plasma cholesterol is carried in the LDL-C fraction similar to humans.

Also, increases in plasma cholesterol associated with dietary changes occur in the LDL-C fraction (Hegsted & Gallagher, 1967). A problem inherent in using the gerbil as a model is their small size. Blood collection is minimal, thus limiting measurable parameters.

In summary, the animal studies reviewed show conflicting results. Omega-3s appear to have more of an effect on triglycerides than other lipid parameters but results are inconsistent. These discrepancies are due in part to differences in species, amount of fat fed, and the type of fat fed in the comparison group (i.e., PUFA or SFA).

Human studies. There have been many studies conducted using both normo- and hyperlipidemic human subjects (see Table 2). As was true with the animal studies, the human feeding trials sometimes yielded inconsistent results, especially for total cholesterol levels. This lack of consistency may be partially attributed to differences in methodology, duration of the study period, variability in diet composition, and preexperimental serum cholesterol levels of the subjects. The present review is organized by type of test ingredient (i.e., fatty fish, cod liver oil, or MaxEPA oil).

Several studies have fed fatty fish as the n-3 PUFA source. Triglycerides and VLDL-C were the plasma lipids most sensitive to n-3 PUFA consumption. Even feeding levels as low as an average of 317 gm of fatty fish per week was associated with a slight decrease (6%) in plasma triglycerides (Fehily, Phillips, & Deadman, 1983). Feeding higher levels to both normo- and hypocholesterolemics produced even

greater reductions in plasma triglycerides (Harris, Connor, & McMurry, 1983; Phillipson, Rothrock, Connor, Harris, & Illingsworth, 1985; Singer, Jager, Wirth, Voight, Nauman, Zimonthkowski, Hajdu, & Goedicke, 1983; von Lossonczy, Ruiter, Bronsgust-Schoute, van Gent, & Hermus, 1978). Very low density lipoprotein cholesterol levels were reduced significantly when at least 200 gm/day (6 oz.) of fatty fish were fed (Harris et al., 1983; von Lossenczy et al., 1978). Total cholesterol levels were also reduced significantly when at least 200 gm of fatty fish were fed per day (von Lossenczy et al., 1978); however, when low levels of fatty fish were fed (317 gm/week) total cholesterol was not significantly reduced (Fehily et al., 1983).

The response of HDL-C and LDL-C to fatty fish feeding is more variable. High density lipoprotein cholesterol levels were increased slightly in females in one study (von Lossonczy et al., 1978), decreased in Type IIb hyperlipidemics (Phillipson et al., 1985), and were unchanged in all others. A decrease in LDL-C was observed in only two studies, one with hyperlipidemic patients fed salmon (amount not reported) (Phillipson et al., 1985), and when Harris et al. (1983) fed very large amounts of salmon and MaxEPA oil (1 lb. and 3-6 T/day, respectively).

Cod liver oil, a relatively rich source of n-3 PUFA, has been fed as a supplement in two human feeding trials. Sanders, Vickers, and Hains (1981) observed significant decreases in triglycerides and increases in HDL-C with daily supplementation of 20 ml of the oil. Brongeest-Schoute, van Gent, Cuten, and Ruiter (1981) supplemented cod

liver oil in varying amounts to provide between 1.4 and 8.2 gm of n-3 PUFA/day. No significant changes in total cholesterol, HDL-C, or LDL-C were reported. Triglycerides and VLDL-C levels were decreased, but only when the highest dosage was given.

MaxEPA oil, a man-made oil rich in EPA, has also been used in human studies. Studies feeding levels from 5 gm/day to 30% energy (approximately 66 gms) from MaxEPA oil have observed a significant decrease in triglycerides (Illingsworth, Harris, & Connor, 1984; Nester, Connor, Reardon, Connor, Wong, & Boston, 1984; Sanders & Hochlano, 1983; Sanders & Roshani, 1983; Saynor, Verel, & Gillot, 1984). However, a significant decrease in total cholesterol was observed only when at least 20 gm MaxEPA oil was fed (Sanders & Roshani, 1983).

A study by Sanders and Roshani (1983) fed MaxEPA oil in three amounts: 5, 10, and 20 gm/day. Only the 20 gm supplement was associated with a decrease in both triglycerides, total cholesterol, and an increase in HDL-C; triglycerides responded in a dose response manner to the three different levels fed. Saynor et al. (1984) fed 20 ml supplements for periods of one month to two years and the results suggested that the beneficial effects on plasma may be compounded over the two-year period. These findings suggest that to observe favorable changes in the lipid bearing fractions, supplementation must be relatively high (20 gm) and/or for a longer duration than has previously been fed.

In summary, the human studies reviewed consistently observed a lowering of triglycerides associated with n-3 PUFA feeding. Indeed, the change in triglycerides associated with consumption of n-3 PUFA may be due to a hepatic suppression of VLDL-C formation and catabolism to LDL-C, subsequently reducing cholesterol deposition in the tissues and arteries (Ballard-Barbash & Calloway, 1987; Herold & Kinsella, 1986). Other lipid parameters appear to be more resistant in response to n-3 PUFAs. For example, total cholesterol requires a relatively large dose to observe a clinically significant change. Based on findings reported in the studies reviewed, n-3 PUFA may be beneficial in reducing triglycerides but may be required in unrealistic amounts to affect other lipid parameters.

Bleeding Time and Prothrombin Time

Consumption of high amounts of n-3 PUFAs by the Eskimos not only resulted in lower plasma lipids but also a favorable change in platelet function, which is associated with a decreased risk of CHD. Prolonged bleeding time results from a decrease in platelet aggregation, suggesting that the Eskimos' arteries were protected by something that alters platelet function (Dyerberg et al., 1982).

Current research on arachidonic acid (AA) metabolism and its role as a precursor for prostaglandins provides a possible mechanism for n-3 PUFA action on bleeding time. Figure 1 outlines the scheme of LAs conversion to AA and its subsequent conversion to prostaglandins. Arachidonic acid is formed from dietary LA by desaturation and elongation and by the action of cyclooxygenase to form the

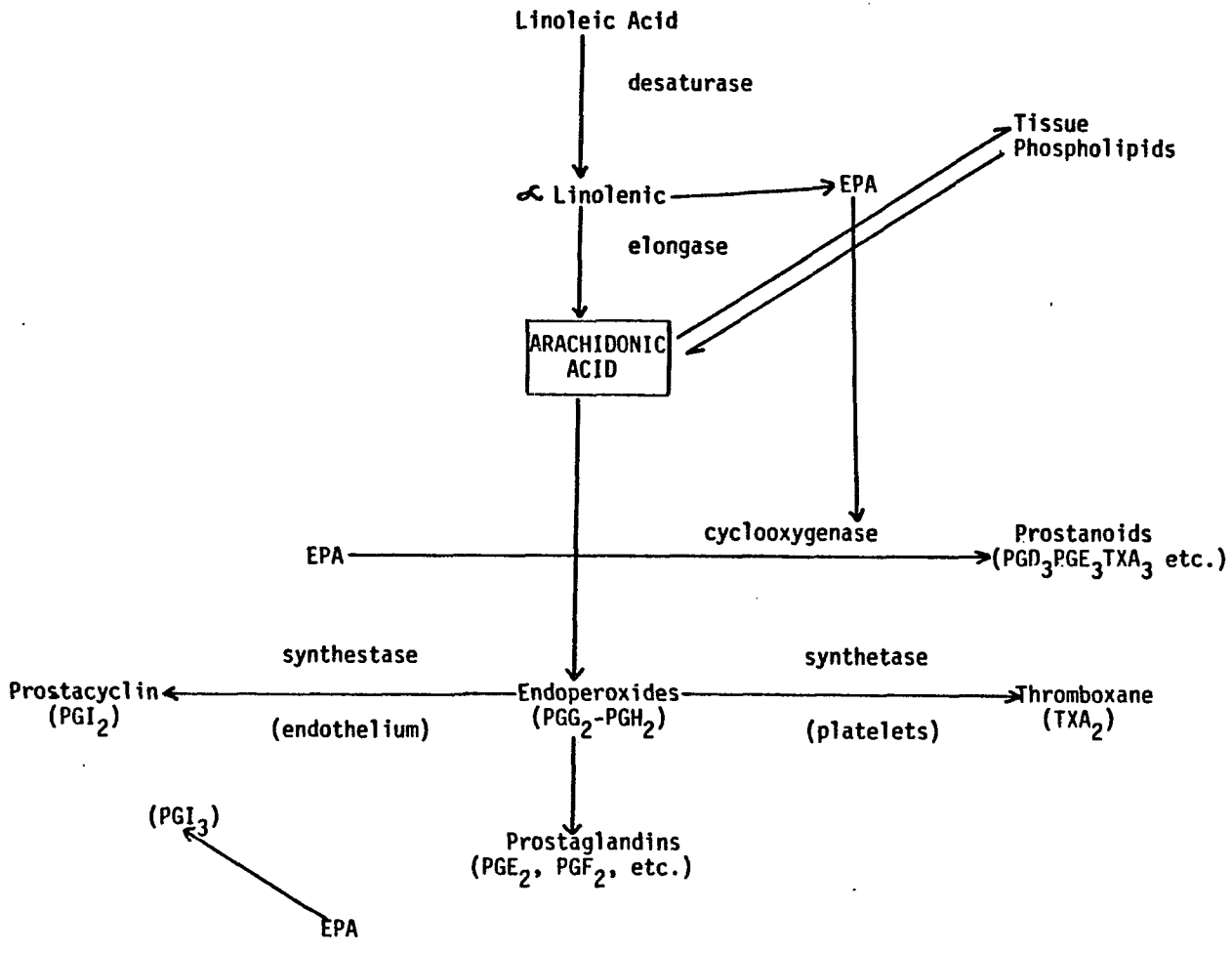

Figure 1. Roles of Linoleic Acid and Arachidonic Acid in Prostaglandin Synthesis.

endoperoxides PGG2 and PGH2. These endoperoxides are intermediates in prostaglandin synthesis. In the endothelium, the endoperoxides are converted to prostacyclin (PGI2) which is a vasodialator and a strong inhibitor of platelet aggregation. In platelets the endoperoxides are converted to thromboxane A2 (TXA2) which has strong platelet aggregating and vasoconstrictive effects. TXA2 has a short half life and is quickly converted to TXB2. The balanced antagonistic effect of TXA2 and PGI2 regulates the initial step of blood clotting (Dyerberg et al, 1982; Herold et al., 1986; Jorgensen & Dyerberg, 1983; Sanders et al., 1983).

