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INDIVIDUAL FATTY ACIDS AND CARDIOVASCULAR RISK FACTORS

Sameline Grimsgaard

Tromsø 2001

Institute of Community Medicine
University of Tromsø, Norway

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CONTENTS

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Working with this thesis has been an apprenticeship and I am indebted to many who contributed to my education.

First I want to thank the men who spent time and effort to be *participants in the intervention study*. They taught me of the loyalty and pride the Tromsø inhabitants take in the Tromsø Study. I am grateful to *the Nordland Health Study participants* who contributed to a large and unique dataset. We learned a lot about fatty acids in blood from it.

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LIST OF PAPERS

This thesis is based on the following papers:

I Grimsgaard S, Bønaa KH, Hansen JB, Nordøy A. Highly purified eicosapentaenoic acid and docosahexaenoic acids in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. *American Journal of Clinical Nutrition* 1997;66:649-59.

II Grimsgaard S, Bønaa KH, Hansen JB, Myhre ESP. Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans. *American Journal of Clinical Nutrition* 1998;68:52-9.

III Grimsgaard S, Bønaa KH, Jacobsen BK, Bjerve KS. Plasma saturated and linoleic fatty acids are independently associated with blood pressure. *Hypertension* 1999;34:478-83.

IV Grimsgaard S, Bønaa KH, Bjerve KS. Both fatty acid chain length and degree of unsaturation are inversely associated with serum triacylglycerol concentrations. *Lipids* 2000;35:1185-93.

The papers will be referred to by their Roman numerals in the text.

ABBREVIATIONS

Fatty acids:

14:0 = Myristic
16:0 = Palmitic
18:0 = Stearic
20:0 = Arachidic
22:0 = Behenic
24:0 = Lignoceric

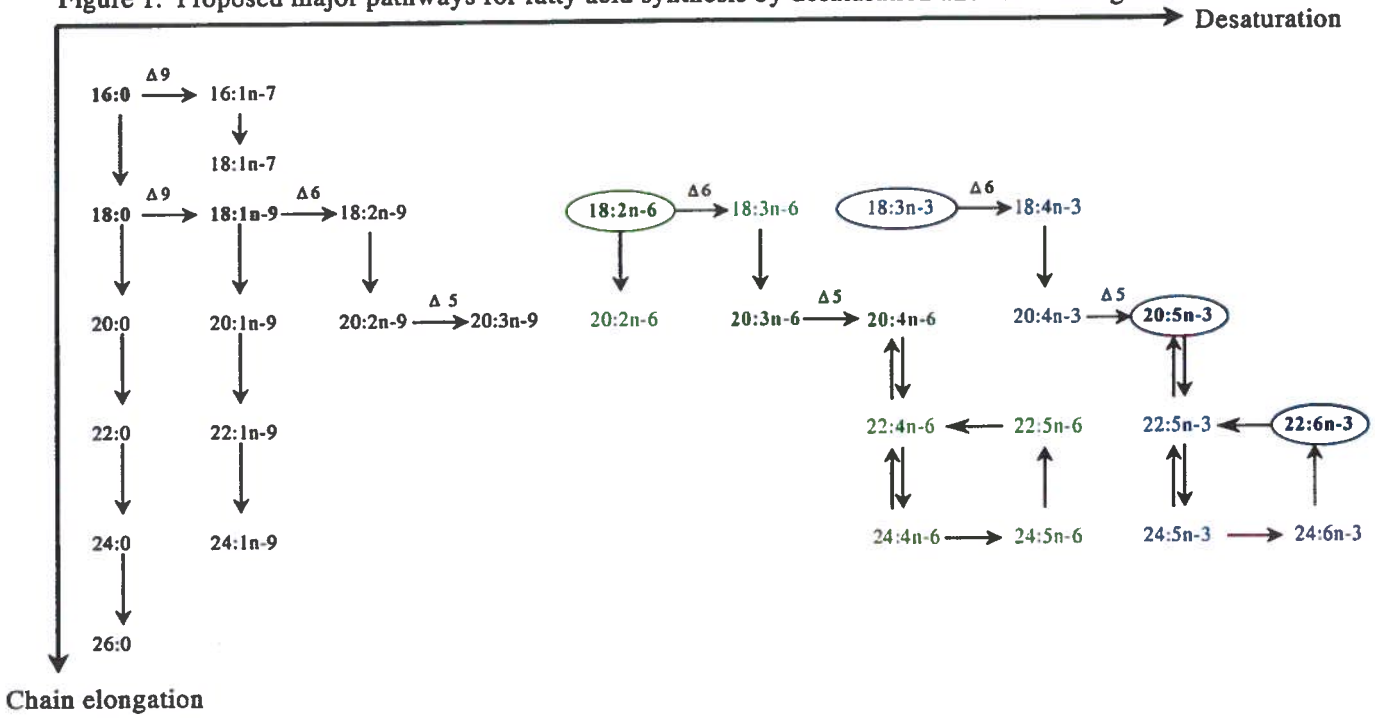
16:1 = Palmitoleic
18:1 = Oleic
20:1 = Gondoic
22:1 = Erucic
24:1 = Nervonic

20:3n-9 = Mead
18:2n-6 = Linoleic
20:2n-6 = Eicosadienoic
20:3n-6 = Dihomo-γ-linolenic
20:4n-6 = Arachidonic
22:4n-6 = Adrenic
22:5n-6 = Docosapentaenoic

18:3n-3 = Linolenic
20:5n-3 = Eicosapentaenoic, EPA
22:5n-3 = Docosapentaenoic
22:6n-3 = Docosahexaenoic, DHA

BMI = Body mass index
BP = Blood pressure
CHD = Coronary heart disease
CI = Confidence interval
CV = Coefficient of variation
HDL = High density lipoprotein cholesterol
LDL = Low density lipoprotein cholesterol
US = United States of America

Figure 1. Proposed major pathways for fatty acid synthesis by desaturation and chain elongation[1]



[1]Boldness of type indicates relative accumulation in plasma phospholipids. Fatty acids considered essential are encircled. Adapted from (1;2).

1. INTRODUCTION

The rise and fall of coronary heart disease (CHD) mortality in Western Europe and the United States (US) in the 20th century has been characterized as a modern epidemic. Atherosclerosis, however, has probably afflicted humans at all times, although to a varying degree. Ancient Egypt mummies show extensive atherosclerosis (3), indicating that CHD was prevalent among affluent people 3-4000 years ago. Descriptions of the symptoms, signs and the course of CHD emerged in European medical literature in the second part of the 18th century (4). The disease gained increasing scientific interest, and in the middle of the 19th century it was recognized that it is a coronary artery occlusion which causes myocardial infarction (4). The etiology of atherosclerosis was a topic of interest among scientists in the early years of the 20th century (5), when Anitschkow performed feeding experiments in rabbits showing that dietary cholesterol raised blood cholesterol levels and induced atherosclerosis (6).

CHD mortality rose dramatically after Second World War in Western Europe and the US. Mortality rates have declined since the 1960s and 1970s, but CHD is still the leading cause of death (7). Secular trends in mortality rates points out life style as a main determinant of the disease, and research has since the 1950s focused the risk factors of disease and how they can be modified (8).

The diet-heart hypothesis

The first prospective epidemiological studies of cardiovascular disease were initiated in the late 1940s. Blood pressure, serum cholesterol level and smoking were pointed out as major and independent risk factors for CHD (9-12). International comparisons revealed large contrasts in both incidence rates and life styles, and generated hypotheses concerning the etiology of cardiovascular disease. The Seven Countries Study demonstrated a positive ecological association between death rates from CHD and percent of dietary calories from saturated fat (12). Experiments by Keys and Hegsted in the 1960s provided evidence that the amount and composition of dietary fat are important determinants of serum cholesterol level (13;14). These observations, together with previous work in animals, and population studies of Japanese migrating to Hawaii and California, gave rise to the "diet-heart hypothesis". According to the classical diet-heart hypothesis, dietary saturated fat and cholesterol increase,

and polyunsaturated fat decrease serum cholesterol levels, thereby determining the development of atheroma leading to myocardial infarction (15).

Observations in special populations contributed to further development of the diet-heart hypothesis. In the 1970s, Bang and Dyerberg found that Greenland Eskimos experienced low mortality from coronary heart disease compared to Danes (16), and that the traditional Eskimo diet was high in very long chain n-3 polyunsaturated, as well as monounsaturated fatty acids (17). It was also reported that Japanese fishing islanders who consumed a diet rich in fish had higher serum levels of n-3 polyunsaturated fatty acids, and lower rates of cardiovascular disease compared to people living on mainland Japan (18). Cohort studies conducted in western populations indicated that moderate fish consumption protected against coronary heart disease (19-21) although the results were not consistent (22;23). Finally, a secondary prevention study reported that modest intake of fatty fish protected against death from recurrent myocardial infarction (24).

Dietary fats

Saturated and monounsaturated fatty acids can be obtained from the diet or produced endogenously, whereas polyunsaturated fatty acids of the n-3 and n-6 classes are essential to humans (Figure 1). Very long chain n-3 polyunsaturated fatty acids are produced by marine phytoplanctons, accumulate in the food chain, and are abundant in fatty fish, fish oils and sea mammals (25). Eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) are the most important marine n-3 fatty acids. They are incorporated in membranes, act as precursors for cellular signal substances (eicosanoids), and have effects on a variety of cellular processes influencing atherosclerosis and thrombosis (26). Since the 1970s, many researchers have focused EPA and DHA as major candidate substances responsible for the potential cardioprotective effects of dietary fish (27).

The most common dietary n-6 fatty acid is linoleic acid (18:2n-6), which is metabolized to arachidonic acid and its longer chain derivatives (Figure 1). Linoleic acid is plentiful in the seeds of most plants, and oils rich in 18:2n-6 include among others sunflower, safflower, soybean and corn oil (25). The n-6 fatty acids are vital components of biological membranes, and arachidonic acid is substrate for eicosanoid metabolism.

Dietary fats and cardiovascular risk factors

The effects of dietary fats on serum cholesterol levels have been extensively studied. Several studies confirmed that saturated fat increases whereas polyunsaturated fat decreases total cholesterol concentrations, as outlined by Keys and Hegsted (15). Dietary studies have extended our knowledge about the specific effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on low density lipoprotein (LDL), high density lipoprotein (HDL) cholesterol and triglyceride levels (28;29). Lately, research has suggested that individual fatty acids within the same fatty acid class may have different effects on lipid metabolism (30). It is now established that individual saturated fatty acids have distinct effects on serum LDL and HDL cholesterol concentrations (31). Dietary supplementation with EPA and DHA decrease serum triglycerides by 25-30%, depending on dose and initial triglyceride level (32), but little is known about the effects of other fatty acids. The relation of serum triglyceride concentrations and risk of coronary heart disease has been controversial (33). Recent studies suggested that serum triglyceride level is an independent risk factor for coronary heart disease (34-36), thus the effects of individual fatty acids on triglyceride metabolism may receive more attention.

