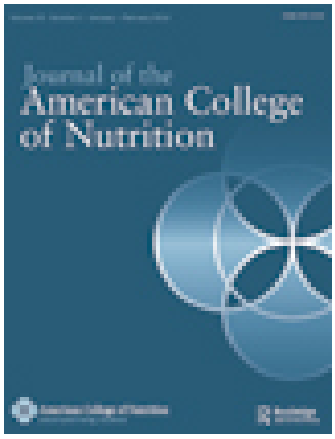

Journal of the American College of Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uacn20

A Comparison of Fish Oil, Flaxseed Oil and Hempseed Oil Supplementation on Selected Parameters of Cardiovascular Health in Healthy Volunteers

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Published online: 14 Jun 2013.

To cite this article: Nalini Kaul PhD, Renee Kreml MD, J. Alejandro Austria, Melanie N. Richard MSc, Andrea L. Edel MSc, Elena Dibrov PhD, Satoru Hirono MD, Marjorie E. Zettler PhD & Grant N. Pierce PhD (2008) A Comparison of Fish Oil, Flaxseed Oil and Hempseed Oil Supplementation on Selected Parameters of Cardiovascular Health in Healthy Volunteers, Journal of the American College of Nutrition, 27:1, 51-58, DOI: 10.1080/07315724.2008.10719674

To link to this article: http://dx.doi.org/10.1080/07315724.2008.10719674

Original Research

A Comparison of Fish Oil, Flaxseed Oil and Hempseed Oil Supplementation on Selected Parameters of Cardiovascular Health in Healthy Volunteers

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Key words: omega-3 fatty acids, cholesterol, polyunsaturated fatty acids, heart disease, platelet aggregation

Objective: The impact of dietary polyunsaturated fatty acids (PUFAs) of the n-6 and n-3 series on the cardiovascular system is well documented. To directly compare the effects of three dietary oils (fish, flaxseed and hempseed) given in concentrations expected to be self-administered in the general population on specific cardiovascular parameters in healthy volunteers.

Design: 86 healthy male and female volunteers completed a 12 week double blinded, placebo controlled, clinical trial. They were randomly assigned to one of the four groups. Subjects were orally supplemented with two 1 gm capsules of placebo, fish oil, flaxseed oil or hempseed oil per day for 12 weeks.

Results: Plasma levels of the n-3 fatty acids docosahexanoic acid and eicosapentanoic acid increased after 3 months supplementation with fish oil. Alpha linolenic acid concentrations increased transiently after flaxseed supplementation. However, supplementation with hempseed oil did not significantly alter the concentration of any plasma fatty acid. The lipid parameters (TC, HDL-C, LDL-C and TG) did not show any significant differences among the four groups. Oxidative modification of LDL showed no increase in lag time over the 12 wk period. None of the dietary interventions induced any significant change in collagen or thrombin stimulated platelet aggregation and no increase in the level of inflammatory markers was observed.

Conclusion: From a consumer's perspective, ingesting 2 capsules of any of these oils in an attempt to achieve cardiovascular health benefits may not provide the desired or expected result over a 3 month period.

INTRODUCTION

Coronary heart disease (CHD), a major cause of morbidity and mortality, is often attributable to atherosclerosis. Atherosclerosis is a complex process involving the progressive accumulation of lipid laden macrophages (foam cells) and smooth muscle cells within the vessel intima [1]. Reactive oxygen species (ROS) released during atherosclerosis are thought to induce the formation of a highly atherogenic molecule, oxidized low density lipoprotein, that accelerates lipid deposition in the evolving plaque [2]. Although lipid deposition is closely associated with the development of atherosclerosis, the observation that Inuit rarely developed CHD despite a high intake of fat prompted investigations for the protective component in their diet. Much emphasis has been placed on polyunsaturated fatty acids (PUFA), namely omega 3 (n-3) and omega 6 (n-6), for limiting atherosclerosis. Three randomized clinical trials have reported benefits from PUFA to patients with pre-existing CHD [3–5]. The most convincing is the GISSI-Prevenzione trial in which patients taking omega 3 fatty acids experienced a 20% reduction in overall mortality and a 45% decrease in the risk of sudden cardiac death [5]. These findings support the view that relatively small intakes of n-3 fatty acids are beneficial. A multiplicity of functional effects of n-3 fatty acids in human physiology, human diseases and animal models has