Increasing dietary n-3 PUFA increases their levels in platelet phospholipids, allowing them to compete effectively with AA for cyclooxygenase. This alters prostaglandin synthesis patterns and may result in a reduction of platelet aggregation and an increased bleeding time (Dyerberg et al., 1982; Jorgensen et al., 1983; Sanders et al., 1983). There is also evidence that EPA may be converted to PGI3, an effective antiaggregatory agent, which accentuates the beneficial effects of n-3 PUFA (Jorgensen et al., 1983).

Rats fed fat-free diets supplemented with alpha-linolenic (an n-3 fatty acid) exhibit a bleeding tendency (Nordoy, Hamlin, Chandler, & Newland, 1968) and prostaglandin production is inhibited in rats fed linseed oil, which is rich in alpha-linolenic acid (Ten Hoor, de Deckere, Haddeman, Hornstra, & Quadt, 1980). These observations can be attributed to the displacement of AA from platelet lipids by EPA, which is synthesized from alpha-linolenic acid (Nordoy et al.,

1968; Ten Hoor et al., 1980). EPA is a precursor for the trienoic prostaglandins. TXA3 is weakly active but PGD3 and prostacyclin PGI3 are potent inhibitors of platelet aggregation (Needleman, Raz, Minkos, Ferrendelli, & Sprecher, 1979). EPA is a more potent inhibitor of platelet aggregation in humans than is n-6 PUFA or DHA (Gryglewski, Salmon, Ubatuba, Weatherley, Moncada, & Vane, 1979).

Several human studies have examined the effects of n-3 PUFA on bleeding time. Goodnight, Harris, and Connor (1981) observed favorable changes in bleeding time when healthy volunteers were fed 454 gm (1 lb.) of salmon and 60-90 ml of salmon oil per day. Sanders and Roshani (1983) found similar results while feeding MaxEPA oil but observed that increased bleeding times were not dose-dependent; that is, increasing MaxEPA consumption from 5 gm/day to 20 gm/day did not further increase bleeding time. Sanders et al. (1981) observed that after only three weeks of supplementation with 20 ml of cod liver oil, the subjects' bleeding times were significantly prolonged. Bleeding times were also prolonged when males were fed moderate amounts (100 gm/day-3 1/2 oz.) of mackerel as compared to meat (100 gm/day) for six weeks (Houwelingen, Nordoy, van der Beck, Houtsmuller, de Metz, & Hornstra, 1987).

Human subjects were fed 2 gm supplements of EPA/day for 3-6 weeks (Nagakawa, Orimo, Haragawa, Morita, Yashiro, & Murota, 1983). The authors observed a significant decrease in platelet aggregation associated with EPA but no significant change in either bleeding time or prothrombin time. However, when Saynor et al. (1984) fed 3.6 gm EPA/day they observed a significant increase in bleeding time.

Very few animal studies have examined n-3 PUFAs effect on bleeding time. Using the rat as the model, Bruckner, Likesh, German, and Kinsella (1984) fed diets containing either 1.5% by weight n-3 PUFA-enriched menhaden oil or 1.09% by weight n-3 PUFA-enriched shark oil. Bleeding times of rats fed either n-3-containing diet were not significantly different from those of rats fed a control diet. Rats consuming a diet that contained 22% by wt. cod liver oil had a 52% increase in bleeding time compared to those fed sunflower seed oil (Hornstra, Crist-Hazelhof, Haddeman, Ten Hoor, & Nugteren, 1981).

Due to the different types of fatty fish or fish oils used in the studies mentioned above, it is difficult to extrapolate from one study to another. However, it is evident that n-3 PUFAs have a pronounced effect on bleeding time in most studies. The effect of prothrombin time has not been frequently studied in association with n-3 PUFA but, in that it deals directly with blood clotting, will provide additional information to the study of n-3 PUFA and CHD.

Liver Cholesterol

Type of dietary fat influences cholesterol metabolism and, thus, may decrease risk of CHD. It is well established that saturated fat is hypercholesterolemic and antherogenic relative to PUFA (Jackson, Tavaton, Morrisett, & Gotto, 1978). Several mechanisms for the hypocholesterolemic effect of PUFA have been suggested including increased cholesterol deposition in body tissues (Jackson et al., 1978). Although marine oils (n-3 PUFAs) differ in their potential for lowering plasma lipids, the mechanism for their action may be

similar. The mechanism for the hypolipidemic (primarily triglycerides) action of n-3 PUFA may be due to a hepatic suppression of VLDL-C synthesis, but has not been thoroughly researched (Ballard-Barbash & Calloway, 1987; Herold & Kinsella, 1986).

A search of the literature did not reveal any studies examining the effect of n-3 PUFA on liver cholesterol. However, there are investigations as to the effects of SFA versus PUFA on this parameter.

In several studies employing the rat as the model, highly unsaturated dietary lipids such as sunflower oil, corn oil, and safflower oil were associated with increased hepatic cholesterol content when compared to lipids high in SFA such as coconut oil or lard (Avigan & Steinberg, 1958; Awad, 1981; Bloomfield, 1964; Grunbaum, Geary, Grande, Anderson, & Glick, 1957; Klein, 1985; Wiggers, Richard, Stewart, Jacobson, & Berger, 1977). Barrows, Heeg, McGilliard, Richard, and Jacobson (1980) observed similar results when calves were fed beef tallow (SFA source) or soybean oil (PUFA source). The soybean oil contining diet was associated with greater liver cholesterol. These results suggest that hepatic cholesterol content may be influenced by the degree of saturation of the dietary fat; the greater the degree of unsaturations being associated with increased liver cholesterol. This may be due in part to a shift in cholesterol from the plasma to the tissues because of increased catabolism of LDL-C (Mayes, 1983). If this is true of n-3 PUFA it may be a partial explanation as to the mechanism of their hypolipidemic effects.

Ratio of n-6 to n-3 PUFA

Researchers suggested that there may be a desirable ratio of n-6 to n-3 dietary PUFAs (approximately 5) that will facilitate a decrease risk of CHD (Budauski & Crawford, 1985). The typical Western diet with its preponderance of seed oils may have a ratio of n-6 to n-3 PUFAs of greater than 10.

The n-3 and n-6 families of fatty acids give rise to different families of eicosanoids which have differing effects on clotting mechanisms (Knapp, Reilly, Alessandrini, & Fitzgerald, 1986; Needleman, Raz, Minkes, Ferrendelli, & Sprecher, 1979). However, the effect of differing ratios of n-6 to n-3 PUFAs with respect to thrombogenic mechanisms (i.e., competition with cyclooxygenase) or plasma lipid levels has not yet been researched.

CHAPTER III

METHODOLOGY

Study Design

A completely randomized design, utilizing the gerbil as the mammalian model, was conducted to assess changes in plasma lipids, bleeding time, prothrombin time, and liver cholesterol resulting from the feeding of diets containing varying amounts of n-3 and n-6 PUFA. This study design allowed evaluation of combinations of n-3 and n-6 PUFA on the parameter under observation.

Adult male gerbils (15 per group) were randomly assigned to one of six dietary treatment groups. The dietary treatments differed only in the type of fat fed; the compositions of the diets are described in Table 4. All diets contained 15% fat by weight, 15% as the test fat, and 1% as safflower oil, a level designed to provide essential fatty acid requirements. Group 1 consumed 100% of their test fat (14% by weight) as lard (SFA); group 2 , 100% as safflower oil (n-6 PUFAO; group 3, 100% as MaxEPA oil (n-3 PUFA); group 4, 25% n-3 PUFA and 75% n-6 PUFA; group 5, 25% n-6 PUFA and 75% n-3 PUFA; and group 6, 50% n-3 PUFA and 50% n-6 PUFA.

Food and water were provided ad libitum. The gerbils remained on the test diets for five weeks and were weighed weekly. After a 12-hour fast, the animals were anesthetized using phenobarbital, the bleeding time measured, blood collected via exsanguination, and the livers removed.

Table 4

Dietary Treatments

Group	Type of Fat[a]
1	100% saturated fatty acids (lard)
2	100% safflower oil (n-6 PUFA)
3	100% MaxEPA oil (n-3 PUFA)
4	25% MaxEPA and 75% safflower oil
5	25% safflower oil and 75% MaxEPA
6	50% MaxEPA and 50% safflower oil

[a]All diets contain 15% fat by weight - 14% as the test fat and 1% as safflower oil to meet essential fatty acid requirements.

Animals

Male Mongolian gerbils (merionis unguiculatus) weighing 45 +/- 10 gm were purchased from Tumblebrook Farms, West Brookfield, MA. The gerbil has been demonstrated to be an appropriate model in the study of diet induced hyperlipidemia (Otken & Scott, 1984; Rich, 1968).

The gerbils were housed in individual solid-bottom cages with shredded paper as bedding. Temperature ($70^{o}F$), humidity, and lighting (12-hour alternating dark-light cycle) in the animal room were controlled.