Do dietary fats influence blood pressure? In ecological data from the Seven Countries Study, there was a positive correlation between dietary saturated fat and systolic blood pressure (12). Observational studies from populations with high intakes of dietary saturated fat indicate that saturated fat is positively associated (37-40) and polyunsaturated fat is inversely associated (38;39) with blood pressure. In reports from Japanese migrant (41) and US populations (42-44), however, there were no associations of dietary fat with blood pressure. Clinical trials provide some support that a low fat diet with high ratio of polyunsaturated to saturated fat decrease blood pressure (45-47). One review of animal and human studies concluded that n-6 polyunsaturated fatty acids decrease blood pressure in hypertensive individuals (48). In contrast, two reviewers of human studies, stated that n-6 polyunsaturated fatty acids do not influence blood pressure (49;50). While the present work was conducted, two meta-analyses concluded that EPA and DHA decrease blood pressure in individuals with hypertension or atherosclerotic disease (51;52).

The effects of EPA and DHA on atherogenesis and clinical disease

A great number of studies have been conducted to identify potential cardioprotective effects of EPA and DHA. Dietary supplementation studies in humans show that total cholesterol levels remain largely unchanged, serum triglycerides decrease, and blood pressure are lowered in selected groups (32;51;52). Experimental data indicate that EPA and DHA can have beneficial effects on atherogenesis (53). In clinical trials, however, dietary n-3 fatty acid supplementation had a modest beneficial effect on coronary artery graft patency (54), and restenosis rate after coronary angioplasty was unaffected (55;56). In the past years the possible antiarrhythmic effects of EPA and DHA have been focused (57). A recent secondary prevention trial (58) suggest that dietary supplementation with a small dose (1 gram daily) of DHA and EPA can protect against fatal cardiac arrhythmias. In conclusion, studies this far have pointed to several pathways by which EPA and DHA can influence cardiovascular disease development, but clinical trials have not demonstrated consistent effects on established cardiovascular risk factors.

Most studies of dietary n-3 fatty acid supplementation used mixtures of EPA and DHA. The studies varied with respect to the selection of study participants and their background diet, the duration of the supplementation period, the total daily dose of n-3 fatty acids, and the ratio of EPA to DHA provided in the dietary supplements. Thus, the question arose whether EPA and DHA have different pharmacological and physiological effects. When this work was conducted, the studies addressing separate effects of EPA and DHA were few and small (59-61), mainly because of is the limited availability of highly purified fatty acids.

Biomarkers of dietary fats

Dietary fat intake is difficult to measure. The effects of individual fatty acids on cardiovascular risk factors can be studied by supplementing the diet with the particular fatty acid of interest. An alternative approach, and feasible in population studies, is to investigate the relationship of blood levels of individual fatty acids with cardiovascular risk factors. Individual fatty acid composition can be determined in lipid subfractions of plasma, in erythrocyte and platelet membranes, and in adipose tissue. It is generally thought that adipose tissue reflects long term (years) dietary fatty acid intake, plasma triglycerides reflect short time intake (days), and the plasma cholesterol ester, phospholipids and blood cell membranes reflect medium term intake (weeks, months) (62). The plasma fractions differ with respect to the relative contributions of fatty acid classes and individual fatty acids. The phospholipid

fraction reflects dietary intake of very long chain n-3 fatty acids well (63;64), and was therefore chosen for fatty acid determinations in the present studies. Measurements of individual fatty acid concentrations are too complex and time consuming to be part of routine biochemistry in the near future, but can be feasible in population studies as measures of diet.

Extended knowledge about the effects of dietary fats on cardiovascular risk factors can enable us to refine dietary advice to high-risk individuals and to the general population. By combining the results from epidemiological and clinical studies with basic biomedical research, we may gain insight into the mechanisms by which dietary fats influence disease processes. These mechanisms can subsequently be targeted by interventions to lower the risk of disease.

2. AIM OF STUDY

This thesis focuses on the relationship of individual fatty acids in blood phospholipids with blood pressure, serum cholesterol, triglycerides and apolipoproteins. A clinical trial and a observational study address the following questions:

1. What are the effects of dietary supplementation with high doses (4 grams daily) of highly purified EPA and DHA on serum lipids (triglycerides, total, LDL and HDL cholesterol, apolipoprotein A and B), serum phospholipid fatty acid concentrations, blood pressure, heart rate and left ventricular performance in middle aged healthy men?

2. What is the cross-sectional relationship of plasma phospholipid fatty acids concentrations with blood pressure, serum triglycerides and total cholesterol among 40-42 year old men who participated in a population study?

3. SUBJECTS AND METHODS

3.1 The intervention trial

3.1.1 The source population

The third cardiovascular survey in Tromsø (Tromsø III) was conducted from August trough April 1986/87 by the University of Tromsø, Tromsø Health Council and the National Health Screening Service. The organization and conduct of the screening has been described in detail (65). The survey included residents of the municipality of Tromsø: All men born between 1925 and 1966, all women born between 1930 and 1966, a 10% random sample of youths born between 1967 and 1974 and the families of high risk men participating in a family intervention study. Of 28 847 invited subjects, 49 had died and 2 203 had moved or were living outside Tromsø during the screening period. Thus, the adjusted eligible population was 26 595, and 21 647 subjects (81.4%) participated.

The screening procedure included a questionnaire (Appendix I), measurements of height, weight, blood pressure and heart rate, and a non-fasting blood sample. The questionnaire covered previous and present cardiovascular disease, symptoms suggesting angina pectoris, coronary heart disease among first degree relatives, leisure time physical activity, the use of salt and fat in the diet, coffee consumption, smoking habits, and work situation. A nurse checked the questionnaire for incomplete items and logical inconsistencies at the screening, and also asked about time since last meal. Blood pressure and heart rate were measured automatically (66). The blood sample was analyzed for total cholesterol, HDL cholesterol and triglycerides at the Department of Clinical Chemistry, Institute of Medical Biology, University of Tromsø (66).

A second questionnaire (Appendix II) was handed out at the screening site and was returned by mail by 92.0% of the participants. Questions covered chronic diseases other than cardiovascular disease, the use of drugs and health care services, social support, diseases among first-degree relatives, and dietary habits including habitual fish consumption. Altogether 13 970 (adjusted eligible number) men were invited to Tromsø III, of whom 10 882 attended (77.9%) the survey and 9 997 (91.9%) returned the second questionnaire.

3.1.2 The study participants

In 1993 a randomized double blind placebo controlled study with parallel design was conducted to evaluate possible separate effects of highly purified EPA and DHA in healthy men. Study participants were recruited among 2 159 eligible men who attended Tromsø III (denoted screening in figures 2 and 3). Primary end points were blood pressure and serum triglyceride concentrations. The secondary end points were serum total cholesterol, HDL-cholesterol, apolipoprotein A-I, apolipoprotein B, plasma fibrinogen, coagulation factor VII and plasminogen activator inhibitor (PAI-I). The trial started with a 4-month run-in period. At baseline the participants were randomized to dietary supplementation with 3.8 g/day ethyl ester concentrate of EPA, 3.6 g/day ethyl ester concentrate of DHA, or 4g/day of corn oil (placebo) for seven weeks.

The study was conducted at the Institute of Community Medicine, University of Tromsø. At the first visit, a physician performed a clinical examination. At the third visit, a certified clinical nutritionist assessed the nutrient intake. At all other visits, the participants met fasting with the same investigator (a physician or a specially trained nurse), at the same hour of the day (between 8.10 and 11.05 am), and in the same room. The investigator measured blood pressure and obtained information on intercurrent disease, side effects and compliance. Afterwards the participants went to an adjacent room for drawing of a venous blood sample. Echocardiographic measurements were recorded at baseline and after the supplementation period in a subsample of participants. A cardiologist performed the measurements at the Regional Hospital in Tromsø, which is located nearby the university.

Figure 2 shows a flow diagram of the subjects and the reasons for exclusions and dropouts. The study design is outlined in figure 3, and table 1 displays the schedule of investigational events.

3.1.3 Selection of study participants

The inclusion criteria were as follows:

-Participant in Tromsø III (screening).

-Nonsmoking male, 35-55 years old in 1992.

-Non-fasting serum triglycerides < 2.5 mmol/L at screening and mean fasting serum triglycerides < 5.00 mmol/L during the run-in period.

-Serum total cholesterol < 8.00 mmol/L at screening and serum total cholesterol < 9.5 mmol/L during the run-in period.

-Diastolic blood pressure (DBP) < 95 mm Hg and systolic blood pressure (SBP) < 160 mm Hg at screening. DBP < 100 mm Hg and SBP < 170 mm Hg measured on two occasions during the run-in period, and the DBP difference between the two measurements < 15 mm Hg.

-Written informed consent.

The exclusion criteria were:

-Evidence of myocardial infarction, stroke, diabetes mellitus, liver or renal disease, bleeding disorder, or other disease that can affect blood pressure, lipid metabolism, or hemostasis, based on medical history, routine biochemistry or clinical examination.

-Symptoms suggesting angina pectoris or intermittent claudication.

-Regular use of prescribed or non-prescribed drugs.

-Consumption of more than three dishes of fish per week in the usual diet, or regular use of fish oil capsules or cod liver oil during the run-in period.

-Dieting.

-Mental illness, alcohol or drug abuse.

-Expected poor compliance for any reason.

-Expected or planned change in life-style, diet or physical activity the month before or during the intervention period.

3.1.4 Power calculations

The study was designed to have 90% power to detect a difference in change between two groups (by two-sample t-test) of 3.0 mm Hg diastolic blood pressure (assuming a standard deviation of 5.1 mm Hg) at a significance level of 5%. Based on these assumptions, a total of 61 evaluable subjects was needed in each of the three treatment groups ($n = [(z_\beta + z_\alpha)^2 \times (SD_1^2 + SD_2^2)] / \Delta\bar{x} = [(1.28 + 1.96)^2 \times (5.1^2 + 5.1^2)] / 3^2 = 61$). With 61 subjects in each treatment group, the study allowed detection of a 0.17 mmol/L difference between two groups in change in serum triglyceride concentration, (assuming a standard deviation of 0.28 mmol/L). The standard deviations were estimated from a pilot study and previous studies conducted at Institute of Community Medicine (67;68). A total of 234 subjects were randomized to allow for dropouts.