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Journal of the American College of Nutrition, Vol. 27, No. 1, 51–58 (2008)
Published by the American College of Nutrition

been demonstrated [6–9]. These include effects on plasma lipids and lipoproteins, eicosanoid metabolism, platelet lipid composition and platelet function, platelet/vessel wall interactions, blood viscosity, arterial blood pressure, coagulation, cytokines and growth factors [6–15].

Despite the encouraging results, these earlier studies have some important limitations. First, many studies employed inadequate controls, lacked a placebo group, did not control for supplement usage or failed to document diet records. This points to the need for further work using more careful controls to determine the effects of this oil. More importantly, the majority of the previous studies administered relatively large amounts of fish oil (3–10g/day). This is not a practical dosage for most people in everyday life. Fish oil is not always palatable and often causes complaints of eructation. Thus, although it may provide health related benefits, if it is not administered in the general population in high dosages, it may not achieve the desired health benefits. Because of the problems regarding the palatability of fish oil, there is a need for alternative sources of omega 3 fatty acids. Two plant based sources of PUFA include flaxseed and hempseed. Flaxseed oil is comprised of 50% PUFA and is one of the richest vegetarian sources of ALA [16,17]. A growing body of evidence supports flaxseed as a beneficial dietary supplement for maintaining healthy cholesterol levels [17–24]. Modest reductions in both total cholesterol and LDL levels without changes in HDL-C may be achieved in healthy volunteers when incorporated in the diet [21,24]. Another vegetarian source of omega-3 fatty acid is hempseed oil. Although hempseed oil contains the same fatty acids as flaxseed, the optimum ratio of omega 6 to omega 3 fatty acids (3:1) is found in hempseed oil [25]. Therefore, it may offer nutritional advantages. Unfortunately, there are no data available regarding the impact of hempseed oil on any cardiovascular parameters in humans.

The purpose of the present investigation, therefore, was two fold. First, it was important to determine if the lower concentrations of PUFA supplements that could be expected to be administered by the general population would have effects on biochemical parameters associated with cardiovascular disease. In view of recent work documenting compliance problems with administration of multiple pills or capsules [26], we chose a dosage of two large 1 g capsules per day as a reasonable expectation for the general population to self administer. Second, it was our goal to conduct a double blinded, placebo controlled trial to compare the relative effects of three different sources of PUFAs, fish oil, flaxseed oil and hempseed oil supplementation on a number of indices of cardiovascular disease in healthy volunteers.

SUBJECTS AND METHODS

Study Design

The study design was approved by the Institutional Review Board of the University of Manitoba. After being recruited locally, without restriction to gender, race or socioeconomic status, all subjects provided informed consent to participate in the study. Eligibility of the participants was determined by the following preset criteria: fasting total cholesterol levels below 5.2 mM, non-smokers, consumption of fish not more than once a week, consumption of no more than 1oz. of alcohol per day, no use of aspirin, ibuprofen or any other non-steroidal, anti-inflammatory medications, no use of thyroid, anticoagulant or lipid lowering medications, pre-menopausal, and no chronic illness.

Participants were requested to follow their usual diet. Subjects were asked to perform only moderate exercise throughout the study period. Post menopausal women were excluded from the study. Once eligibility was established, the participants were randomized to one of the four study groups by a computer generated schedule. The groups included a placebo group (2g/d sunflower oil), fish oil group (2g/d), flax seed oil group (2g/d) and hemp seed oil group (2g/d). Sunflower oil is used frequently in the literature as a control oil in fatty acid nutritional interventions. Therefore, it is a good reference point for our study. Its composition also contrasts well with the other dietary interventions used in this study because it is highly enriched in omega-6 fatty acids with some monounsaturated fats as well. Dietary supplements were administered as two 1 gm capsules per day with a meal. Capsule levels of n-3 and n-6 fatty acids are shown in Table 1. Compliance with the treatment protocol was determined by counting the remaining pills and periodic telephone conversations.