Diets

The composition of each of the six test diets is shown in Table 5. All diets contained by weight 18% protein as casein and

Table 5

Diet Composition-Recorded as Percent by Weight[a]

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Casein[b]	18.0	18.0	18.0	18.0	18.0	18.0
Dextrose	40.9	40.9	40.9	40.9	40.9	40.9
Cornstarch	15.0	15.0	15.0	15.0	15.0	15.0
Lard	14.0	-	-	-	-	-
Safflower Oil	1.0	15.0	1.0	11.5	4.5	8.0
MaxEPA Oil	-	-	14.0	3.5	10.5	7.0
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin Mix[c]	1.0	1.0	1.0	1.0	1.0	1.0
Choline	0.2	0.2	0.2	0.2	0.2	0.2
Mineral Mix[d]	5.0	5.0	5.0	5.0	5.0	5.0
Cholesterol	0.1	0.1	0.1	0.1	0.1	0.1
Total Percent	100.0	100.0	100.0	100.0	100.0	100.0

[a]% calories as fat, 31.4%; carbohydrate, 51.9%; protein 16.7%

[b]Vitamin-free test

[c]American Institute of Nutrition vitamin mix (addition of choline needed) - see Appendix A for composition of vitamin mix

[d]American Institute of Nutrition mineral mix - see Appendix A for composition of mineral mix

55.7% carbohydrate with 40.7% as dextrose and the remaining 15.0% as cornstarch. All diets contained 15% fat by weight, 1% as safflower oil and the other 14% as a test fat. The diets were isocaloric and estimated to provide 31.4% calories as fat, 16.7% as protein, and 51.9% as carbohydrate. All diets provided a cholesterol (0.1% by weight) challenge. The dietary cholesterol was fed to switch the majority of plasma cholesterol from the HDL-C fraction to the LDL-C fraction. The diets were prepared on site at UNCG using test materials purchased from Tecklad Incorporated (Madison, WI) and were designed to meet all known nutritional requriements for the gerbil (American Institute of Nutrition, 1977). MaxEPA oil was kindly donated by R.P. Sherer Corporation. The diets were refrigerated throughout the study.

Blood and Tissue Collection

The gerbils were fasted for 12 hours, then anesthetized with sodium phenobarbital (5 mg/100 gm body wt). Firstly, a bleeding time measurement was obtained and secondly, blood samples were collected for plasma lipid and prothrombin time analysis from the vena cava using 18 gauge hypodermic needles. The blood was centrifuged in glass tubes for 15 minutes at 2500 rpm. An aliquot of fresh serum was used for prothrombin time and lipoprotein fraction separation. The remaining serum was stored in polyethylene vials and frozen at -20^{o}C. At sacrifice, the livers were removed, washed in cold saline, and frozen at -20^{o}C for future cholesterol analysis.

Plasma Lipid Analysis

Alpha (HDL-C) and beta (LDL-C and VLDL-C) fractions were separated by passing fresh serum over heparin-bound agarose columns (Lipo-Sep, Isolab Inc., Akron, OH). Total cholesterol and the cholesterol in the alpha and beta fractions were analyzed by the enzymatic colorimetric assay (Isolab Inc., Akron, OH). These cholesterol measures (i.e., total cholesterol, alpha fraction, and beta fraction) were done on 10 of the 15 gerbils in each dietary treatment group. Total triglycerides were measured on the remaining five animals in each group using a Sigma diagnostic kit (Sigma Inc., St. Louis, MO).

Bleeding Time and Prothrombin Time

While the animals were anesthetized a 2 mm slice was taken from the tip of the tail. The incision was blotted every 5-6 seconds. Bleeding time was recorded as the number of seconds elapsed between the cut and complete clotting (cessation of blood flow). Prothrombin time was measured on five gerbils per group and analyzed using an assay kit purchased from Sigma Incorporated.

Liver Cholesterol

Whole rat livers were homogenized in chloroform/methanol (2/1 by volume) according to the method of Folch, Leed, and Stanley (1956) to obtain liver lipid extracts. The extract was then filtered and the filtrate analyzed for cholesterol using the acid method described by DeHoff, Davidson, and Kritchevsky (1978).

Statistical Analysis

The general linear model procedure from Statistical Analysis System (SAS) was used to fit a fixed-effects linear model to the data; this procedure was used to test the overall effect of dietary treatment on the parameters measured in the study. If overall effect was statistically significant at the $p < .05$ level, Tukey's multiple comparison test was employed to find which pairs of dietary treatments were significantly different. Statistically significant treatment effects were found with both weight change and ending weight. The statistical analysis of the lipid and blood clotting variables was repeated with weight change included as a covariate. The assumption of homogeneity of slope was tested and found to hold and the appropriately reduced model was fitted.

CHAPTER IV

RESULTS AND DISCUSSION

The results and discussion of this study are presented in four sections; weight gain in section one, the response of plasma lipids; including triglycerides, total cholesterol, and alpha and beta cholesterol in section two, changes in bleeding time and prothrombin time in section three, and lastly, the response of liver cholesterol. Raw data for each individual animal are presented in Appendix B.

Weight Gain

Means and standard deviations for beginning weight, ending weight, and weight change for each dietary treatment are presented in Table 6. The sample sizes vary among diets due to the loss of several animals prior to the end of the study period.

Beginning weights were not significantly different among dietary treatments ($F = 0.15$, $p = 0.98$). There were statistically significant differences among diets for ending weight and weight change ($f = 4.14$, $p = 0.0021$; $F = 4.99$, $p = 0.0005$, respectively). Tukey's multiple comparison test revealed statistically significant differences ($p < .05$) between the 100% n-3 PUFA containing diet and those diets containing 100% n-6 PUFA, 25% n-3 PUFA 75% n-6 PUFA, and 50% n-6 PUFA 50% n-3 PUFA for both ending weight and weight change.

The animals consuming the 100% n-3 PUFA containing diet gained the smallest amount of weight (5.9 gm). Gerbils fed the other diets

Table 6

Means and Standard Deviations for Beginning Weight, Ending Weight, and Weight Change for Each Dietary Treatment

Dietary Treatment	Beginning Weight	Ending Weight	Weight Change
	gm		
100 % SFA (n=13)	56.4 ± 4.9	67.7 ± 9.9	11.3 ± 10.9
100% n-6 PUFA (n=16)	55.1 ± 4.3	71.8 ± 7.3[b]	16.6 ± 7.8[e]
100% n-3 PUFA (n=15)	55.5 ± 5.1	61.5 ± 7.7[a,b,c]	5.9 ± 4.8[d,e,f]
25% n-3 PUFA 75% n-6 PUFA (n=16)	55.5 ± 4.8	73.5 ± 7.4[a]	18.0 ± 6.5[d]
50% n-3 PUFA 50% n-6 PUFA (n=15)	55.9 ± 4.5	71.5 ± 9.1[c]	15.7 ± 8.0[f]
75% n-3 PUFA 25% n-6 PUFA (n=15)	55.1 ± 4.7	68.1 ± 8.6	13.0 ± 7.5

[a-e] means values with alike superscripts are significantly different ($p < .05$)

exhibited weight gains ranging from 11.3 to 18 gms. Those consuming the 25% n-3 PUFA 75% n-6 PUFA containing diet gained the most weight; however, this was not statistically different from any other treatment group with the exception of the 100% n-3 PUFA group.

One possible explanation for the overall low weight gain of the animals may be an infection incurred during the second week of the study. Some of the animals (approximately 30%) began having slight nose bleeds indicating an upper respiratory infection. The animals were then treated with low doses of tetracycline added to the drinking water. After one week of treatment the nose bleeds stopped. As the infected animals were not confined to one specific diet but randomly occurred across all six diets; a link between the dietary treatment and the infection was disregarded. However, the low weight gain observed in those gerbils consuming the 100% n-3 PUFA containing diet may more likely be a consequence of the gerbils expressing an aversion to that particular diet. Actual food intake was not measured, but weight gain may serve as a proxy.

The gerbils did not appear to adapt to n-3 PUFA feeding possibly due to its fishy smell and taste. Gerbils consuming diets containing 25, 50, or 75% n-3 PUFA did not appear to have this aversion to the diet as indicated by their higher weight gain. The n-6 PUFA contained in these diets may have served to mask the fishy smell and taste of the n-3 PUFA. As this is the first known research to be conducted investigating n-3 PUFA using the gerbil as the model, there is no basis for comparison. However, the gerbil does appear to be

sensitive to food components; a study comparing different protein sources, reported similar problems of failure to gain weight in several aminals consuming one of the protein sources (Forsythe, 1983).

Plasma Lipids

The least square means, adjusted for weight change, for triglycerides, total cholesterol, alpha and beta cholesterol, and the ratio of the alpha fraction to total cholesterol are presented in Table 7. The adjustment for weight change decreased the variability about the mean estimate.

Triglycerides. The only statistically significant effect of dietary treatment on any lipid measure was for triglycerides (F = 2.87, p = 0.04). The R^2 suggested that 47.5% of the variability in triglyceride levels could be accounted for by diet. When Tukey's multiple comparison test was employed, the pairs which were significantly different ($p < .05$) were 100% n-6 PUFA and 25% n-6 PUFA 75% n-3 PUFA, and 100% n-6 PUFA and 50% n-6 PUFA 50% n-3 PUFA. The animals consuming the 100% n-6 PUFA containing diet exhibited the highest mean triglyceride level (132.1 +/- 14.5 mg/dl). The animals fed the 50% n-6 PUFA 50% n-3 PUFA containing diet (54.0 +/- 15.4 mg/dl) and the animals fed the 25% n-6 PUFA 75% n-3 PUFA containing diet (67.5 +/- 13.8 mg/dl) exhibited the lowest mean triglyceride levels.