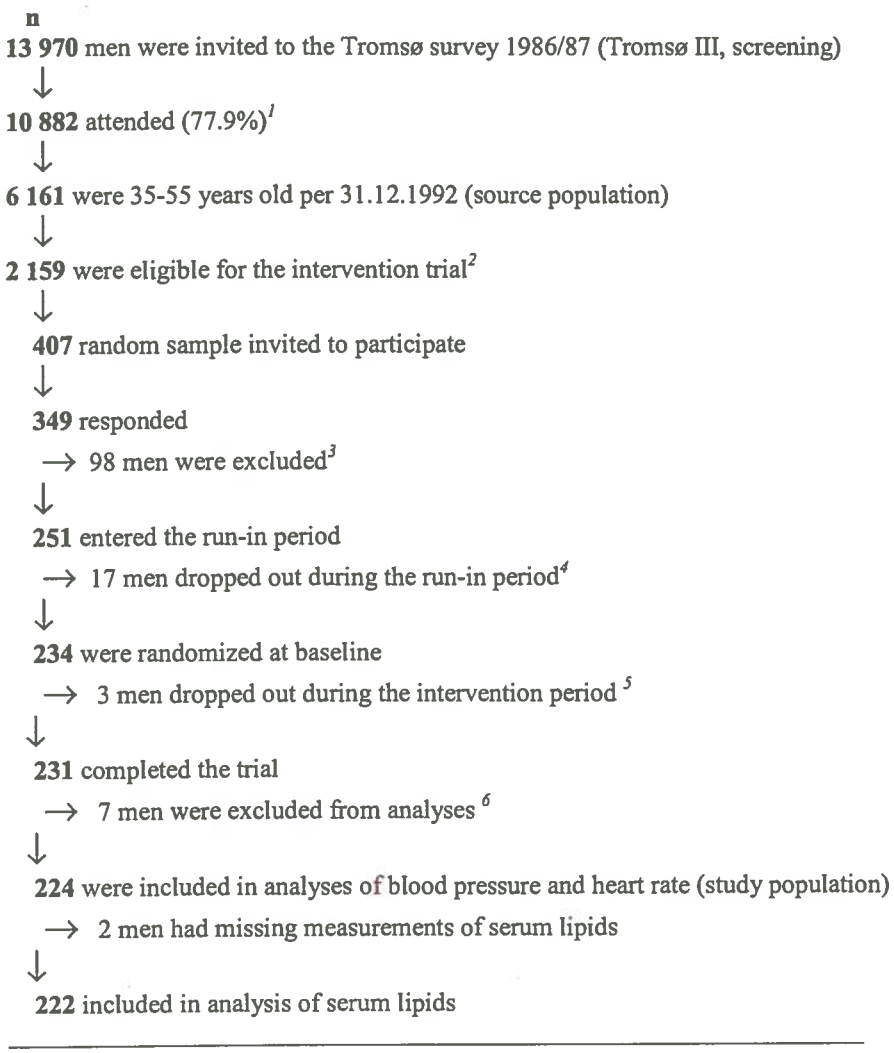
3.1.5 Randomization

The subjects were allocated to one of three treatment groups by computer generated random numbers in blocks of six. Professor Egil Arnesen (Institute of Community Medicine), who was unaware of the trial management, generated the numbers by using a computer program. He kept one code list, and mailed one code list to Pronova Biocare A/S, that was responsible for packing and labeling of the dietary supplements according to National Norwegian Guidelines. Three boxes each containing 75 capsules and labeled with identical randomization numbers were prepared for every participant. Pronova mailed a set of sealed envelopes each containing the individual randomization code to the investigator, in case of serious adverse event requiring the code to be broken during the trial. Pronova confirmed that the envelopes were still sealed upon trial termination. The participants received ascending randomization numbers with corresponding boxes in the sequence at which they met to visit 5, and the investigator wrote the randomization numbers in the case report forms. The participants returned leftover boxes and capsules to the investigator at the end of the trial.

3.1.6 Measurements

The methods for measurements of height, weight, blood lipids, serum phospholipid fatty acids and dietary assessment are presented in paper I. Methods for measurements of blood pressure, heart rate, echocardiography, routine biochemistry and plasma active renin are presented in paper II.

FIGURE 2. Men participating in a randomized clinical trial investigating the effects of highly purified EPA and DHA



[1] Of whom 9 997 returned questionnaire 2.

[2] Men who returned questionnaire 2 and had the following characteristics at screening: nonsmoker, systolic blood pressure < 160 mm Hg, diastolic blood pressure < 95 mm Hg, fish for dinner meals < 4 times weekly serum total cholesterol < 8.00 mmol/L and serum triglycerides < 2.5 mmol/L.

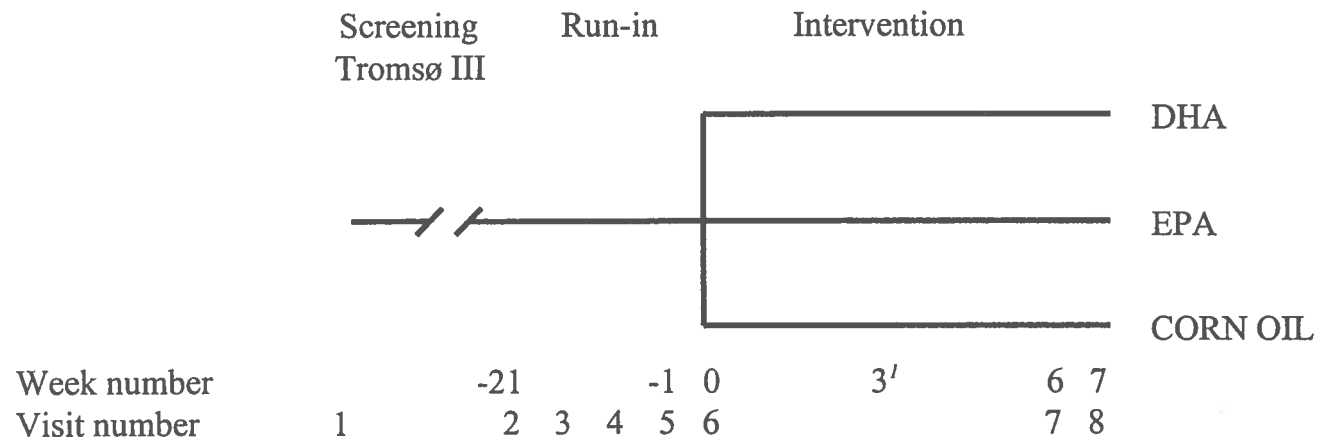
[3] Excluded before the run-in period: 31 men used fish for dinner or bread spread >3 times weekly, 20 used prescribed drugs, 20 were unable to participate due to work hours, 18 were smokers, 6 refused to participate, 1 did not agree to abstain from fish oil during the trial, 1 was a female and 1 dropped out for unknown reasons.

[4] Excluded during the run-in period: 7 men could not participate due to work hours, 2 used vasoactive drugs, 2 were smokers, 1 had decreased glucose tolerance, 1 had serum triglyceride levels > 2.5 mmol/L during the observation period, 1 consumed too much fish, 1 was on a waiting list for surgery, 1 withdrew and 1 dropped out for unknown reasons.

[5] Dropped out during intervention: 1 man withdrew due to abdominal discomfort (cholecystectomy), 1 suffered vertigo and vomiting and 1 developed diarrhea due to fish intolerance.

[6] Excluded from analyses: 3 changed their dietary habits and level of physical activity during the intervention period, 1 initiated vasoactive medication, 1 underwent cancer surgery, 1 had hematuria and 1 had irregular lifestyle and clinic visits.

FIGURE 3. Study design of a randomized clinical trial investigating the effects of highly purified EPA and DHA



[1] Telephone interview

TABLE 1. Schedule of investigational events in a randomized clinical trial investigating the effects of highly purified EPA and DHA

Visit number	1 [1]	2	3	4	5	6		7	8
Week number		-21			-1	0		6	7
Clinical examination		*							
Weight		*				*			*
Height	*	*							
Blood pressure/heart rate	*	*		*	*	*		*	*
Echocardiography [2]						*			*
Dietary history			*						
Dietary questionnaire						*			*
Side effects							* [3]		*
Compliance							* [3]		*
Capsule count									*
Serum									
Triglycerides	*	*		*	*	*		*	*
Total cholesterol	*	*			*	*		*	*
HDL-cholesterol	*				*	*		*	*
Apolipoprotein A-1					*	*		*	*
Apolipoprotein B					*	*		*	*
ALAT		*				*			*
ALP		*				*			*
GGT		*				*			*
Bilirubin						*			*
Albumin		*				*			*
Creatinine		*				*			*
Na^+, K^+		*				*			*
C- reactive protein		*				*			*
Fatty acid profile						*			*
Plasma									
Fibrinogen					*	*		*	*
Factor VII					*	*		*	*
PAI-1						*			*
Active renin						*			*
Glucose						*			*
Blood									
Hemoglobin						*			*
Blood cell count						*			*

[1] Visit 1 refers to screening (Tromsø III 1986/87)
[2] Subsample of 20 men from each treatment group
[3] Telephone interview

3.2 The observational study

The National Health Screening Service in Norway has since 1985 screened individuals aged 42-42 years in 18 out of 19 counties in Norway (69). The aims of the screening are to monitor cardiovascular disease risk, do epidemiological research, educate health care personnel, do primary prevention of cardiovascular disease through population and high-risk strategies and secondary prevention through early diagnosis, and finally to do supplementary studies.

The Nordland Health Study in 1988/89 was conducted in collaboration with the local health authorities. The design and conduct and has been described (70). All subjects aged 40-42 years, and all residents in a small fishing community aged 14-49 years were invited. In June 1989, a reminder was sent and a second visit was made in 12 selected areas where attendance rates were low. Altogether 10 497 individuals aged 40-42 years were invited, of whom 8 612 participated (82.0%).

The screening procedure was similar to that described for the Tromsø survey, and all other county surveys in Norway (71). A questionnaire (Appendix III) was included in the letter of invitation, and the participants handed it in at the screening site. A second questionnaire (Appendix IV) was handed out at the screening and 7 506 participants (87.2%) returned it by mail. The questions covered information about health and illness, diseases within the family, psychological problems, social network, use of health care services, work environment, physical activity, and dietary habits.