Table 1. Omega-3 and -6 Fatty Acid Levels in a 1 gm Capsule

Subject group	DHA n-3 (mg)	EPA n-3 (mg)	ALA n-3 (mg)	LA n-6 (mg)	GLA n-6 (mg)	Σ n-6 (mg)	Σ n-3 (mg)	n-6:n-3
Placebo	0	0	15	680	7	687	15	46:1
Fish oil	121	176	6	13	2	15	303	0.05:1
Flaxseed oil	0	0	511	140	9	149	511	0.3:1
Hempseed oil	0	0	186	572	26	598	186	3:1

Capsules were only analyzed once as manufacturer's fatty acid levels were in agreement with our data.

N-3 = omega-3 fatty acid, n-6 = omega-6 fatty acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, ALA = alpha linolenic acid, LA = linoleic acid, GLA = gamma linolenic acid.

Blood samples (60 ml) were collected by venupuncture after the subjects had fasted for 10–12 h, on three visits (0, 6 and 12 weeks). Blood was collected in tubes containing 1 mg EDTA/ml for determination of complete blood count and plasma lipids. Serum and plasma were separated from blood by low speed centrifugation (800g×15 min) at 4°C. Platelet aggregation studies were done immediately on fresh blood samples. Some aliquots of plasma were stored at −80°C until the end of the study when lipid profile and LDL isolation and oxidation could be performed. All samples were numbered consecutively so that sample identity and treatment assignment were not apparent. Furthermore, the laboratory staff was blinded to treatment assignments.

Analytical Procedures

Lipid and lipoprotein levels were quantified in a routine manner at the Clinical Chemistry Laboratory at the Health Sciences Center, Winnipeg, Canada. LDL (d 1.019–1.063 g/mL) was isolated by rapid two-step ultracentrifugation using a modification of the method of Kleinveld et al. [27]. All salts had EDTA (1 mg/mL) to prevent oxidation during the isolation of LDL. The final LDL fraction was obtained by passing it through a Sephadex G-25 column to eliminate EDTA before oxidation. LDL (100 μg/mL) was oxidized in a cell-free system using 5μM $CuSO_4$ at 37° C for 5 hours. Conjugated dienes were measured every 10 minutes by continuous absorption spectrophotometry at 234 nm [28]. From the time course curve, lag phase of oxidation was computed [28].

Plasma fatty acids were determined by gas liquid chromatography, following extraction and transmethylation as previously described using C17:0 as an internal standard (Nu-chek Prep, MN, USA) [29]. Data was expressed in μg/ml.

For platelet aggregation, 5 ml of whole blood was collected with minimal trauma in a tube containing sodium citrate to prevent coagulation. Platelet rich plasma (PRP) was isolated from the sample by low centrifugation at 100×g for 15 min. Platelet aggregation was measured in a Chrono-log Aggregometer, model 490 (Chrono-log Corp.), as described [30,31]. Aggregation was carried out in 500 μl of PRP. After equilibration at 37°C for 5 minutes, the aggregate was added (collagen or thrombin in final concentrations of 2 ug/ml and 0.3 units, respectively).

C-Reactive Protein (CRP) and Tumor Necrosis Factor-alpha (TNFα) were used as inflammatory markers. CRP and TNFα levels in the serum were determined by ELISA systems (CRP ELISA Kit, Alpha Diagnostic International; High sensitivity TNFα Elisa system, Biotrak, Amersham).

Diet Analyses

Daily intake of energy was analyzed from 3 day diet records using food analysis software (Food Processor version 7.7) (ESHA Research, Salem, OR).

Statistical Analyses

Statistical analyses were undertaken to assess the significance of the parameters tested. Subjects were included in the statistical analyses if they had been at least 80% compliant with taking the capsules as required, had given three blood samples and had been 90% compliant with diet and concomitant medication restrictions as instructed within the time frames required. One way ANOVA was used to assess the differences between the placebo and the supplemented groups at baseline, 6 wks and 12 wks of supplementation. All data was expressed as mean ± S.E.M. The level of significance was set at $p < 0.05$.