Triglyceride determinations were made on a sub-sample of gerbils within each dietary group, sample size ranging from 3 to 5 animals, therefore, the estimate for total triglycerides may not be

Table 7

Least Square Means for Triglycerides, Total Cholesterol, Alpha and Beta Cholesterol, and Alpha to Total Cholesterol Ratio for Each Dietary Treatment, Covaried for Weight Change

Dietary Treatment	Triglycerides	Total Cholesterol	Alpha	Beta	Alpha/TC Ratio
		mg/dl			
100% SFA	94.1 ± 15.7 (n=4)	254.9 ± 22.1 (n=8)	85.5 ± 7.1 (n=8)	133.7 ± 20.9 (n=8)	.34
100% n-6 PUFA	132.1 ± 14.5[a,b] (n=5)	188.5 ± 25.6 (n=6)	72.1 ± 6.4 (n=10)	138.0 ± 18.7 (n=10)	.38
100% n-3 PUFA	93.2 ± 19.2 (n=3)	174.3 ± 37.8 (n=3)	83.9 ± 7.5 (n=9)	118.8 ± 22.0 (n=9)	.48
25% n-3 PUFA 75% n-6 PUFA	95.5 ± 14.0 (n=5)	162.3 ± 25.5 (n=6)	58.9 ± 6.5 (n=10)	82.3 ± 19.1 (n=10)	.36
50% n-3 PUFA 50% n-6 PUFA	54.0 ± 15.4[b] (n=5)	196.4 ± 26.6 (n=6)	68.3 ± 6.9 (n=9)	94.5 ± 20.0 (n=9)	.35
75% n-3 PUFA 25% n-6 PUFA	67.5 ± 13.8[a] (n=5)	182.9 ± 28.0 (n=5)	74.1 ± 6.7 (n=9)	94.7 ± 19.7 (n=9)	.41

[a,b]Mean values with alike superscripts are significantly different ($p < .05$)

very stable. The high value observed for the 100% n-6 PUFA diet was unexpected and not consistent with the literature on the effects of dietary fat composition on plasma triglycerides (Glueck, 1979; Stamler, 1979). In general, polyunsaturated fats lower triglycerides in comparison to saturated fats. The unexpected observation of high value for the 100% n-6 PUFA group makes it difficult to evaluate the impact of the n-3 PUFA containing diets against the n-6 PUFA containing diets.

Several studies have demonstrated that n-3 PUFA elicit a greater lypotriglyceridemic response than n-6 PUFA containing diets. The significantly lower triglyceride levels observed in the animals consuming diets containing 75% n-3 PUFA and 50% n-3 PUFA is in agreement with these studies. Ruiter et al. (1978), who fed pigs diets containing either 10% by wt. mackerel oil or 10% by wt. olive oil (monounsaturated fatty acid), observed a significant decrease in triglyceride levels associated with n-3 PUFA consumption. When Wong et al. (1984) fed rats 15% by wt. MaxEPA oil or 15% by wt. safflower oil, he too observed a significant decrease in triglyceride levels associated with n-3 PUFA feeding.

Morisaki et al. (1983) fed rats 100 mg of EPA or DHA per day compared to a basal diet and observed no significant decrease in triglyceride levels as a result of n-3 PUFA supplementation. In the present study, the 75% n-3 PUFA containing diet contained approximately 500 mg EPA per day (assuming an average food consumption rate of 5 gm per day) and the 50% n-3 PUFA containing diet contains approximately 350 mg EPA per day. Perhaps concentrations greater than 100 gm per

day of EPA may be necessary to observe a significant decrease in triglyceride levels in rodent populations.

Researchers also observed a decrease in triglyceride levels associated with MaxEPA oil feeding in human subjects (Illingworth et al., 1984; Nestel, Connor, Reardon, Connor, Wong, & Boston, 1984; Sanders & Hochlano, 1983; Sanders & Roshani, 1983). These studies, however, used MaxEPA oil as a supplement and not as a substitute for other types of fat in the diet. Levels of supplementation as low as 5 gm MaxEPA oil per day were associated with a significant decrease in triglyceride levels (Sanders & Roshani, 1983).

Although comparison is difficult due to the differences in study design and species, another study conducted by Sanders and Hochlano (1983) observed a decrease in triglyceride levels associated with 10 gm of MaxEPA oil compared to 10 gm corn oil (n-6 PUFA). This is similar to the findings of the present study where n-3 PUFA feeding was associated with a decrease in triglyceride levels when compared to a diet containing n-6 PUFA.

Total cholesterol. The mean total cholesterol level for the animals consuming the 25% n-3 PUFA 75% n-6 PUFA containing diet (174.3 +/- 37.8 mg/dl) was the lowest of the six dietary treatment while the animals fed the 100% SFA containing diet exhibited the highest mean total cholesterol level (254.9 +/- 22.1 mg/dl). However, these differences did not reach statistical significance. Saturated fatty acid containing diets are typically hypercholesteroleic as compared to PUFA containing diets (Keys, Anderson, & Grande, 1957). The

animals consuming the five diets containing different combinations of n-3 and n-6 PUFA exhibited similar total cholesterol levels as they were all 100% PUFA. There was no apparent effect of feeding n-3 PUFA as compared to n-6 PUFA with respect to total cholesterol.

A study conducted by Kobatake, Hirahara, Innami, and Nishiole (1983) observed a significant decrease in total cholesterol levels when rats were fed 5% squid liver oil (n-3 PUFA) compared to 5% lard (SFA). Although the results did not reach statistical significance, the animals consuming the n-3 PUFA containing diets did exhibit lower total cholesterol levels than those consuming the 100% SFA containing diet. When Wong, Nestel, Trimble, Storere, Illman, and Topping (1984) compared MaxEPA oil and safflower oil in the rat (fed at a level consistent with the present study - 15% by wt.) there were no significant differences in total cholesterol levels. This is similar to the findings in the current study that n-3 PUFA have no significant effect on total cholesterol levels when compared to n-6 PUFA.

Two other studies employing the rat as a model also observed no significant change in total cholesterol when 100 mg of EPA per day was fed in comparison to a basal diet (Morisaki et al., 1983). Ruiter et al. (1978) alos observed no significant decrease in total cholesterol levels associated with feeding pigs a 10% mackerel oil diet as compared to a 10% olive oil diet.

Human feeding trials have shown conflicting results with respect to the effect of n-3 PUFA on total cholesterol levels. Studies

consistent with the findings observed in the present study are as follows. When von Lossonczy et al. (1978) compared cheese (SFA) to mackerel (n-3 PUFA), a significant decrease in total cholesterol was associated with n-3 PUFA consumption. A study conducted by Sanders and Roshani (1983) observed no effect on total cholesterol when n-3 PUFA (MaxEPA oil) was compared to n-6 PUFA (corn oil).

Research by Singer, Jaeger, Wirth, Voigt, Nauman, Zimontkowski, Hajdu, and Goedicke (1983) contradicts the findings of the above reported studies. Subjects consumed either 280 gm per day of herring or 280 gm per day of mackerel. Although the predominant fatty acids in both fish are PUFA, there was a significant decrease in total cholesterol levels associated with consumption of mackerel (n-3 PUFA). Possibly a factor other than the n-3 PUFA in the fish caused the decrease in total cholesterol.

In summary, n-3 PUFA may exhibit a hypocholesterolemic effect when compared to SFA but this decrease is not significantly greater than that observed with n-6 PUFA feeding. The predominant PUFA in the American diet is n-6 PUFA, and may be a more feasible hypocholesterolemic agent than n-3 PUFA for the general population.

Alpha fraction. The alpha fraction measured in the present study represents the HDL-C level. There were no significant differences among the dietary treatments with respect to HDL-C levels; however, a trend was observed in the HDL-C data. The highest HDL-C level was observed in the animals consuming the 100% SFA containing diet. The animals consuming the 100% n-3 PUFA containing diet

exhibited the second highest HDL-C level. This observation is consistent with the findings of Sanders and Hochlano (1983) who observed an increase in HDL-C levels when humans were fed 10 gm of MaxEPA oil compared to 10 gm olive or corn oil. Studies by Sanders and Roshani (1983) and Saynor, Verel, and Gillott (1984) also observed an increase in HDL-C when the diets of human subjects were supplemented with 20 gm MaxEPA oil per day. Other studies using normolipidemic subjects found no significant differences in HDL-C levels when n-3 PUFA were fed (Bronsgeest et al., 1981; Fehily et al, 1983; Harris et al., 1983; Singer et al., 1983).

Animal studies employing the rat as a model also found conflicting results with respect to n-3 PUFA consumption and HDL-C levels. Kobatake et al. (1983) observed a significant increase in HDL-C levels when n-3 PUFA feeding was compared to SFA feeding. Another study in the rat conducted by Morisaki et al. (1983) found no significant change in HDL-C when EPA was fed but a significant increase in HDL-C when DHA was fed. The predominant n-3 PUFA fed in the present study was EPA; this may provide a partial explanation for the nonsignificant findings for HDL-C in the present study.

The findings with respect to HDL-C levels and n-3 PUFA feeding are clearly inconsistent. However, accumulation of data to date, suggest that even though n-3 PUFA may not significantly decrease total cholesterol they may favorably change the way cholesterol is carried in the plasma.

One measure of the atherogenecity of a diet is the HDL-C (alpha) to total cholesterol ratio; higher ratios being more desirable. Although there was no statistical difference among diets, the trend was as expected with the animals consuming the 100% SFA containing diet exhibiting the lowest ratio and the animals fed the n-3 PUFA and n-6 PUFA containing diets exhibiting higher ratios.

Beta fraction. There was no significant effect of dietary treatment on the beta fraction, however, the response to the different diets was greater and more variable in the beta fraction than was observed in the alpha fraction. The beta fraction is composed of both the LDL-C and VLDL-C lipoproteins but the VLDL-C fraction is the major carrier for triglycerides. The significant differences in triglyceride levels in three of the dietary treatments might suggest an expected difference in VLDL-C levels since VLDL-C is carrying primarily triglyceride. However, if there was a decrease in VLDL-C associated with the dietary treatments it was not large enough to be detected in the total beta fraction.