The methods for measurements of height, weight, blood pressure and heart rate are described in paper III. A non-fasting venous blood sample was analyzed for serum total cholesterol and serum triglyceride concentrations. Plasma phospholipid fatty acids were measured in a sub-study of the men, as described in paper IV.

We assessed the relationship of plasma phospholipid fatty acids with blood pressure, serum total cholesterol and triglycerides in a sub-study of the men aged 40-42 years. Altogether 5 492 men 40-42 years old were invited, 4 302 participated in the screening (78.3%), and 3 722 men (86.5%) returned the second questionnaire. Figure 4 shows a flow diagram of the men included in the study, and the reasons for exclusion from the analyses.

FIGURE 4. Men 40-42 years old participating in the Nordland Health Study 1988/89

n

5 492 men were invited to the Nordland Health Study

↓

4 302 attended (78.3%) [1]

→ 144 men had missing measurements of plasma phospholipid fatty acids [2]

↓

4 158 were included in analysis of relationship of plasma phospholipid fatty acids with serum triglycerides and total cholesterol (75.7%) [3]

→ 1 man had no blood pressure measurement

→ 19 men had previous myocardial infarction

→ 98 men used blood pressure medication

→ 7 men had both previous myocardial infarction and present blood pressure medication

↓

4 033 were included in analysis of relationship of plasma phospholipid fatty acids with blood pressure (73.4%) [4]

---

[1] Of whom 3722 returned questionnaire 2
[2] Of whom 6 men used blood pressure medication
[3] Of whom 3590 men returned questionnaire 2
[4] Of whom 3483 men returned questionnaire 2

4. RESULTS

Paper I

Dietary supplementation with 4 grams daily of highly purified DHA and EPA for seven weeks in a randomized double blind placebo controlled clinical trial showed that both fatty acids lowered serum triglyceride levels in healthy middle-aged men. Although not statistically significant, the net decreases in serum triglycerides were larger in the DHA group compared to the EPA group at all levels of baseline triglycerides. The results further indicated that EPA and DHA have separate effects on lipoprotein and fatty acid metabolism in humans. There was a slight increase in serum HDL cholesterol in the DHA-group. Small decreases in serum total cholesterol and apolipoprotein A-1 levels were observed in the EPA group. Serum phospholipid levels of both DHA and EPA increased in the DHA group, indicating that DHA is retroconverted to EPA (Figure 1). In the EPA group, serum phospholipid EPA increased and DHA levels decreased, indicating that EPA is not elongated to DHA in humans.

Paper II

Dietary supplementation with 4 grams daily of highly purified DHA decreased heart rate, whereas supplementation with 4 grams daily of EPA increased heart rate in a randomized double blind placebo controlled clinical trial. The changes in heart rate were associated with changes in serum phospholipid fatty acid concentrations of DHA and EPA. Blood pressure remained unchanged following dietary supplementation with DHA and EPA. Baseline blood pressure was positively associated with serum phospholipid concentrations of saturated fatty acids. A pooled analysis showed that left ventricular diastolic filling improved in the DHA and EPA groups. A decrease in serum phospholipid concentrations of saturated fatty acids was associated with improved left ventricular diastolic filling.

Paper III

Plasma concentrations of total fatty acids and saturated fatty acids showed positive linear associations with blood pressure in a cross-sectional analysis of 4033 men 40-42 years old in the Nordland Health Study. Polyunsaturated linoleic acid (18:2n-6) was inversely and linearly associated with blood pressure. The associations of plasma fatty acids with blood pressure were independent in multivariate regression analyses.

Paper IV

Individual plasma phospholipid fatty showed different associations with serum total cholesterol and non-fasting triglyceride levels in a cross-sectional analysis of 4158 men 40-42 years old in the Nordland Health Study. Linoleic (18:2n-6) was the only fatty acid inversely associated with total cholesterol. Very long chain (20 carbon atoms and more) saturated, monounsaturated and n-3 polyunsaturated fatty acids displayed significant inverse associations with non-fasting triglycerides, whereas shorter fatty acids within these classes were positively associated with triglycerides. The associations of serum triglycerides with individual plasma fatty acid concentrations depended both on fatty acid chain length and the degree of unsaturation.

5. DISCUSSION

5.1 Methodological considerations

The objective of the intervention study was to provide accurate estimates of the effects of dietary supplementation with highly purified EPA and DHA on blood lipids, blood pressure and heart rate in humans. The accuracy of a study depend on the precision (lack of random error in measurement and estimation) and the validity (lack of systematic error in estimation) of the study (72). Study precision can be improved by increasing study size (n), or by increasing size efficiency, which is the amount of statistical information from a given number of study subjects. Precision was attended to by actions taken to maximize size efficiency, since it is not feasible to include a large number of subjects in an intervention trial.

The validity of a study is usually separated into two components. Internal validity refers to whether results are valid ("true") pertaining to the study population in from which the data arose. Confounding, selection bias and information bias can detract from internal validity. External validity refers to whether the study results are applicable to groups other than the study population. The present study was a phase II trial (initial clinical investigation for treatment effect), in which internal validity was the main focus, rather than external validity. This priority had consequences for both the study design and how the measurements were obtained.

5.1.1 The intervention trial: subjects

Power calculations were performed to determine study size, which is the number of individuals needed to answer a specified research question. Study power is the statistical probability of detecting a difference between the groups under study, if there is one. The power of a study can be increased by 1) increasing the number of study subjects, or by 2) increasing the number of observations for each subject, or by 3) actions taken to decrease measurement error. All three strategies were applied in the present study.

Table 2. Calculated study power to detect protocol specified changes in the randomized clinical trial at p< 0.05 (two-sided) [1]

Primary end-point	DHA-group SD_1	EPA-group SD_2	Study group n	Power (%)
	mm Hg			
Diastolic BP				
Protocol estimate	5.1	5.1	61	90
One observation	6.3	5.8	72	85
Two observations	4.6	4.2	72	98
	mmol/L			
S-triglycerides				
Protocol estimate	0.28	0.28	61	90
One observation	0.42	0.45	72	65
Two observations	0.31	0.40	72	81

[1] According to protocol, and the use of one and two observations at baseline and at end of the study.

Table 2 shows the calculated protocol power and actual study power for the primary end-points (difference between groups of 3.0 mm Hg diastolic blood pressure and 0.17 mmol/L serum triglycerides). According to the protocol, 61 individuals were needed in each study group, and more than 70 men were included to allow for potential dropouts.

Power increased when the number of observations of each individual increased. Two observations of 72 individuals in each study group provided 98% power to detect the protocol specified change in diastolic blood pressure. It can be calculated from table 2 that a 90% increase in study size (n=137 per study group) was necessary to achieve identical power by the use of only one observation for each individual. Actual study power was less for serum triglycerides because the variability in change (SD) was larger than anticipated. Two observations of 72 individuals in each study group provided 81% power to detect the protocol specified change in serum triglyceride concentration. Calculations show a 47% increase in study size (n=106 per study group) was necessary to achieve identical power by the use of one observation for each individual. In conclusion, increasing the number of observations per individual from one to two (at baseline and at end of intervention) increased power more effectively than increasing study size.

The power calculations of the present study were based on a two-sample t-test of pairwise comparisons. We performed, however, a one-way analysis of variance, and contrasted groups

in the SAS general linear models procedure when the overall F-test p was less than 0.05 (Papers I and II). In the present study, these two analytical strategies yielded almost identical p-values, supporting that the power calculations were satisfactory.

We did not adjust for multiple comparisons (Papers I and II). There is no agreement among statisticians on whether or how to adjust for multiple comparisons in hypothesis testing. Strategies to adjust for multiple comparisons reduce the possibility of type I errors in statistical testing (reject a true null hypothesis), but simultaneously, the probability of type II errors (accept a false null hypothesis) increases. In other words, study power decreases. Rothman recommends not adjusting for multiple comparisons, because this will lead to fewer errors of interpretation when the data under evaluation are actual observations (73).

The size efficiency of a clinical trial can be improved by restricting eligibility criteria. This strategy controls confounding, but may reduce the generalizability of the study results. The source population, from which the study participants were recruited into the present study, were all men aged 35-55 years per 31.12.1992 and who were examined at Tromsø III (n=6161, figure 2 and table 3). Eligibility was restricted on the basis of blood pressure, serum triglycerides and total cholesterol concentrations. We also aimed to recruit men with moderate fish intakes, because a previous study indicated that the blood pressure lowering effect of DHA and EPA may be limited to individuals with low dietary intakes and blood levels of DHA and EPA (67). Nonsmokers were selected, because smokers showed larger variability in change in blood pressure and were less compliant compared with non-smokers in a previous intervention trial (65). Table 3 shows screening characteristics of the source and study population.

Table 3. Source and study population in the randomized clinical trial. Characteristics at screening (Tromsø III 1986/87)

Variable	Source population (n=6161)	Study population (n=224)
	Mean ±SD	
Systolic BP (mm Hg)	128.3 ± 13.0 [1]	127.4 ± 11.5
Diastolic BP (mm Hg)	76.6 ±10.2 [1]	75.2 ± 8.0
Total cholesterol (mmol/L)	5.93 ± 1.19 [2]	5.56 ± 0.97
HDL-cholesterol (mmol/L)	1.36 ± 0.34 [3]	1.35 ± 0.29
Non-fasting triglycerides (mmol/L)	1.66 ± 1.07 [2]	1.48 ± 0.75
Body mass index (kg/m^2)	24.8 ± 3.0 [1]	24.5 ± 2.4
Present smokers (%)	47	0
Leisure time physical activity (%) [4]		
Sedentary	24	20
Moderate	49	50
Active	27	30

[1] n=6158; [2] n=6153; [3] n=6152; [4] n=6160

The study population had a slightly lower mean diastolic blood pressure, serum total cholesterol and serum triglyceride levels than the source population from which they were recruited (by 2%, 6% and 11% respectively) (Table 3). Leisure time physical activity level was somewhat higher than observed in the source population. They consumed less fat in their usual diet than reported for Norwegian households in 1991-92 (30% vs. 34% of total energy from fat) (Paper I) (74). Their baseline dietary intakes of DHA and EPA were higher than reported among US (64) and Dutch (75) men, and comparable to Norwegian participants in clinical trials (76).