RESULTS

The characteristics of the subjects at baseline are shown in Table 2. Out of the 88 people who completed the study, 34 were males and 54 were females. No significant differences between the groups with regard to age and body mass index were observed. There were no adverse effects documented. Subject compliance was 85–88% based on capsule count records. There was no significant difference in the patient compliance between groups. Average intake of protein, fat and carbohydrates in the diet did not change appreciably during the supplementation period nor was it different between groups.

Fish oil is enriched in the n-3 fatty acids, EPA and DHA. Flaxseed oil is enriched in ALA and hempseed oil is enriched in ALA and the n-6 fatty acid, linoleic acid (LA). We would expect the concentrations of these fatty acids to increase in a specific fashion in subjects supplemented with the respective oils. A significant increase in plasma levels of EPA and DHA in the fish oil supplemented group was observed at 6 and 12 weeks as compared to baseline ($p < 0.05$) (Table 3). The flaxseed supplemented group showed a significant difference in ALA at 6 wk as compared to baseline ($p < 0.05$) (Table 3). Conversely, the hempseed group and the placebo group did not

Table 2. Baseline Characteristics of the Subjects

Group	Placebo	Flaxseed	Fish	Hempseed
Age (years)	32.93 ± 1.99	34.70 ± 1.69	34.44 ± 1.82	34.98 ± 1.73
BMI (kg/m^2)	24.41 ± 0.82	24.28 ± 0.73	25.06 ± 0.64	24.14 ± 0.77

Values are mean ± SEM.

BMI = body mass index.

Table 3. Plasma Fatty Acid Profiles for Study Participants at 0, 6 and 12 Weeks of Dietary Intervention

GROUP	DHA mean ± sem	EPA mean ± sem	ALA mean ± sem	LA mean ± sem
Placebo 0wk	56.8 ± 4.3	22.2 ± 2.6	14.0 ± 2.3	1057 ± 60
Placebo 6wk	56.5 ± 5.0	22.4 ± 2.7	16.4 ± 2.8	1063 ± 40
Placebo 12wk	56.8 ± 4.4	22.6 ± 2.6	14.9 ± 2.8	1055 ± 41
Fish 0wk	66.1 ± 6.5	22.6 ± 3.0	19.5 ± 3.4	1027 ± 47
Fish 6wk	90.1 ± 5.6*	36.9 ± 3.7*	19.9 ± 3.4	1064 ± 49
Fish 12wk	95.4 ± 7.0*	35.0 ± 3.0*	20.4 ± 3.4	1063 ± 48
Flax 0wk	65.0 ± 4.9	23.9 ± 2.8	19.7 ± 3.1	1014 ± 35
Flax 6wk	64.9 ± 4.4	25.7 ± 2.8	27.7 ± 3.5*	1001 ± 36
Flax 12wk	64.2 ± 6.1	25.6 ± 3.0	25.1 ± 3.5	989 ± 43
Hemp 0wk	66.3 ± 7.2	26.8 ± 3.4	14.8 ± 2.5	1089 ± 42
Hemp 6wk	64.8 ± 6.5	23.4 ± 3.7	17.5 ± 2.9	1049 ± 48
Hemp 12wk	64.3 ± 7.3	24.7 ± 3.7	15.8 ± 3.0	1108 ± 56

Values are mean ± SEM and are given in ug/ml.

DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, ALA = alpha linolenic acid, LA = linoleic acid.

* $p < 0.05$ vs respective baseline value at 0 week within same group. All other comparisons were not significantly different ($p > 0.05$).

GLA: gamma-linolenic acid levels are not shown as there were no significant differences within each group at 0, 6 and 12 weeks nor between any of the groups (average level was 14 mg).

show any significant differences in EPA, DHA, LA or ALA levels at 6 wk and 12 wk as compared to baseline (Table 3).

Total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol levels were measured. There were no significant differences observed in any of the lipid parameters amongst the four groups at any of the time points examined in this study (Table 4).