Few studies have measured either LDL-C or VLDL-C. Ruiter et al. (1978) observed a significant decrease in VLDL-C in pigs fed 10% mackerel oil compared to 10% olive oil. The decrease in VLDL-C was in conjunction with a significant decrease in triglyceride levels. This VLDL-C level was, however, not measured chemically but by using a formula, which is not consistent with the means of measurement of the present study. Morisake et al (1983) observed no change in LDL-C or VLDL-C levels when rats were fed 100 gm of EPA per day. This study

also observed no change in total cholesterol levels. These findings are consistent with the nonsignificant findings with respect to the beta fraction of the current study (i.e., nonsignificant differences in total cholesterol and the beta fraction). Human studies measuring LDL-C and/or VLDL-C that observed no significant change in total cholesterol also observed no significant change in LDL-C or VLDL-C associated with n-3 PUFA feeding (Bronsgeest et al., 1981; Fehily et al., 1983).

Bleeding Time and Prothrombin Time

Means for bleeding time and prothrombin time for each dietary treatment, adjusted for weight change, are presented in Table 8. No overall effect of dietary treatment on bleeding time or prothrombin time (F = 1.94, p = 0.086; F = 0.85, p = 0.55, respectively) was observed.

Omega-3 PUFA have been shown to effect prostaglandin production in a way that favorably alters platelet aggregation and increases bleeding time (Dyerberg et al., 1932; Herold et al., 1986; Jorgensen & Dyerberg, 1983; Sanders & Hochlano, 1983). The present study was designed to assess the level of n-3 PUFA associated with a favorable change in bleeding time. However, there were no significant differences across the six dietary treatments with respect to bleeding time nor was any trend in the data observed. Unexpectedly, the gerbils fed the 100% n-3 PUFA containing diet exhibited the lowest bleeding time. This is in disagreement with studies conducted in both humans and other animal models.

Table 8

Least Square Means for Bleeding Time and Prothrombin Time for Each Dietary Treatment, Covaried for Weight Change

Dietary Treatment	Bleeding Time[a]	Prothrombin Time[b]
100% SFA	3.04 ± 0.43 (n=12)	19.64 ± 2.38 (n=4)
100% n-6 PUFA	2.98 ± 0.42 (n=13)	21.16 ± 2.19 (n=5)
100% n-3 PUFA	2.20 ± 0.49 (n=11)	17.06 ± 2.92 (n=3)
25% n-3 PUFA 75% n-6 PUFA	2.87 ± 0.39 (n=16)	21.39 ± 2.12 (n=5)
50% n-3 PUFA 50% n-6 PUFA	4.02 ± 0.43 (n=12)	17.74 ± 2.71 (n=4)
75% n-3 PUFA 25% n-6 PUFA	2.78 ± 0.39 (n=15)	23.56 ± 2.09 (n=5)

[a]recorded in minutes and fractions of minutes

[b]recorded in minutes

Human studies consistently observed significant increases in bleeding time associated with n-3 PUFA consumption. Goodnight et al. (1981) fed 1 lb of salmon and 60-90 ml salmon oil per day and observed a significant increase in bleeding time. Two studies feeding fish oil also observed a significant increase in bleeding time (Sanders & Roshani, 1983; Sanders et al., 1981). Feeding even moderate amounts of fatty fish (100 gm per day) produced significant prolonged bleeding times in healthy males (Houwelingen, Nordoy, van der Beek, Houtsmuller, de Metz, & Hornstra, 1987).

Rats fed a diet with 45% of the total calories from cod liver oil exhibited a significant increase in bleeding time (Hornstra et al., 1981). Another study feeding fish oil observed no effect of n-3 PUFA on leeding time (Bruckner et al., 1984). However, the level fed was very low (1.5% by wt.) compared to the level fed in the present study (3.5 - 14.0% by wt.).

A possible reason for the disagreement in the results of this study and those reviewed may be due to the methodology used to measure the bleeding time. The method used in rats of exposing the major artery in the leg, cutting the artery and measuring clotting time, was not feasible due to the small size of the gerbil. Alternate ways were pilot tested, the most successful being cutting the tail at 2 mm and timing the clotting of the cut. In preliminary tests, this method appeared to be the most reliable in providing adequate blood flow. In order to reduce any experimental bias, both the person timing and the person determining the point of clotting were blinded to the dietary treatment. Difficulty was encountered in consistently cutting at exactly the same point on the tail; this may have introduced measurement variability. Possibly cutting a longer segment of the tail than 2 mm would provide a more precise measure of bleeding time. However, this might compromise collection of a maximum volume of blood for other analyses due to excessive blood loss from the bleeding time procedure.

Bleeding time reflects the effectiveness of the platelet plug formation at site of vascular injury. Prothrombin time is a test of

the extrinsic pathway and also the final common pathway of clotting, the latter involves the activity of thrombin. Thrombin is actively controlled via two mechanisms; one is the existence of a thrombin antagonist antithrombin III, the other involves the inactive thrombin zymogen prothrombin. Plasma levels of antithrombin III have been demonstrated to increase on feeding 10 gm supplements of either fish or vegetable oil (Mortensen, Schmidt, Nielsen, & Dyerberg, 1983). Thus, suggesting that no only platelet function but also fibrin clot formation may be altered by n-3 PUFA feeding.

These findings are in disagreement with the present study which observed no significant effects of dietary treatment on prothrombin time. However, a study conducted by Nagakawa, Orimo, Harasawa, Morita, Kashirok, and Murota (1983) also observed no change in prothrombin time when human subjects were fed 2 gm EPA/day. Due to conflicting results in the relatively few studies measuring the effects of n-3 PUFA on prothrombin time, the degree to which n-3 PUFA alter prothrombin time is yet to be fully understood.

Liver Cholesterol

Means for liver cholesterol for each dietary treatment, adjusted for weight change, are presented in Table 9. There was no overall effect of dietary treatment on liver cholesterol (F = 1.91, p = 0.089). Although it did not reach statistical significance, the animals fed the 100% n-3 PUFA containing diet exhibited the lowest liver cholesterol level (19.67 +/- 2.31 mg/gm). The other polyunsaturated fat containing diets consistently showed higher liver

cholesterol levels, greater than that seen with the SFA containing diet. The literature reports that SFA consumption is associated with lower hepatic cholesterol when compared to PUFA consumption (Avigan & Steinberg, 1958; Awad, 1981; Barrows et al., 1980; Bloomfield, 1964; Grunbaum et al., 1957; Klein, 1958; Wiggers et al., 1977).

Table 9

Least Square Means for Liver Cholesterol for Each Dietary Treatment, Covaried for Weight Change

Dietary Treatment	Liver Cholesterol mg/dl
100% SFA	20.89 ± 2.31 (n=13)
100% n-6 PUFA	22.64 ± 2.10 (n=16)
100% n-3 PUFA	19.67 ± 2.31 (n=15)
25% n-3 PUFA 75% n-6 PUFA	22.57 ± 2.13 (n=16)
50% n-3 PUFA 50% n-6 PUFA	23.81 ± 1.11 (n=15)
75% n-3 PUFA 25% n-6 PUFA	21.01 ± 2.14 (n=15)

If the 100% n-3 PUFA containing diet is eliminated from the analysis, the other four diets that contain all PUFA had very similar liver cholesterol levels. This suggests that n-6 PUFA and n-3 PUFA may have similar effects on liver cholesterol. Increased deposition of cholesterol in tissue is a proposed mechanism for the hypocholesterolemic action of PUFA (Jackson et al, 1978).

Concluding Comments

Two objectives of the study were to assess the effect of differing levels of n-3 and n-6 PUFA fed in combination and the effects of n-3 PUFA, n-6 PUFA, and SFA on plasma lipids, bleeding time, prothrombin time, and liver cholesterol. The dietary treatments had no significant effects on any parameter measured with the exception of plasma triglycerides. The gerbils consuming the 100% n-6 PUFA exhibited significantly ($p < .05$) lower triglyceride levels than those animals consuming the 50% n-6 PUFA 50% n-3 PUFA and the 75% n-3 PUFA 25% n-6 PUFA containing diets.

Another objective of the present study was to determine at what combination of n-3 and n-6 PUFA there was a hypolipidemic effect without compromising the beneficial reduction in platelet adhesiveness, as measured by bleeding time. As mentioned above, the only significant effect on plasma lipids was observed in triglycerides. The 50% n-6 PUFA 50% n-3 PUFA containing diet exhibited the lowest triglyceride levels. As there were no significant changes in any other plasma lipid parameter, nor any significant changes or trends in the bleeding time data, it is not possible to suggest a ratio of n-3 to n-6 PUFA that would favorably effect plasma lipids and bleeding time simultaneously.

A final objective was to determine the usefulness of the gerbil as a model in the study of diet and CHD risk factors. Several problems were encountered in using the gerbil, including infection; aversion to the 100% n-3 PUFA diet; low weight gain; and low blood

volume available for analysis. However, the fact remains that the gerbil is a more suitable model than the rat for the study of lipid metabolism due to the way the gerbil carries cholesterol in plasma (closely resembles humans when challenged with dietary cholesterol). If the limitations of using the gerbil are addressed by the study design (i.e., increased animal numbers to increase total blood collected, sterile environment, etc.), the gerbil could be an acceptable model to assess effect of diet on chronic disease risk factors.

Hypothesis Tests

Hypothesis 1 stating that there will be no significant difference in plasma triglycerides across the six dietary treatments was rejected.

Hypothesis 2 stating that there will be no significant difference in total cholesterol across the six dietary treatments was accapted.

Hypothesis 3 stating that there will be no significant difference in HDL cholesterol across the six dietary treatments was accepted.

Hypothesis 4 stating that there will be no significant difference in LDL and VLDL cholesterol across the six dietary treatments was accepted.