The run-in period lasted for approximately 4 months and served several purposes. It was a washout period for the men who used fish oil supplements when they were recruited into the trial. The run-in period gave the investigator an opportunity to confirm that inclusion criteria were present and exclusion criteria were absent, and allowed for dropout of non-compliant individuals. Finally, the run-in period controlled for regression to the mean. When repeated measurements of some variable result in values drifting towards the mean of the distribution, the phenomenon is called regression to the mean. It is a common problem in clinical trials and it is most frequently encountered if individuals are sampled at the upper (or lower) end of the population frequency distribution of the variable. Some of the high (or low) values among

these individuals are due to random intra-individual and measurement variation of the variable.

Table 4. Blood pressure and serum triglyceride concentration during the run-in period in the randomized clinical trial (n=224)

Variable	Visit 2	Visit 4	Visit 5
		Mean ± SD	
Diastolic BP (mm Hg)	77.8 ± 8.0	77.2 ± 7.4	77.2 ± 7.8
S-triglycerides (mmol/L)	1.29 ± 0.70	1.25 ± 0.58 [1]	1.25 ± 0.63 [2]

[1] n=197; [2] n=222

Table 4 shows that there were negligible changes in major end-points (diastolic blood pressure and serum triglyceride concentration) during the run-in period. In conclusion, regression to the mean was not operating in the clinical trial, mainly because the study participants were sampled within the normal range of the serum triglyceride and blood pressure distributions. The lack of regression to the mean could also be expected from the data in table 3, showing that the trial participants were fairly representative of the source population from which they were recruited.

The purpose of randomization is to ensure comparability of the study groups. Randomization is the main strategy, but no guarantee, to avoid confounding in a clinical trial. Confounding can be thought of as a mixing of the effect of the exposure (e.g. study group receiving highly purified EPA) on the outcome (e.g. diastolic BP) with that of a third factor (e.g. physical activity). The third factor (physical activity) must be associated with exposure (EPA-group), and independent of the exposure, be associated with the outcome (BP). Thus, confounding is in essence a confusion of effects, which can distort internal validity. The DHA, EPA and corn oil groups were well balanced with respect to baseline characteristics (Paper I), indicating that the randomization was successful. Confounding may also be introduced during the intervention period, however. To prevent confounding, all study participants were asked to maintain their life styles during the course of the whole trial. The men were monitored with respect to factors known to influence serum lipids and blood pressure. As a result, seven individuals were excluded from the analyses (Figure 2). In summary, it seems unlikely that confounding was introduced at baseline or throughout the intervention period of the clinical trial.

Several measures were taken to ensure compliance. We tried to select study participants who were highly motivated. They received a schedule for their clinic visits, and a written reminder prior to the final two visits. A telephone interview was conducted in the middle of the intervention period to monitor compliance and side effects. Participants were offered an economical compensation for loss of work-hours upon finishing the trial, but the amount (NOK 500) was probably too small to influence compliance. Measures of compliance were based on determination of serum fatty acids, the number of capsules returned at the end of intervention, and a self report of number of missed doses (Paper I).

The analyses were restricted to the 224 men who adhered to the protocol ("per protocol analysis"), rather than the 234 men who were randomized ("intention to treat analysis"). The former strategy was chosen because the scope of the study was to assess the efficacy (effect under ideal conditions) of highly purified DHA and EPA on the study end-points. This approach can introduce selection bias in a clinical trial, if non-adherence to the protocol is unevenly distributed between the study groups. Ten men who were excluded from the analyses were evenly distributed in the study groups (DHA n=3; EPA n=4; corn oil n=3), thus the choice of analytical strategy did not introduce selection bias.

The generalizability of study results (external validity) was not the main focus in the present study. The participants were healthy and fairly representative of the source population from which they were recruited (Table 3). Strictly, the results of the present clinical trial applies to healthy middle aged men consuming a western diet with relatively high levels of n-3 fatty acids. There are no data indicating that sex interact with dietary n-3 fatty acid supplementation in the effects on blood pressure and blood lipid levels. It seems plausible that the study results apply also to women, who with hindsight could have been included in the clinical trial.

5.1.2 The intervention trial: measurements

Measurement error can hamper the precision of a study. In the clinical trial, we made considerable effort to reduce measurement errors due to 1) the subjects being examined, 2) the instruments being used for measurements, and 3) the observers. The quality of a measurement rests on its reliability and validity. Reliability refers to whether a measurement is reproducible (repeatable), whereas validity refers to the correctness of the measurement.

Both the primary end-points, diastolic blood pressure and serum triglycerides, show considerable intra-individual variation (77;78). Blood pressure shows circadian variation (79), and we therefore examined the study participants at the same time at each visit during the

intervention period. After the run-in period they were familiar with the observer and the standardized measurement procedure during which three recordings were made. Intra-individual serum triglyceride levels vary considerably with time since last meal, and they are also influenced by alcohol consumption. The trial participants were asked to fast for 12 hours, and to abstain from alcohol for 48 hours prior to clinic visits. Non-fasting men were asked to return to the clinic the following day for a fasting blood sample. Minor dietary intakes were allowed for (up to one glass of semi-skimmed milk), and these men were categorized as non-fasting. Thus, 92-99% of the men gave fasting blood samples, and 87-89% abstained from alcohol for 48 hours prior to clinic visits at baseline and throughout the intervention period. The protocol deviations were uncommon and the frequency of deviations did not differ between the three study groups. It is unlikely that non-fasting and alcohol consumption among study participants introduced measurement error of serum triglycerides that biased the study results.

Each participant was assigned to one observer and one Dinamap^{TM} automatic blood pressure recorder throughout the study. The Dinamap is used in many trials evaluating effects of blood pressure interventions. A search on Dinamap in the Medline database 1990 through 1999 gave 129 hits, indicating that it is frequently used. Regrettably, the Dinamap recorder received low grades when evaluated by the British Hypertension Society (80). The deviations from manually recorded blood pressure with a standard mercury sphygmomanometer were larger for diastolic, compared to systolic blood pressure. The article states that accuracy was better in the low blood pressure range (<130/80 mm Hg), which applies to most men in the present trial (mean blood pressure recorded at baseline was 122/77 mm Hg). A report from the Norwegian County Studies concluded that the Dinamap measured slightly lower diastolic blood pressure when compared to a sphygmomanometer (81). In the present study, calibration of the two Dinamap recorders showed that there was no drift in measurements during the trial. This observation supports the reliability and hence the validity of the blood pressure results in the clinical trial.

Table 5. Baseline blood pressure and heart rate by observers and Dinamap recorders in the randomized clinical trial

Variable	Observer 1 Machine 1 (n=119)	Observer 2 Machine 2 (n=105)
	mean ± SD	
Systolic BP (mm Hg)	120.6 ± 9.9	124.2 ± 9.8 [1]
Diastolic BP (mm Hg)	76.5 ± 7.5	77.7 ± 7.4
Heart rate (bpm)	63.3 ± 9.2	63.5 ± 7.1
Body mass index (kg/m^2)	24.6 ± 2.7	25.5 ± 2.8 [2]

[1] $p<0.01$ vs. observer 1; [2] $p<0.05$ vs. observer 1

Standardized and automatic blood pressure recordings were important to reduce observer bias. Table 5 shows that observer 2 (nurse) measured significantly higher systolic blood pressure than observer 1 (physician) at baseline. The difference persisted throughout the trial (data not shown). After adjustment for differences in body mass index by those who were measured by observers 1 and observers 2, there was no significant difference between systolic blood pressures measured by the two observers. It is therefore unlikely that observer bias influenced measurement validity.

Routine blood chemistry, i.e. blood tests of liver and kidney function, electrolytes, hemostatic variables, C-reactive protein, blood glucose, hemoglobin and blood cell count were measured at baseline and at end of intervention (Table 1). The blood tests of kidney function are presented in paper II. The remaining data are presented in tables 6 and 7. Overall, the changes in routine blood chemistry were small and clinically insignificant. Serum alkaline phosphatase (ALP) levels decreased by 4% and 5% in the EPA and DHA groups respectively, significantly different from the corn oil group (Table 6). Reduced ALP levels after dietary supplementation with DHA and EPA has been observed previously (65). Serum albumin decreased by 1% in the EPA group, significantly different from the DHA group. Factor VII increased by 4% in the corn oil group, significantly different from the EPA and DHA groups. In all three study groups there were small changes in blood cell count, that probably reflect laboratory drift (Table 7). In the EPA group (relative to DHA and corn oil groups), there were

minor decreases in blood hemoglobin, erythrocytes and hematocrit that were statistically significantly different from the DHA and corn oil groups.

It is beyond the scope of this presentation to discuss the effects of n-3 polyunsaturated fatty acids on hemostasis and fibrinolysis. Nevertheless, in this group of healthy men, no clinically significant effects of highly purified EPA and DHA were observed on fibrinogen, factor VII, platelet count (Tables 6 and 7) or PAI-1 activity (82).

Table 6. Blood tests at baseline and change after 7 weeks of dietary supplementation with highly purified DHA, EPA or corn oil

	DHA (n=72)		EPA (n=75)		CORN (n=77)			Contrasts between groups p		
Variable	Baseline	Change	Baseline	Change	Baseline	Change	F-test P [1]	DHA vs. EPA	DHA vs. CORN	EPA vs. CORN
Serum										
ALAT (U/L) [2]	24.5 ± 10.4 [3]	3.8 ± 10.3 [4]	25.7 ± 9.4	1.5 ± 10.1	21.0 ± 8.3	2.1 ± 6.4 [4]	0.3			
ALP (U/L) [6]	163 ± 45	-7 ± 17 [4]	161 ± 33	-8 ± 17 [6]	150 ± 40	-2 ± 15.0	0.04	0.6	0.06	0.01
GGT (U/L) [7]	33.5 ± 27.5	0.2 ± 12.6	32.9 ± 21.5	-1.5 ± 15.9	26.3 ± 15.6	0.5 ± 6.5	0.6			
Bilirubin (µmol/L)	10.6 ± 4.8	0.5 ± 2.8	12.2 ± 6.0	-0.1 ± 3.3	13.0 ± 6.8	-0.1 ± 3.4	0.4			
Albumin (g/L)	45.2 ± 1.6	0.3 ± 1.4	45.3 ± 2.1	-0.6 ± 1.5 [6]	45.2 ± 2.0	-0.3 ± 1.4	0.0004	0.0001	0.009	0.2
C-reactive protein (mg/L)	5.00 ± 0.00	0.03 ± 0.24	5.00 ± 0.00	0.67 ± 5.43	5.49 ± 3.10	-0.49 ± 3.10	0.2			
Plasma										
Glucose (mmol/L)	5.23 ± 0.44	0.04 ± 0.29	5.24 ± 0.46	0.09 ± 0.39	5.22 ± 0.43	0.02 ± 0.32	0.5			
Fibrinogen (g/L)	3.15 ± 0.58	-0.11 ± 0.45 [8]	3.25 ± 0.53	-0.09 ± 0.65	3.27 ± 0.76	-0.20 ± 0.59 [4]	0.4			
Factor-VII (%)	112 ± 25	1 ± 12	115 ± 24	-2 ± 13	117 ± 26	5 ± 11 [6]	0.005	0.3	0.03	0.001
PAI-1 (IU/ml) [9]	7.87 ± 6.02	1.07 ± 6.84	8.40 ± 6.07	2.36 ± 6.29 [4]	6.88 ± 5.23	1.49 ± 5.68 [8]	0.4			