LDL oxidation by $CuSO_4$ was examined in an in vitro setting using lag time as a measurement parameter. No significant change in lag time was observed in any of the groups over the 12 wk period (Fig. 1).

CRP and TNFα were measured as markers of inflammation within each group at each time point (Table 5). Statistical analysis of the results indicated that there were no significant changes.

Collagen and thrombin-induced platelet aggregation was also examined in the 4 groups. No statistical difference in platelet aggregation with collagen was detected amongst the four groups after oral supplementation with the oils at 6 wk and 12 wk when compared to baseline (Table 6). Thrombin induced platelet aggregation was also similar amongst the 4 groups (Table 6).

Table 4. Lipid Profile of Subjects Receiving Placebo(Sunflower Oil), Fish Oil, Flaxseed Oil or Hempseed Oil

Lipid Parameter	Placebo	Fish oil	Flax oil	Hemp oil
Total Cholesterol (TC) (mMol/L)				
Baseline	4.6 ± 0.2	4.9 ± 0.1	4.7 ± 0.2	4.8 ± 0.2
6 weeks	4.7 ± 0.2	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.2
12 weeks	4.9 ± 0.2	5.1 ± 0.2	4.9 ± 0.1	5.0 ± 0.2
LDL Cholesterol (mMol/L)				
Baseline	2.6 ± 0.1	2.7 ± 0.2	2.5 ± 0.2	2.8 ± 0.2
6 weeks	2.6 ± 0.1	2.7 ± 0.2	2.7 ± 0.1	2.8 ± 0.2
12 weeks	2.8 ± 0.1	2.9 ± 0.2	2.8 ± 0.2	2.9 ± 0.2
HDL Cholesterol (mMol/L)				
Baseline	1.5 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.5 ± 0.1
6 weeks	1.4 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
12 weeks	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Triglycerides (mMol/L)				
Baseline	1.4 ± 0.2	1.5 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
6 weeks	1.4 ± 0.2	1.4 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
12 weeks	1.4 ± 0.2	1.5 ± 0.2	1.1 ± 0.1	1.3 ± 0.2
TC/HDL Ratio (mMol/L)				
Baseline	3.5 ± 0.2	3.4 ± 0.2	3.0 ± 0.1	3.4 ± 0.2
6 weeks	3.5 ± 0.2	3.4 ± 0.1	3.1 ± 0.2	3.2 ± 0.3
12 weeks	3.6 ± 0.3	3.6 ± 0.2	3.1 ± 0.1	3.5 ± 0.3

Results are presented as mean ± SEM.

All values were $p > 0.05$ vs control.

LDL = low density lipoprotein, HDL = high density lipoprotein, TC = total cholesterol.

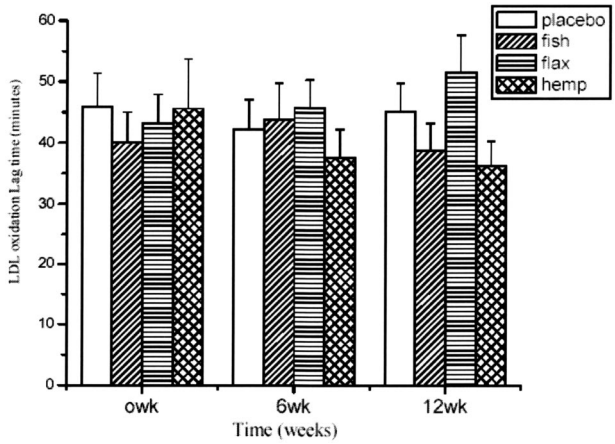

Fig. 1. Lag times for conjugated diene generation in LDL exposed to copper sulfate in subjects receiving placebo, fish oil, flaxseed oil or hempseed oil supplementation. Values are presented as mean ± SEM LDL (100 μg protein/ml) was incubated with 5 μM copper sulfate for 5 hrs at 37°C and monitored continuously at 234 nm for the formation of conjugated dienes. All values were $p > 0.05$.