Hypothesis 5 stating that there will be no significant difference in bleeding time across the six dietary treatments was accepted.

Hypothesis 6 stating that there will be no significant difference in prothrombin time across the six dietary treatments was accepted.

Hypothesis 7 stating that there will be no significant difference in liver cholesterol across the six dietary treatments was accepted.

CHAPTER V

RECOMMENDATIONS FOR FUTURE RESEARCH

Currently research concerning n-3 PUFA have concentrated on the risk factors of plasma lipids and platelet function. Research is needed in several areas related to n-3 PUFA. Few studies have been conducted in animals other than rodents. The pig or primate would be appropriate models to explore n-3 PUFA mechanisms of action on bleeding time and plasma lipids as they more closely resemble humans in physiology. These models could also be employed to assess the effects on n-3 PUFA when fed in varying combinations with n-6 PUFA to ascertain an optimal ratio for decreasing risk factors of CHD. Using repeated measures would allow the researcher to assess the effects of n-3 PUFA feeding and a combination of n-3 and n-6 PUFA feedings on bleeding time and plasma lipids over the course of time.

Research efforts are also needed to assess the effects of n-3 PUFA at the cellular level. Cyclooxygenase acts on AA to form endoperoxides, which are intermediate compounds in the synthesis of the prostaglandins known to have strong platelet aggregating and vasoconstricting effects (Dyerberg et al., 1982; Herold et al., 1986; Jorgensen & Dyerberg, 1983; Sanders et al., 1983). Studies measuring the effect of n-3 PUFA fed in combination with varying levels of n-6 PUFA on the AA synthesis pathway enzymes and tissue levels of AA would provide information on levels of n-3 PUFA required to favorably alter prostaglandin synthesis and may partially explain mechanisms involved.

Evidence indicates that n-3 PUFA may decrease the risk of CHD because of their possible effects on monocytes. Monocytes adhere to the artery wall in atherosclerosis and are then transformed into macrophages, which accumulate lipid material from circulating lipoproteins (Ross, Bowen-Pope, Raines, & Faggiotto, 1982). Fish oil feeding may increase the EPA content in the membrane of monocytes and favorably alter leukotriene production (i.e., increase B5 and decrease B4 leukotrienes) which could decrease adherence of the monocyte to the artery wall (Lands, 1986). Research is needed to determine the possibility of this mechanism and how n-6 PUFA may compete with n-3 PUFA for lipoxygenase, an enzyme in the leukotriene production pathway.

Additional research is needed in the area of fish oil supplementation (i.e., fish oil capsules) in order to ascertain both beneficial qualities and possible long-term side effects. Comparison studies examining consumption of fish oil capsules versus fatty fish on plasma lipids and platelet function would prove helpful to isolate which component of fatty fish is associated with favorable changes in CHD risk factors.

It is not feasible for Americans to consume the amounts of fatty fish ingested by Eskimo populations, as they consume upwards of 1 lb. of fatty fish per day (whale blubber and other marine oils). The average per capita consumption of fish in the United States is less than 1 oz. per day, and this is primarily fish which contributes little n-3 PUFA (Dyerberg, 1986). Thus, data are needed in the areas of long-term consumption of low levels of fatty fish and

supplementation with n-3 PUFA in order to assess the likelihood of using n-3 PUFA as a preventive measure for CHD. Such research would provide useful information to health professionals so that they may suggest appropriate recommendations to the American population with regard to n-3 PUFA.

BIBLIOGRAPHY

American Institute of Nutrition. (1977). Report of the American institute of nutrition ad hoc committee on standards for nutritional studies. American Journal of Clinical Nutrition, 107, 1340-1348.

Anonymous. (1984). Number one killers: Cardiovascular diseases in developed world, respiratory diseases in developing. World Health, pp. 30-31.

Avigan, J., & Steinberg, D. (1958). Effects of saturated and unsaturated fat on cholesterol metabolism in the rat. Proceedings for the Society of Experimental Biological Medicine, 97, 814-816.

Awad, A. B. (1981). Effect of dietary lipids on composition and glucose utilization by rat adipose tissue. Journal of Nutrition, 111, 34-39.

Ballard-Barbash, R., & Callaway, C. W. (1987). Marine fish oils: Role in prevention of coronary heart disease. Mayo Clinic Proceedings, 62, 113.

Bang, H. O., & Dyerberg, J. (1972). Plasma lipids and lipoproteins in Greenlandic west-coast Eskimos. Acta Medical Scandinavia, 192, 85.

Bang, H. O., & Dyerberg, J. (1980). The bleeding tendency in Greenland Eskimos. Danish Medical Bulletin, 27, 202.

Bang, H. O., Dyerberg, J., & Nielsen, A. B. (1971). Plasma lipid and lipoprotein pattern in Greenlandic west-coast Eskimos. Lancet, 1, 1143.

Barrows, K. K., Heeg, T. R., McGilliard, A. D., Richard, M. J., & Jacobson, N. L. (1980). Effect of type of dietary fat on plasma and tissue cholesterol in calves. Journal of Nutrition, 110, 335-342.

Bloomfield, D. K. (1964). Cholesterol metabolism. 3. Enhancement of cholesterol absorption and accumulation in safflower oil fed rats. Journal of Laboratory and Clinical Medicine, 164, 613-623.

Bronsgeest-Schoute, H. C., van Gent, C. M., Cuten, J. B., & Ruiter, A. (1981). The effects of various intakes of w-3 fatty acids on the blood lipid composition in healthy human subjects. American Journal of Clinical Nutrition, 34, 1752-1757.

Bruckner, G. G., Likesh, B., German, B., & Kinsella, J. E. (1984). Biosynthesis of prostanoids, tissue fatty acid composition and thrombotic parameters in rats fed diets enriched with docoahexaeroic and eicosapentaenoic acid. Thromboxane Research, 34, 479-497.

Budowski, P., & Crawford, M. A. (1985). ∝Linolenic acid as a regulator of the metabolism of arachidonic acid: Dietary implications of the ratio, n-6:n-3 fatty acids. Proceedings of the Nutrition Society, 44, 221-229.

Clifford, A. J., Smith, L. M., Creveling, R. K., Hamblin, C. L., & Clifford, C. K. (1986). Effect of dietary triglycerides on serum and liver lipids and sterol excretion of rats. Journal of Nutrition, 116, 944-956.

Culp, B. R., Lands, W. E. M., Luches, B. R., Pitt, B., & Romson, J. (1980). The effect of dietary supplementation of fish oil on experimental myocardial infarction. Prostaglandins, 20, 1021-1031.

DeHoff, J. L., Davidson, L. M., & Kritchevsky, D. (1978). An enzymatic assay for determining free and total cholesterol in tissue. Clinical Chemistry, 24, 433-435.

Drersen-Schade, D. A., Richard, M. J., Beitz, D. C., & Jacobson, N. L. (1986). Plasma tissue and fecal cholesterol of young pigs fed restricted or liberal amounts of beef, soy or conventional diets. Journal of Nutrition, 116, 2086-2095.

Dyerberg, J. (1986). Linoleate derived polyunsaturated fatty acids and prevention of atherosclerosis. Nutrition Reviews, 44, 125.

Dyerberg, J., & Bang, H. O. (1982). A hypothesis on the development of acute myocardial infarction in Greenlanders. Scandinavian Journal of Clinical Investigation, 42, 7-13.

Dyerberg, J., Bang, H. O., & Hjorne, N. (1975). Fatty acid composition of the plasma lipids in Greenland Eskimos. American Journal of Clinical Nutrition, 28, 958.

Fehily, A. M., Phillips, K. M., & Deadman, N. M. (1983). The effect of fatty fish on plasma lipid and lipoprotein concentrations. American Journal of Clinical Nutrition, 38, 349-351.

Folch, J., Lees, M., & Stanley, G. H. S. (1956). A simple method for the isolation of total lipids from animal tissues. Journal of Lipid Research, 8, 497-509.

Forsythe, W. A. (1983). Dietary protein source effects on plasma lipid and hormone concentrations in the gerbil. Federation Proceedings, 42, 1057.

Glueck, C. J. (1979). Dietary fat and atherosclerosis. American Journal of Clinical Nutrition, 32, 2703-2711.

Goodnight, S. H., Harris, W. S., & Connor, W. E. (1981). The effects of dietary w-3 fatty acids on platelet composition and function in man: A prospective controlled study. Blood, 58, 880-885.

Grunbaum, B. W., Geary, J. R., Grande, F., Anderson, J. T., & Glick, D. (1957). Effect of dietary lipid on rat serum and liver cholesterol and tissue mast cells. Proceedings for the Society of Experimental Biological Medicine, 94, 613-617.

Gryglewski, R. J., Salmon, J. A., Ubatuba, F. B., Weatherley, B. C., Moncada, S., & Vane, J. R. (1979). Effects of all cis-5,8-11, 14,17-eicosapentaenoic acid and PGI_3 on platelet aggregation. Prostaglandins, 19, 453-478.

Harris, W. S., Connor, W. E., & McNurry, M. P. (1983). The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: Salmon oil versus vegetable oils. Metabolism, 32, 179-184.

Hartog, J. M., Lamers, J. M. J., Montfoort, A., Becker, A. E., Klompe, M., Morse, H., Ten Cate, F. J., van der Werf, L., Hulsmann, W. C., Hugenholtz, P. G., & Verdouw, P. D. (1987). Comparison of mackerel oil and lard fat enriched diets on plasma lipids, cardiac membrane phospholipids, cardiovascular performance, and morphology in young pigs. American Journal of Clinical Nutrition, 46, 258-266.

Hartog, J. M., Verdouw, P. D., Klompe, M., & Lamers, J. M. J. (1987). Dietary mackerel oil in pigs: Effect on plasma lipids, cardiac sarcolemmal phospholipids and cardiovascular parameters. Journal of Nutrition, 117, 1371-1378.