[1] ANOVA for between-group comparisons of change; [2] Alanine aminotransferase; [3] $\bar{x}$ ± SD; [4,6,8] One-sample t-test of difference between baseline and 7 wk: [4] $p < 0.01$, [6] $p < 0.001$, [8] $p < 0.05$; [5] Alkaline phosphatase; [7] Gamma-glutamyl transferase; [9] Plasminogen activator inhibitor type 1. See also (82)

Table 7. Blood cell count, hemoglobin and platelets at baseline and change after 7 weeks of dietary supplementation with highly purified DHA, EPA or corn oil

Variable	DHA (n=72)		EPA (n=75)		CORN (n=77)		F-test P [1]	Contrasts between groups p		
	Baseline	Change	Baseline	Change	Baseline	Change		DHA vs. EPA	DHA vs. CORN	EPA vs. CORN
Leucocytes (10^9/L)	5.49 ± 1.29 [2]	-0.18 ± 1.08	5.47±1.16	0.08 ± 1.03	5.50 ± 1.40	-0.26 ± 1.28	0.2			
Granulocytes (%)	51.3 ± 8.2	-1.3 ± 6.5	52.1 ± 7.4	0.4 ± 7.6	51.0 ± 8.5	-0.5 ± 9.1	0.5			
Lymphocytes (%)	34.8 ± 7.0	1.4 ± 5.4 [3]	35.0 ± 7.0	-0.3 ± 6.4	35.4 ± 8.0	0.0 ± 7.3	0.3			
Monocytes (%)	9.36 ± 2.09	-0.30 ± 1.42	9.15 ± 2.13	-0.42 ± 1.57 [3]	9.44 ± 1.82	-0.04 ± 1.67	0.3			
Eosinophils (%)	3.70 ± 2.11	0.03 ± 1.29	2.93 ± 1.76	0.21 ± 1.45	3.34 ± 2.21	0.33 ± 2.24	0.6			
Basophils (%)	0.82 ± 0.43	0.16 ± 0.48 [4]	0.86 ± 0.43	0.16 ± 0.63 [3]	0.79 ± 0.43	0.18 ± 0.46 [4]	0.9			
Hemoglobin (g/dl)	15.0 ± 0.8	0.0 ± 0.5	15.1 ± 0.8	-0.2 ± 0.5 [5]	14.9 ± 0.8	0.0 ± 0.5	0.005	0.002	0.6	0.009
Erythrocytes (10^{12}/L)	4.97 ± 0.35	0.05 ±0.21 [3]	4.99 ± 0.28	-0.04 ± 0.18	4.94 ± 0.33	0.05 ± 0.19 [3]	0.004	0.004	0.9	0.004
Hematocrit (%)	43.5 ± 2.4	0.7 ± 1.9 [4]	43.7 ± 2.6	0.0 ± 1.7	43.1 ± 2.3	0.7 ± 1.9 [4]	0.03	0.02	0.9	0.02
MCV (fl) [6]	87.5 ± 3.7	0.5 ± 1.2 [5]	87.6 ± 3.0	0.7 ± 1.2 [5]	87.4 ± 4.1	0.5 ± 1.3 [5]	0.4			
MCH (pg) [7]	30.2 ± 1.4	-0.3 ± 0.6 [5]	30.2 ± 1.1	-0.2 ± 0.6 [4]	30.3 ± 1.7	-0.3 ± 0.6 [5]	0.2			
MCHC (g/dl) [8]	34.5 ± 0.7	-0.5 ± 0.8 [5]	34.5 ± 0.8	-0.5 ± 0.8 [5]	34.6 ± 0.9	-0.6 ± 0.8 [5]	0.7			
Platelets (10^9/L)	250 ± 54	-11 ± 29 [5]	252 ± 52	-11 ± 25 [5]	253 ± 49	-8 ± 22 [4]	0.7			
Mean platelet volume (fl)	8.57 ± 0.94	-0.09 ± 0.50	8.54 ± 0.70	-0.14 ± 0.37 [4]	8.57 ± 0.94	-0.05 ± 0.43	0.4			

[1] ANOVA for between-group comparisons of change; [2] $\bar{x}$ ± SD; [3-5] One-sample t-test of difference between baseline and 7 weeks: [3] $p < 0.05$, [4] $p < 0.01$, [5] $p < 0.001$;
[6] Mean corpuscular volume; [7] Mean corpuscular hemoglobin; [8] Mean corpuscular hemoglobin concentration

5.1.3 The observational study: subjects

A cross-sectional study design can be used to describe associations between exposures and outcomes, and to generate hypotheses. In the Nordland Health Study in 1988/89, a sub-study was originally planned to be a prospective study of the relationship between plasma fatty acid concentrations and cardiovascular disease. Due to financial constraints, limited capacity for analyses, and because men are at higher risk of developing cardiovascular disease than women, men were chosen for the study.

Study size is an important contributor to the precision of a population study, where (compared to clinical trials) less effort can be taken to reduce measurement error. The large sample size provided power to examine thoroughly the cross-sectional associations of plasma fatty acid concentrations with blood pressure and serum triglycerides and total cholesterol.

The overall attendance rate of 78% was acceptable. Some non-attenders lived temporarily outside their home, had died, or moved out of the county before or during the screening period (70). In the Tromsø III survey in 1986/87, the adjusted eligible number was 5.5% less than the crude number of men 40-44 years old who were invited (65). Applied on the Nordland Health Study, these data give an adjusted attendance rate of 83% (5492 invited, 5190 adjusted eligible men, 4302 attended).

Self-selection bias can operate in population studies, and the direction, but not the magnitude can be indicated. In the Oslo Study, attenders had lower incidence of coronary heart disease and were more likely to be married compared to non-attenders (83). In the Norwegian county studies, death rates from most causes including coronary heart disease and hypertension were lower among attenders compared to non-attenders (84). In the Nordland Health Study, attenders were more likely to be married than non-attenders, and there were no differences in attendance rates between rural and urban areas (70). Previous studies indicate that an adverse cardiovascular risk profile is more frequent among non-attenders compared to attenders. We have no information about phospholipid fatty acid levels among non-attenders, but it is unlikely that the associations under study were different among non-attenders. The analyses of the associations of plasma phospholipid fatty acids with blood pressure and serum lipids were adjusted for potential confounding factors (Papers III and IV). In multivariate analyses n was restricted to the men who returned questionnaire 2. Table 8 shows that these men had slightly lower levels of serum total cholesterol, total plasma phospholipid fatty acids, 16:1 and 20:3n-9, and slightly higher levels of 20:5n-3 and 22:6n-3 compared with non-responders. Exclusion of the non-responders to questionnaire 2 did not alter the associations under study.

Table 8. Blood pressure, serum total cholesterol, triglycerides and selected fatty acids according to response rate to questionnaire 2. The Nordland Health Study 1988/89, 4158 men 40-42 years old

Variable	Returned questionnaire 2	
	Yes (n=3590)	No (n=568)
	mean ± SD	
Systolic BP (mm Hg) [1]	133 ± 14	133 ± 15
Diastolic BP (mm Hg) [1]	80.5 ± 10.0	81.2 ± 10.3
s-Total cholesterol (mmol/L)	6.10 ± 1.15	6.22 ± 1.20 [2]
s-Triglycerides (mmol/L)	1.97 ± 1.22	2.03 ± 1.24
Individual fatty acids (mol%)		
16:0	26.6 ± 1.5	26.7 ± 1.6
18:0	13.9 ± 0.9	13.9 ± 1.0
22:0	1.87 ± 0.58	1.87 ± 0.59
16:1	0.37 ± 0.18	0.39 ± 0.20 [2]
22:1	0.15 ± 0.13	0.16 ± 0.12
24:1	1.40 ± 0.54	1.37 ± 0.51
20:3n-9	0.11 ± 0.07	0.12 ± 0.07 [2]
18:2n-6	23.4 ± 3.7	23.6 ± 3.6
20:3n-6	2.46 ± 0.67	2.46 ± 0.66
20:5n-3	2.41 ± 1.79	2.25 ± 1.51 [2]
22:6n-3	6.57 ± 1.68	6.37 ± 1.63 [3]
Total fatty acids (µmol/L) [4]	4522 ± 683	4616 ± 737 [3]

[1] n=3589; [2] $p<0.05$ vs. men who returned questionnaire 2
[3] $p<0.01$ vs. men who returned questionnaire 2
[4] Total fatty acids include 14:0, 16:0, 18:0, 20:0 22:0, 24:0, 16:1, 18:1 20:1, 22:1, 24:1, 20:3n-9, 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3

Measurements of plasma phospholipid fatty acids were missing for 144 men. The reasons for missing data were difficulties in drawing an extra blood sample for preparation of plasma, or because samples were lost or damaged during transport or analysis. Men for whom fatty acid measurements were missing did not differ from men for whom measurements were present with respect to baseline characteristics (data not shown). It is unlikely that loss of these men in the analyses influenced the relationship of plasma fatty acids with blood pressure, serum triglycerides and total cholesterol.

Previous myocardial infarction and current blood pressure medication can theoretically confound the relationship of fatty acids with blood pressure. Both conditions influence blood pressure and may also influence dietary habits and fatty acid levels. Altogether 124 men with previous myocardial infarction, present blood pressure medication, or both were excluded from the blood pressure analyses (Figure 4). These men accounted for only 3% of the eligible population (n=4158), however, and including them in the analyses did not influence the associations under study.