DISCUSSION

The present study was designed to evaluate whether supplementation with fish oil, flaxseed oil or hempseed oil has any effect on lipid profile, LDL oxidation, inflammatory markers or platelet aggregation. This study is the first to directly compare the effects of these three different oils. Each of these oil sources provides very different PUFAs. LA and ALA are considered the precursor fatty acids of the n-6 and n-3 series of PUFAs [32]. They are essential fats in the diet for healthy individuals. However, long chain PUFAs like GLA, EPA and DHA often provide benefits that the precursor essential fatty acids do not and in some cases may be essential in the diet as well [8–10,32].

When the subjects received 2 × 1 gm oil capsules, all of the experimental groups received a comparable amount (~700 mg) of very different selected PUFAs in each capsule. The fish oil group had lower levels of PUFA supplementation than any of the other groups (~300 mg/capsule). It was surprising, therefore, that the fish oil supplemented subjects obtained the largest stimulation of plasma PUFAs of all of the interventions. Supplementation with flaxseed oil resulted in a significant but modest increase in ALA whereas the hempseed supplementation did not significantly affect plasma levels of ALA or LA. There can be several reasons for this. The ALA and LA may not be absorbed from the gastrointestinal tract as well as EPA and DHA. We have observed similar effects when rabbits are supplemented with hempseed (unpublished data). A higher level of ALA and LA supplementation may be required to produce a meaningful increase within the 3 month time frame of our study. Alternatively, the ALA and LA may be absorbed but might be metabolized faster within the body to clear it from the blood stream more rapidly. Without tissue measurements of PUFA content, this cannot be directly evaluated. Finally, another possible reason for the unusually low plasma PUFA levels could be poor subject compliancy. However, there was no reason to believe poor subject compliancy was a factor given the capsule count was excellent. It is possible that the hempseed oil did not modify the plasma fatty acid profile because of an insufficient amount of LA and a dilution of the omega-6 levels with other omega-6's in the diet. We can reasonably conclude that there are intrinsic differences in the absorption and/or metabolism of these fatty acids within the human body.

The three oils failed to show any significant effects on lipid profile, LDL oxidation or platelet aggregation over a 12 week period. Our data are in agreement with previous work using fish oil supplements. Earlier studies of platelet aggregation indicate that doses of n-3 fatty acids greater than 6 g for more prolonged time periods are required to affect platelet function [32]. Our results are also consistent with an earlier study in which there were essentially no changes in the lipid profile in normolipidemic individuals after supplementation with omega 3 fatty acids [33] and the work of Higgins et al [34] who demonstrated that the dietary intake of a similar concentration of n-3 PUFA did not significantly influence LDL oxidation in vitro. Our

Table 5. CRP Levels (A.) in ug/dL and TNFα Levels (B.) in pg/ml in Serum from Study Subjects

CRP: Group	Placebo	Fish	Flax	Hemp
A.				
0wk	314 ± 69	499 ± 136	319 ± 65	311 ± 66
6wk	240 ± 191	508 ± 125	335 ± 55	329 ± 69
12wk	202 ± 166	589 ± 152	326 ± 50	295 ± 73
B.				
TNFα: Group	Placebo	Fish	Flax	Hemp
0wk	0.55 ± 0.11	0.55 ± 0.15	0.33 ± 0.08	0.39 ± 0.10
6wk	0.27 ± 0.02	0.27 ± 0.05	0.36 ± 0.06	0.30 ± 0.06
12wk	0.58 ± 0.14	0.47 ± 0.15	0.62 ± 0.19	0.40 ± 0.08

Values represented mean ± SEM for 22 subjects in each group.

$p > 0.05$ between all groups.

CRP = C-Reactive Protein (CRP), TNFα = Tumor Necrosis Factor-alpha.