Haug, A., & Hostmark, A. T. (1987). Lipoprotein lipases, lipoproteins and tissue lipids in rats fed fish oil or coconut oil. Journal of Nutrition, 117, 1011-1017.

Hegsted, D. M., & Gallaghen, A. (1967). Dietary fat and cholesterol and serum cholesterol in the gerbil. Journal of Lipid Research, 8, 210-214.

Hepburn, F. N., Exler, J., & Weihrauch, J. L. (1986). Provisional tables on the content of omega-3 fatty acids and other fat components of selected foods. Journal of the American Dietetic Association, 86, 788-793.

Herold, P. M., & Kinsella, J. E. (1986). Fish oil consumption and decreased rish of cardiovascular disease: A comparison of findings from animal and human feeding trials. American Journal of Clinical Nutrition, 43, 566.

Hornstra, G., Christ-Hazelhof, E., Haddeman, E., Ten Hoor, F., & Nugteren, D. H. (1981). Fish oil feeding lowers thromboxane and prostacyclin production by rat platelet and aorta and does not result in the formation of prostaglandin I_3. Prostaglandins, 21, 727-738.

Houwelingen, R., Nordoy, A., vander Beek, E., Houtsmuller, U., de Metz, M., & Hornstra, G. (1987). Effect of moderate fish intake on blood pressure, bleeding time, hematology and clinical chemistry in healthy males. American Journal of Clinical Nutrition, 46, 424-436.

Illingworth, D. R., Harris, W. S., & Connor, W. E. (1984). Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. Arteriosclerosis, 4, 270-275.

Jackson, R. L., Tavaton, O. D., Morrisett, J. D., & Gotto, A. M. (1978). The role of dietary polyunsaturated fat in lowering blood cholesterol in man. Circulation Research, 42, 447-453.

Jorgensen, K. A., & Dyerberg, J. (1983). Platelets and atherosclerosis. Advanced Nutrition Research, 5, 57-75.

Keys, A., Anderson, J. T., & Grande, F. (1957). Essential fatty acids degree of unsaturation and effects of corn (maize oil) on the serum cholesterol level in man. Lancet, 1, 66.

Klein, P. D. (1958). Linoleic acid and cholesterol metabolism in the rat. I. The effect of dietary fat and linoleic acid esters in liver and plasma. Archives of Biochemistry and Biophysics, 76, 56-64.

Knapp, H. R., Reilly, I. A. G., Alessandrini, P., & Fitzgerald, G. A. (1986). In vitro indexes of platelet and vascular function during fish oil administration in patients with atherosclerosis. New England Journal of Medicine, 314, 937-942.

Kobatake, Y., Hirahara, F., Innami, S., & Nishiole, E. (1983). Dietary effect of w-3 type polyunsaturated fatty acids on serum and liver lipid levels in rats. Journal of Nutrition Science Vitaminol, 29, 11-21.

Lands, W. E. M. (1986). Fish and human health. Orlando, FL: Academic Press.

Mayes, P. A. (1983). Metabolism of lipids: II. Role of the tissues. In D. W. Martin, P. A. Mayes, V. W. Rodwell, & D. K, Granner (Eds.), Harper's review of biochemistry (pp. 232-256). Los Altos, CA: Lange Medical Publications.

Morisaki, N., Shinomiya, M., Matsuoka, N., Saito, Y., & Kumagai, A. (1983). In vivo effects of cis-5,8,11,14,17-20:5 (n-3) and cis-4,7,10,1316,19-22:6 (n-3) on serum lipoproteins, platelet aggregation and lipid metabolism in the aorta of rats. Tohoku Journal of Experimental Medicine, 141, 397-405.

Mortensen, J. Z., Schmidt, E., Nielsen, A. H., & Dyerberg, J. (1983). The effect of n-6 and n-3 polyunsaturated fatty acids on hemostasis, blood lipids and blood pressure. Thrombin Haemostasis, 50, 543-546.

Nagakawa, Y., Orimo, H., Harasawa, M., Morita, I., Kashirok, & Murota, S. (1983). Effect of eicosapentaenoic acid on the platelet aggregation and composition of fatty acid in man. Atherosclerosis, 47, 71-75.

Needleman, P., Raz, A., Mindes, M. S., Ferrendelli, J. A., & Sprecher, H. (1979). Triene prostaglandins: Prostacyclin and thromboxane biosynthesis and unique biological properties. Proceedings of the National Academy of Science USA, 76, 944-948.

Nestel, P. J., Connor, W. E., Reardon, M. F., Connor, S., Wong, S., & Boston, R. (1984). Suppression by diets rich in fish oil of very low density lipoprotein production in man. Journal of Clinical Investigation, 74, 82-89.

Nordoy, A., Hamlin, J. T., Chandler, A. T., & Newland, H. (1968). The influence of dietary fat on plasma and platelet lipids and ADP induced platelet thrombosis in the rat. Scandinavian Journal of Haematology, 5, 458-472.

Otken, C. C., & Scott, C. E. (1984). Feeding characteristics of mongolian gerbils (meriones unguiculatus). Laboratory Animal Science, 34, 181-184.

Phillipson, B. E., Rothrock, D. W., Connor, W. E., Harris, W. S., & Illingworth, D. R. (1985). Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. New England Journal of Medicine, 312, 1210-1218.

Rich, S. T. (1968). The Mongolian gerbil (meriones unguiculatus) in research. Laboratory Animal Care, 18, 235-243.

Ross, R., Bowen-Pope, D., Raines, W., & Faggiotto, A. (1982). Endothelial injury: Blood vessel wall interactions. Annals of New York Academy of Science, 401, 260-264.

Ruiter, A., Jongblood, A. W., van Gent, C. M., Danse, L. H. J. C., & Metz, S. H. M. (1978). The influence of dietary mackerel oil on the condition of organs and on blood lipid composition in the young growing pig. American Journal of Clinical Nutrition, 31, 2159-2166.

Sanders, T. A. (1983). Dietary fat and platelet function. Clinical Science, 65, 343-350.

Sanders, T. A. B., & Hochlano, M. C. (1983). A comparison of the influence on plasma lipids and platelet function of supplements of w-3 and w-6 polyunsaturated fatty acids. British Journal of Nutrition, 50, 521-529.

Sanders, T. A. B., & Roshani, F. (1983). The influence of different types of w-3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. Clinical Science, 64, 91-99.

Sanders, T. A. B., Vickers, M., & Haines, A. P. (1981). Effect of blood lipids and haemostasis of a supplement of cod liver oil, rich in eicosapentaenoic and docosahexaenoic acids, in healthy young men. Clinical Science, 61, 317-324.

Saynor, R., Verel, D., & Gillott, T. (1984). The long-term effect of dietary supplementation with fish lipid concentrate on serum lipids, bleeding time, platelets and angina. Atherosclerosis, 50, 3-10.

Singer, P., Jaeger, W., Wirth, M., Voigt, S., Nauman, E., Zimontkowski, S., Hajdu, I., & Goedicke, W. (1983). Lipid and blood pressure-lowering effect of mackerel diet in man. Atherosclerosis, 49, 99-108.

Stamler, J. (1979). Population studies. In R. I. Levey, B. M. Rifkind, B. H. Dennis, & N. D. Ernst (Eds.), Nutrition, lipids and coronary heart disease: A global view (pp. 25-88). New York: Raven.

Ten Hoor, F., de Deckere, E. A. M., Haddeman, E., Hornstra, G., & Quadt, J. F. A. (1980). Dietary manipulation of prostaglandin and thromboxane synthesis in heart, aorta, and blood platelets of the rat. Advances in Prostaglandin and Thromboxane Research, 8, 1771-1781.

VasDias, F. W., Gibney, M. J., & Taylor, T. G. (1982). The effect of polyunsaturated fatty acids of the n-3 and n-6 series on platelet aggregation and platelet and aortic fatty acid composition in rabbits. Atherosclerosis, 43, 245-247.

von Lossonczy, T. O., Ruiter, A., Bronsgust-Schoute, H. C., van Gent, C. M., & Hermus, R. J. J. (1978). The effect of a fish diet on serum lipids in healthy human subjects. American Journal of Clinical Nutrition, 31, 1340-1346.

von Shacky, C., Fischer, S., & Weber, P. C. (1985). Long-term effects of dietary marine w-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosaroid function in humans. Journal of Clinical Investigations, 76, 1626-1631.

Wiggers, K. D., Richard, M. J., Stewart, J. W., Jacobson, N. L., & Berger, P. J. (1977). Type and amount of dietary fat affect relative concentration of blood and other tissues of rat. Atherosclerosis, 27, 27-34.

Wong, S. H., Nestel, P. J., Trimble, R. P., Storere, G. B., Illman, R. J., & Topping, D. L. (1984). The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver. Biochemical Biophys Acta, 792, 103-109.