The possibility of confounding was discussed in papers III and IV, specifically related to residual confounding by physical activity and alcohol consumption. Time since last meal may potentially confound the relationship of individual plasma phospholipid fatty acids with blood pressure and serum lipids. Time since last meal was associated with both fatty acid levels, blood pressure and serum lipids (Table 9). Concentrations of fatty acids believed to reflect dietary saturated fat (16:0, 16:1, 20:3n-9 and 20:3n-6) and DHA increased, whereas 18:2n-6 concentration decreased significantly with time since last meal. Diastolic blood pressure and serum total cholesterol increased, and serum triglycerides decreased significantly according to time since last meal. The observed associations of plasma phospholipid fatty acids with blood pressure, serum total cholesterol and triglycerides did not change in adjusted or in stratified analyses, thus time since last meal was not a confounder in the present data.

Table 9. Mean of selected plasma phospholipid fatty acids, blood pressure, serum total cholesterol and serum triglycerides according to time since last meal. The Nordland Health Study 1988/89, 4158 men 40-42 years old

TSSM[6] (hours)	n	16:0	22:0	16:1	18:1	22:1	24:1	20:3n-9	18:2n-6	20:3n-6	22:6n-3	Total FA[1]	SBP[2]	DBP[3]	t-Chol[4]	TG[5]
		(mol%)										(µmol/L)	(mmHg)		(mmol/L)	
0	850	26.6	1.87	0.36	8.44	0.15	1.39	0.10	23.9	2.43	6.45	4535	132	79.2	6.04	2.11
1	1204	26.6	1.86	0.37	8.50	0.15	1.36	0.11	23.8	2.45	6.42	4524	133	80.1	6.07	2.08
2	792	26.6	1.86	0.37	8.45	0.15	1.36	0.11	23.7	2.48	6.46	4556	133	80.8	6.12	2.04
3	509	26.6	1.86	0.39	8.57	0.15	1.42	0.12	23.1	2.47	6.72	4517	132	81.0	6.14	1.88
4	375	26.8	1.87	0.39	8.65	0.14	1.45	0.12	22.9	2.46	6.65	4550	134	82.8	6.16	1.75
5	178	26.7	1.93	0.36	8.50	0.14	1.44	0.11	23.0	2.44	6.84	4528	133	81.8	6.27	1.60
6	75	27.0	1.88	0.41	8.76	0.15	1.49	0.12	21.9	2.53	6.68	4443	134	83.5	6.05	1.40
7	22	27.1	1.68	0.45	8.86	0.14	1.36	0.13	23.2	2.44	6.23	4555	138	84.2	6.36	1.26
8	11	27.1	1.76	0.41	8.72	0.15	1.44	0.13	19.8	2.54	7.81	4766	133	82.0	6.89	1.82
9	128	27.2	1.94	0.44	8.70	0.15	1.51	0.12	20.9	2.61	7.16	4596	133	81.6	6.65	1.66
Missing	14	26.3	1.79	0.44	8.77	0.17	1.29	0.13	22.3	2.64	7.16	4354	134	84.1	5.89	2.30
Total	4158	26.6	1.87	0.37	8.52	0.15	1.39	0.11	23.4	2.46	6.54	4535	133	80.6	6.12	1.98
p-values																
Equality[7]		0.0001	0.6	0.0001	0.03	0.6	0.01	0.004	0.0001	0.3	0.0001	0.8	0.5	0.0001	0.0001	0.0001
Trend[8]		0.0001	0.4	0.0001	0.0006	0.5	0.003	0.0001	0.0001	0.001	0.0001	0.5	0.14	0.0001	0.0001	0.0001

[1] See table 8 for individual fatty acids included in total fatty acids; [2] Systolic blood pressure; [3] Diastolic blood pressure; [4] Serum total cholesterol; [5] Serum triglycerides; [6] Time since last meal; [7] F-test for difference between groups (TSSM); [8] F-test for linear trend

An intermediate variable is one that occurs on the causal pathway from the exposure to the outcome. Adjustment for an intermediate variable in the analysis will erroneously attenuate the association between exposure and outcome. There are data indicating that the type of dietary fat influence weight gain in animals (85;86). If so, BMI is an intermediate variable in the relationship of dietary fats (as expressed in plasma phospholipid fatty acids) with blood pressure and serum lipids. On the other hand, BMI may by itself influence plasma phospholipid fatty acid concentrations. BMI is a powerful determinant of blood pressure (87), and it seems unlikely that the association between BMI and blood pressure rely solely on dietary fat. It can thus be argued that BMI is both an intermediate and a confounder in the relationship of individual plasma fatty acids with blood pressure and serum lipids. We presented both unadjusted and BMI-adjusted analyses in papers III and IV.

The results of the present cross-sectional analysis applies to middle aged men with relatively high intakes of very long chain polyunsaturated n-3 fatty acids. There are data indicating that for each level of reported dietary fat intake, women have lower levels (mol%) of plasma saturated fatty acids and higher levels of 18:2n-6 compared with men (63;64). The differences may be related to differential reporting of dietary intake, and we can speculate that sex influence fatty acid metabolism. Thus, the study results need confirmation in women and other age groups.

5.1.4 The observational study: measurements

In the Nordland Health Study three automatic blood pressure recordings (Dinamap^{TM}) were made at one occasion. The lowest blood pressure recording was used in the analysis, because this was considered to be closest to the "usual" blood pressure. We can also reduce blood pressure measurement error by using the mean of two or more recordings, which reduces within-person variation.

Table 10. Blood pressure variability according to one and two recordings. The Nordland Health Study 1988/89, 4033 men 40-42 years old

Blood pressure recording	Systolic BP (mm Hg)	CV [1] (%)	Diastolic BP (mm Hg)	CV (%)
Lowest recording	132.6 ± 14.0	10.6	80.4 ± 10.0	12.4
Mean of recording 2 and 3	135.6 ± 14.0	10.3	82.3 ± 9.9	12.0

[1] Coefficient of variation

Table 10 shows that the blood pressure variability (expressed by the coefficient of variation, CV) was similar whether one or the mean of two blood pressure recordings was the unit of analysis. Crude and multivariate associations between plasma phospholipid fatty acids and blood pressure remained unchanged when the analysis was performed on the mean of recording 2 and 3 as compared to the lowest recorded blood pressure.

5.2 Plasma phospholipid fatty acids

While some clinical trials evaluating effects of dietary interventions with DHA and EPA reported absolute concentrations (μmol/L, mg/L) of plasma fatty acids (56;67;88), studies relating plasma fatty acids to diet have focused relative concentrations (mol%) (63;64;89). In the clinical trial (Papers I and II) we addressed absolute concentrations of individual fatty acids, under the assumption that it was the absolute number of fatty acid molecules, rather than relative contribution of a particular fatty acid which was important. In the population study (Paper III) we evaluated the associations of both absolute and relative concentrations of fatty acids with blood pressure. Absolute concentrations displayed larger variability (Table 11), and stronger associations with blood pressure than did relative concentrations of fatty acids (Table 12). Absolute concentrations of most fatty acids were intercorrelated, and highly correlated with total fatty acids (Table 13). Thus, independent associations of absolute concentrations of individual fatty acids with any variable are hampered by collinearity problems. Absolute concentrations of saturated fatty acids in particular will reflect total fatty acids. The intercorrelations between relative concentrations of individual fatty acids were weaker (Table 13).

Table 11. Absolute (μmol/L) and relative (mol%) concentrations of selected plasma phospholipid fatty acids.
The Nordland Health Study 1988/89, 4033 men 40-42 years old

	Absolute conc.		Relative conc.	
Fatty acid	Mean ± SD	CV [1]	Mean ± SD	CV
	μmol/L		*mol%*	
Saturated fatty acids [2]	1996 ± 314	16	44.1 ± 1.3	3
Palmitic, 16:0	1206 ± 203	17	26.6 ± 1.5	6
Stearic, 18:0	628 ± 104	17	13.9 ± 0.9	7
Monounsaturated fatty acids [3]	486 ± 108	22	10.7 ± 1.3	12
Palmitoleic, 16:1	17.3 ± 10.5	61	0.37 ± 0.18	49
Oleic acid, 18:1	387 ± 93	24	8.52 ± 1.24	15
Eicosatrienoic acid, 20:3n-9	5.13 ± 3.58	70	0.11 ± 0.07	64
n-6 fatty acids [4]	1571 ± 273	17	34.8 ± 3.6	10
Linoleic, 18:2n-6	1059 ± 211	20	23.5 ± 3.6	15
Dihomo-γ-linolenic, 20:3n-6	112 ± 38	34	2.45 ± 0.67	27
Arachidonic, 20:4n-6	356 ± 82	23	7.87 ± 1.40	18
n-3 fatty acids [5]	465 ± 164	35	10.3 ± 3.3	32
Linolenic, 18:3n-3	8.43 ± 4.59	54	0.18 ± 0.09	50
Eicosapentaenoic, 20:5n-3	107 ± 81	76	2.37 ± 1.73	73
Docosahexaenoic, 22:6n-3	294 ± 85	29	6.52 ± 1.66	25
Total fatty acids [6]	4523 ± 682	15		

[1] Coefficient of variation
[2] Saturated fatty acids include 14:0, 16:0, 18:0, 20:0, 22:0, 24:0
[3] Monounsaturated fatty acids include 16:1, 18:1, 20:1, 22:1, 24:1
[4] n-6 fatty acids include 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6
[5] n-3 fatty acids include 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3
[6] See table 8 for fatty acids included in total fatty acids

Table 12. Relationship of systolic and diastolic blood pressure with absolute and relative concentrations of selected plasma phospholipid fatty acids.
The Nordland Health Study 1988/89, 4033 men 40-42 years old [1]

	Systolic BP		Diastolic BP	
Fatty acid	µmol/L	mol%	µmol/L	mol%
16:0	0.24	0.09	0.29	0.16
18:0	0.19	-0.03	0.21	-0.03
16:1	0.22	0.17	0.26	0.21
18:1	0.19	0.06	0.23	0.10
20:3n-9	0.14	0.10	0.19	0.14
18:2n-6	0.07	-0.12	0.05	-0.17
20:3n-6	0.18	0.09	0.26	0.18
20:4n-6	0.16	0.02	0.20	0.05
18:3n-3	0.09	0.02	0.08	0.00
20:5n-3 [2]	0.12	0.07	0.10	0.05
22:6n-3	0.15	0.03	0.15	0.02
Total fatty acids [3]	0.23		0.25	

[1] Pearson correlation coefficients. $|r| > 0.13$, 0.15 and 0.18 are significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively
[2] Logtransformed
[3] See table 8 for individual fatty acids included in total fatty acids