Table 6. Effects of PUFA Supplementation with Placebo (Sunflower Oil), Fish Oil, Flaxseed Oil or Hempseed Oil on Platelet Aggregation

Subject group	Collagen-induced platelet aggregation (%)			Thrombin-induced platelet aggregation (%)		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
Placebo	71.3 ± 2.2	68.6 ± 2.2	67.1 ± 2.8	80.1 ± 1.9	76.5 ± 4.3	80.1 ± 2.9
Fish oil	73.9 ± 3.2	72.8 ± 1.4	70.6 ± 2.5	80.1 ± 3.7	81.8 ± 2.5	81.8 ± 2.5
Flaxseed oil	72.4 ± 2.0	73.3 ± 2.0	71.1 ± 2.9	81.0 ± 2.4	84.0 ± 1.8	80.4 ± 3.9
Hempseed oil	74.7 ± 2.1	71.8 ± 3.0	72.8 ± 2.7	81.9 ± 3.1	78.7 ± 5.0	80.5 ± 5.4

Results are presented as mean ± SEM.
All values were $p > 0.05$ vs control.

present results now extend this to flaxseed oil and hempseed oil supplementation.

In our study, there are several factors that may have contributed to the results that we obtained. First, we used healthy volunteers with no underlying pathology in our study. The effects of n-3 fatty acids on total cholesterol concentrations may be more marked had we used a hypercholesterolemic population [35]. For example, n-3 fatty acid intake has been shown to affect plasma lipids and cells involved in atherosclerosis in patients at high risk of atherosclerosis [36–38]. Another confounding factor may have been the concentration of supplement chosen to use in this study. Studies of fish oil (which is the most intensely studied oil out of the four used in our study) have used relatively large amounts of oils containing n-3 fatty acids to achieve measurable effects on plasma or cells [38,39]. For example, several studies using either fish oil (9–12g/day) or flaxseed oil (20–40g/day) have shown significant effects on the plasma lipid profile, a decrease in platelet aggregation and a decrease in circulating levels of inflammatory mediators [24,40–45]. However, these dosage levels would have necessitated the ingestion of 9–40 capsules per day. The ingestion of this large number of capsules is not practical for use in the general population. On a daily basis, we would argue that few *healthy* subjects are likely to consume more than 2 capsules/day of any one given compound [26]. Furthermore, higher doses might not be tolerated well by the general public. Ingestion of large capsules, poor taste and reflux (eructation) are some factors resulting in poor compliance [46–48]. In our study, ~50% of the subjects in the fish oil group complained of aftertaste, burping and the size of the capsules. We used two 1 g capsules/day for each subject. This dosage would be expected to be better tolerated in the general population but our results would suggest that this dosage may have been too low to be beneficial. Therefore, a higher concentration of PUFA correlating to a larger dosage regimen may be necessary to see a remarkable change in the parameters studied in our investigation.

It is important to emphasize that our results do not negate the potential significance of n-3 fatty acids as therapeutic agents for CHD. A decrease in CHD mortality has been reported to occur when consuming relatively small amounts of fish and/or fish oil similar to those used in the present study (i.e. equivalent to 0.3g EPA/day compared to our value of 0.4 mg EPA and 0.2 mg DHA) over a much longer period of time [3,33]. In addition, the Lyon diet intervention reported that increases in ALA consumption were associated with significant changes in cardiovascular morbidity without changes in plasma cholesterol [49]. However, our work emphasizes the necessity of identifying an appropriate dosage that will have health related benefits and discovering an appropriate vehicle for this dosage that will ensure compliancy if we are to introduce these compounds to the general public for treating CHD. It is important for consumers to know that ingesting two capsules of these oils/day in an attempt to lower lipid levels, reduce LDL oxidation and alter platelet aggregation may not be enough within three months to obtain the desired or expected results. However, in the case of hempseed oil, it is difficult to argue that 2 g/day will ever have a physiological impact upon the body if the PUFA content in the blood is not increased in response to this dietary regimen.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Canadian Institutes for Health Research and St. Boniface Hospital & Research Foundation. The technical assistance of Ms. Xuemei Wang is gratefully appreciated. The authors would also like to thank BiOriginal Food and Science Corporation, Canada, and Mr. Shawn Crew of Hemp Oil Canada, Inc. for their supply of dietary capsules used in the study.

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Received October 14, 2005; revision accepted May 3, 2006.