APPENDIX A

COMPOSITION OF AMERICAN INSTITUTE OF NUTRITION VITAMIN AND MINERAL MIX

American Institute of Nutrition Vitamin Mix

(AIN-76A #40077) Composition

Ingredient	gm/Kg
Thiamine HCl	0.6
Riboflavin	0.6
Pyridoxine HCl	0.7
Niacin	3.0
Calcium Pantothenate	1.6
Folic Acid	0.2
Biotin	0.02
Vitamin B-12 (0.1% trituration in mannitol)	1.0
Dry Vitamin A Palmitate (500,000 U/gm)	0.8
Dry Vitamin A Acetate (500 U/gm)	10.0
Vitamin D-3 trituration (400,000 U/gm)	0.25
Menadione Sodium Bisulfite Complex	0.15
Sucrose, Fine Powder	981.08

American Institute of Nutrition Mineral Mix

(AIN-76) Composition

Ingredient	gm/Kg
Calcium	5.1551
Phosphorus	3.9840
Potassium	3.6023
Sodium	1.0188
Chlorine	1.5711
Sulfur	.3374
Iodine	.0002
Iron	.0351
Magnesium	.5067
Zinc	.0314
Copper	.0056
Manganese	.0585
Selenium	.0001
Chromium	.0020
Sucrose	4.13105

APPENDIX B

RAW DATA

Animal Number	Diet	Beginning Weight (gm)	Ending Weight (gm)	Bleeding Time[a]	Liver Cholesterol[c]	Triglycerides[c]	Prothrombin Time[d]	Total Cholesterol[c]	Alpha[c]	Beta[c]	Weight Change (gm)
29	4	47	78	0.91	17.28	103.8	17.0	0.0	0.0	0.0	21
81	4	55	82	1.96	15.74	0.0	0.0	0.0	70.1	57.1	27
41	4	52	77	2.88	37.68	0.0	0.0	0.0	73.1	65.8	25
99	4	54	72	3.65	29.09	48.6	24.5	0.0	0.0	0.0	18
71	4	54	67	2.36	17.06	115.5	19.0	0.0	0.0	0.0	13
47	4	56	79	4.16	26.72	69.9	21.0	0.0	0.0	0.0	23
19	4	58	62	1.51	16.93	0.0	0.0	167.2	84.1	81.1	4
74	4	53	81	2.66	9.29	0.0	0.0	0.0	35.5	30.5	28
56	4	47	62	2.18	24.24	0.0	0.0	169.3	61.9	119.0	15
25	4	65	75	2.35	19.21	134.1	25.0	0.0	0.0	0.0	10
17	4	57	69	3.49	13.41	0.0	0.0	119.0	46.3	71.4	12
9	4	53	73	5.53	34.50	0.0	0.0	0.0	0.0	0.0	20
60	4	63	86	4.94	25.14	0.0	0.0	123.5	64.2	53.5	23
48	4	61	78	3.16	26.69	0.0	0.0	272.1	92.0	169.2	17
43	4	48	63	1.70	31.94	0.0	0.0	0.0	57.1	80.1	15
70	4	55	72	3.83	34.12	0.0	0.0	121.6	37.6	68.8	17
30	6	57	73	5.15	0.0	52.5	14.5	0.0	0.0	0.0	16
24	6	64	64	1.05	23.55	0.0	0.0	144.4	62.6	69.1	0
5	6	57	82	8.62	21.34	0.0	0.0	232.7	87.1	121.5	25
51	6	58	83	2.33	27.10	0.0	0.0	132.1	63.6	59.0	25
10	6	63	85	2.99	18.76	0.0	0.0	183.7	69.0	74.0	22
1	6	0	0	0.00	18.41	130.6	17.0	0.0	0.0	0.0	0
23	6	52	77	3.10	31.41	59.9	22.0	0.0	0.0	0.0	25
91	6	53	65	5.12	16.09	0.0	0.0	0.0	0.0	0.0	12
72	6	47	61	1.46	42.24	0.0	0.0	0.0	75.2	97.2	14
100	6	55	76	3.94	20.69	0.0	0.0	318.3	95.7	197.7	21
80	6	61	81	0.00	26.00	0.0	0.0	0.0	51.0	25.2	20
36	6	53	75	0.00	24.40	0.0	0.0	153.3	80.2	61.2	22
11	6	55	64	8.88	14.34	60.8	16.5	0.0	0.0	0.0	9
64	6	56	65	3.15	20.08	41.7	0.0	0.0	0.0	0.0	9
38	6	52	56	0.00	22.06	0.0	0.0	0.0	0.9	0.0	4
45	6	55	66	2.87	32.64	0.0	0.0	0.0	55.5	114.9	11
68	3	58	61	1.50	11.55	0.0	0.0	203.9	68.0	151.5	3
83	3	48	53	0.93	20.44	0.0	0.0	166.8	52.7	86.5	5
92	3	52	52	1.16	13.33	63.0	14.5	0.0	0.0	0.0	0
52	3	53	56	2.90	8.74	0.0	0.0	0.0	53.8	58.7	3
53	3	52	62	0.00	28.59	0.0	0.0	0.0	0.0	0.0	10
102	3	56	70	2.01	20.62	0.0	0.0	0.0	77.1	106.1	14
46	3	55	56	0.92	9.65	0.0	0.0	0.0	0.0	0.0	1
98	3	55	61	3.76	21.47	0.0	0.0	0.0	88.0	249.9	6
62	3	66	72	1.45	19.17	126.8	20.5	0.0	0.0	0.0	6
88	3	61	70	0.00	35.40	99.4	17.0	0.0	0.0	0.0	9

Animal Number	Diet	Beginning Weight (gm)	Ending Weight (gm)	Bleeding Time[a]	Liver Cholesterol[c]	Triglycerides[c]	Prothrombin Time[d]	Total Cholesterol[c]	Alpha[c]	Beta[c]	Weight Change (gm)
85	3	63	75	3.42	14.46	0.0	0.0	163.0	67.1	69.0	12
13	3	55	52	0.00	10.59	0.0	0.0	0.0	73.2	135.9	- 3
16	3	56	67	2.53	27.39	0.0	0.0	0.0	97.4	99.1	11
44	3	47	55	0.00	15.14	0.0	0.0	0.0	0.0	0.0	8
54	3	56	60	1.80	10.03	0.0	0.0	0.0	110.9	166.4	4
58	2	55	57	2.70	18.11	0.0	0.0	192.4	42.2	142.7	2
59	2	53	73	2.48	31.85	0.0	0.0	243.3	78.5	132.4	17
4	2	53	73	0.00	8.02	158.0	22.0	0.0	0.0	0.0	20
33	2	59	78	1.98	21.03	109.0	16.5	0.0	0.0	0.0	19
37	2	58	67	1.90	18.61	84.2	21.5	0.0	0.0	0.0	9
34	2	48	75	2.88	25.52	0.0	0.0	0.0	89.6	268.7	27
87	2	53	73	3.52	15.50	0.0	0.9	118.2	67.7	41.4	20
2	2	47	71	1.94	44.17	114.2	21.5	0.0	0.0	0.0	24
77	2	55	67	0.00	19.60	0.0	0.0	0.0	0.0	0.0	12
57	2	56	55	1.69	11.19	0.0	0.0	243.4	53.8	131.6	- 1
82	2	52	73	3.75	12.20	0.0	0.0	122.6	57.7	52.0	21
39	2	63	81	4.69	27.50	0.0	0.0	214.9	73.6	143.3	18
89	2	56	81	0.00	29.39	185.3	23.5	0.0	0.0	0.0	25
90	2	62	76	4.06	33.02	0.0	0.0	0.0	122.9	236.3	14
75	2	54	73	6.45	34.57	0.0	0.0	0.0	63.2	122.9	19
15	2	54	74	1.19	24.39	0.0	0.0	0.0	82.1	100.9	20
86	5	56	76	1.86	23.09	0.0	0.0	166.5	65.9	73.4	20
65	5	58	74	2.98	20.17	0.0	0.0	0.0	97.5	155.2	16
8	5	65	75	3.86	19.85	0.0	0.0	0.0	53.5	48.3	10
96	5	53	58	4.75	20.90	0.0	0.0	98.6	47.6	45.3	5
50	5	48	71	4.54	9.93	31.7	26.0	0.0	0.0	0.0	23
28	5	52	58	2.16	16.00	0.0	0.0	203.6	65.5	94.7	6
94	5	57	73	2.54	13.14	0.0	0.0	0.0	117.7	145.9	16
69	5	53	55	1.05	27.99	110.3	32.5	0.0	0.0	0.0	2
42	5	47	56	3.39	24.32	64.7	27.5	0.0	0.0	0.0	9
93	5	52	75	0.77	23.99	0.0	0.0	227.4	99.5	108.0	23
84	5	54	74	2.96	13.40	75.1	16.5	0.0	0.0	0.0	20
76	5	56	65	2.64	22.65	57.7	15.5	0.0	0.0	0.0	9
66	5	59	60	3.16	24.31	0.0	0.0	0.0	0.0	0.0	1
32	5	55	70	1.73	39.05	0.0	0.0	219.3	53.8	144.5	15
63	5	61	81	3.01	14.43	0.0	0.0	0.0	65.8	36.7	20
73	1	54	61	2.15	33.56	0.0	0.0	210.6	47.0	145.6	7
61	1	57	75	5.05	12.32	0.0	0.0	167.9	83.1	75.6	18
95	1	54	57	3.63	16.21	0.0	0.0	319.6	93.4	114.8	3
49	1	58	81	2.05	21.52	0.0	0.0	301.5	128.4	155.2	23
55	1	61	76	1.29	29.01	0.0	0.0	205.3	85.3	84.0	15
14	1	64	63	3.55	4.44	80.7	15.0	0.0	0.0	0.0	- 1
3	1	65	56	0.00	7.28	0.0	0.0	0.0	0.0	0.0	- 9

Animal Number	Diet	Beginning Weight (gm)	Ending Weight (gm)	Bleeding Time[a]	Liver Cholesterol[c]	Triglycerides[c]	Prothrombin Time[d]	Total Cholesterol[c]	Alpha[c]	Beta[c]	Weight Change (gm)
7	1	53	74	4.94	22.02	0.0	0.0	245.7	88.5	92.4	21
78	1	47	62	2.70	33.22	86.4	18.0	0.0	0.0	0.0	15
97	1	55	83	1.26	16.59	90.3	21.5	0.0	0.0	0.0	28
35	1	53	64	2.87	15.15	0.0	0.0	378.2	58.4	317.2	11
20	1	55	54	3.61	20.17	124.1	24.5	0.0	0.0	0.0	- 1
6	1	57	74	3.20	32.85	0.0	0.0	210.0	98.6	86.0	17

[a]Measured in minutes and fractions of minutes

[b]mg/gm

[c]mg/dl

[d]Seconds