Table 13. Relationship between levels of selected plasma phospholipid fatty acids. The Nordland Health Study 1988/89, 4158 men 40-42 years old [1]

	Fatty acid													
Fatty acid	16:0	18:0	22:0	16:1	18:1	24:1	20:3n-9	18:2n-6	20:3n-6	20:4n-6	18:3n-3	20:5n-3 [2]	22:6n-3	Total fatty acids [3]
16:0		-0.60	-0.14	0.46	0.23	0.06	0.19	-0.35	0.09	0.05	-0.12	0.02	-0.02	0.10
18:0	0.77		-0.12	-0.32	-0.16	-0.18	-0.10	0.14	0.12	-0.03	0.03	-0.02	0.01	0.02
22:0	0.29	0.30		-0.27	-0.31	0.41	-0.17	0.04	-0.15	-0.04	-0.12	-0.14	-0.12	-0.05
16:1	0.66	0.39	-0.02		0.60	-0.17	0.53	-0.35	0.32	0.05	0.30	0.01	-0.08	0.33
18:1	0.78	0.65	0.10	0.75		-0.30	0.59	-0.09	0.24	-0.03	0.18	-0.24	-0.32	0.20
24:1	0.32	0.22	0.49	0.04	0.06		-0.20	-0.46	-0.24	-0.05	-0.19	0.36	0.36	-0.04
20:3n-9	0.50	0.39	0.04	0.62	0.69	-0.01		-0.26	0.45	0.31	0.06	-0.13	-0.26	0.27
18:2n-6	0.49	0.62	0.28	0.12	0.44	-0.13	0.13		-0.08	-0.26	0.17	-0.65	-0.62	-0.15
20:3n-6	0.58	0.59	0.11	0.51	0.56	0.00	0.57	0.34		0.37	0.00	-0.39	-0.34	0.17
20:4n-6	0.61	0.58	0.20	0.39	0.48	0.17	0.52	0.24	0.61		-0.20	-0.18	-0.18	-0.02
18:3n-3	0.32	0.35	0.04	0.42	0.40	-0.05	0.20	0.37	0.21	0.09		-0.06	-0.14	0.10
20:5n-3 [2]	0.28	0.25	-0.01	0.15	0.08	0.42	0.01	-0.30	-0.13	0.05	0.05		0.78	0.06
22:6n-3	0.44	0.44	0.08	0.21	0.19	0.46	0.02	-0.14	0.05	0.18	0.07	0.79		-0.05
Total fatty acids [3]	0.94	0.91	0.37	0.56	0.77	0.32	0.47	0.62	0.60	0.64	0.38	0. 29	0.47	

[1] Numbers to the left of the diagonal are Pearson correlation coefficients between absolute concentrations (µmol/L), and numbers to the right of the diagonal are Pearson correlation coefficients between relative concentrations (mol%) of plasma phospholipid fatty acids. $|r|>0.13$, 0.15 and 0.18 are significant at $p<0.05$, $p<0.01$ and $p<0.001$ respectively

[2] Logtransformed; [3] µmol/L, see table 8 for individual fatty acids included in total fatty acids

Papers III and IV discuss the extent to which plasma phospholipid fatty acid concentrations reflect dietary fat intake. Generally, plasma phospholipid concentrations of essential polyunsaturated fatty acids reflect dietary intake well (63;64). Concentrations of saturated and monounsaturated fatty acids reflect both dietary intake and endogenous synthesis, and correlate less with dietary fatty acids. In the Nordland Health Study, saturated fatty acids concentrations displayed less variability than did monounsaturated and polyunsaturated fatty acids (Table 11), indicating that levels of saturated fatty acids are metabolically regulated. There are data indicating that concentrations of monounsaturated (63;64) and certain polyunsaturated fatty acids (20:3n-9, 20:3n-6) (90;91) may reflect dietary saturated fat.

In the clinical trial, we performed a dietary interview (dietary history, visit 3) 4 months prior to baseline measurements of fatty acid profiles (Paper I). The interview was specially designed to record dietary n-3 polyunsaturated fatty acids, but offers an opportunity to examine the relationship of dietary fatty acids with levels of serum phospholipid fatty acids. Table 14 displays the relationship between dietary fat (% of total fat) and serum concentrations (mol%) of selected fatty acids.

Table 14. Relationship of dietary fatty acids with serum phospholipid fatty acid concentrations among 224 men 35-55 years old[1]

	Serum phospholipid fatty acids (mol%)						
Dietary fat (% of total fat)	16:0	16:1 [2]	20:3n-6	18:2n-6	20:5n-3 [2]	22:6n-3	Total fatty acids (µmol/L)[3]
Saturated fat	0.00	0.06	0.14	-0.02	-0.04	0.02	0.03
16:0	0.02	0.15	0.19	-0.13	-0.02	0.03	0.12
18:2n-6	-0.01	-0.16	-0.13	0.14	-0.01	-0.07	-0.09
20:5n-3 [2]	0.07	0.05	-0.26	-0.23	0.37	0.43	0.21
22:6n-3 [2]	0.06	0.05	-0.25	-0.24	0.38	0.43	0.24

[1] Pearson correlation coefficients. $|r|>0.13$, 0.15 and 0.18 are significant at $p<0.05$, $p<0.01$ and $p<0.001$ respectively.
[2] Logtransformed
[3] Total fatty acids include 16:0, 18:0, 20:0, 22:0, 24:0, 16:1, 18:1, 20:1, 22:1, 24:1, 22:2, 18:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.

Dietary intake of 16:0 was positively and significantly associated with relative concentrations of 16:1 and 20:3n-6 (20:3n-9 was not measured). The data support the

assumption that levels of 16:1 and 20:3n-6 (mol%) partly reflect dietary saturated fat. Dietary intake of 18:2n-6 was also significantly associated with serum 18:2n-6 (mol%). In contrast, dietary saturated fatty acids and 18:2n-6 were not correlated with serum absolute concentrations (μmol/L) of 16:1, 20:3n-6 and 18:2n-6 respectively. The associations between dietary intakes (% of total fat), and serum (mol%) concentrations of DHA and EPA were stronger than reported in paper I (r= 0.39 and r=0.35 respectively, dietary grams/day vs. serum μmol/L). These observations are in agreement with others (76), and support that it is relative rather than absolute concentrations of phospholipid fatty acids, which reflect dietary fat intake.

Other researchers have pointed out that relative concentrations of serum fatty acids indicate relative patterns in intake and do not provide information about absolute differences in fatty acid intake (92). Another problem with relative concentrations is that if dietary intake and blood concentration of one fatty acid increase, the concentrations of some other fatty acids must decrease.

In conclusion, relative concentrations of individual phospholipid fatty acids reflect diet and are not highly intercorrelated. Although relative concentrations of individual fatty acids require careful interpretation, it is the measure of choice when evaluating independent associations of fatty acids with some variable.

5.3 General discussion

The clinical trial evaluated the effects of dietary supplementation with high doses of individual fatty acids on cardiovascular risk factors (Papers I and II). Cross-sectional associations of plasma levels of individual fatty acids with blood pressure and serum total cholesterol and triglycerides were examined in the population study (Papers III and IV). These are different approaches to study the impact of individual fatty acids on cardiovascular risk factors, but the two studies agree on many results.

Dietary supplementation with DHA and EPA lowers serum triglyceride levels (32). DHA had a more pronounced triglyceride-lowering effect than EPA in the clinical trial (Paper I). The finding was new, and opposed studies in rats which indicated that EPA is responsible for the triglyceride lowering effect of very long chain n-3 polyunsaturated fatty acids (93). The results of the clinical trial find support in the population study, where plasma DHA displayed a stronger inverse relationship with serum triglyceride levels than did EPA concentrations (Paper IV). The population study further showed that very long chain saturated and

monounsaturated fatty acids were inversely associated with serum triglycerides (Paper IV). These associations are to our knowledge not described previously. They give rise to the hypothesis that both chain length and the degree of unsaturation determine the association of saturated, monounsaturated and n-3 polyunsaturated fatty acids with serum triglyceride levels. Very long-chain monounsaturated fatty acids are abundant in fish oil (25) and we may speculate that they contribute to the triglyceride lowering effect of fish oil.

EPA and DHA differed in their effects on total- and HDL cholesterol in the clinical trial (Paper I). The effects were modest, and on the basis of this and previous research (32), it seems unlikely that changes in blood cholesterol levels contribute substantially to potential cardioprotective effects of very long chain n-3 fatty acids. The population study showed that plasma phospholipid 18:2n-6 levels were inversely associated with total cholesterol concentrations and agrees with numerous studies showing that increasing dietary 18:2n-6 decreases total cholesterol levels in blood (31). The associations of plasma phospholipid fatty acids with blood cholesterol levels may have been more instructive if HDL cholesterol concentrations were available. Regrettably, HDL cholesterol was not measured in the Nordland Health Study.

The clinical trial showed that heart rate increased in the EPA supplementation group, and decreased in the DHA group (Paper II). The changes were small, but consistent, and find support in a recent study (94). In the population study plasma DHA (mol%) concentration was significantly and inversely associated with heart rate (r=-0.04, p=0.009), whereas EPA concentration was not associated with heart rate. Cell and animal studies indicate that very long chain n-3 polyunsaturated fatty acids have antiarrhythmic effects (57;95). Fish consumption may protect against cardiac arrhythmias in humans (96;97). Secondary prevention studies suggest that dietary fatty fish (24), dietary linolenic acid (18:3n-3) (98), and a modest dose of EPA and DHA protect against cardiac arrhythmias (58). The present work indicates that DHA and EPA have different effects on heart rate. Whether EPA and DHA also differ with respect to antiarrhythmic effects in humans deserves further investigation.

The observational study provided enough power to examine the associations of palmitic (16:0), palmitoleic (16:1) and dihomo-γ-linolenic (20:3n-6) acid with blood pressure (Paper III). These fatty acids were positively associated with blood pressure in previous studies (46;99;100), and paper III can be the first report showing that all three fatty acids may reflect dietary saturated fat. Plasma levels of saturated fat were positively associated, whereas plasma 18:2n-6 levels were inversely associated with blood pressure (Paper III), and the associations

were independent in multivariate analyses. The regression model predicted a 1.4 mm Hg decrease in systolic blood pressure if plasma 16:0 levels decreased by 2 SD, and a further 1.9 mm Hg decrease if plasma 18:2n-6 increased by 2 SD (model I). The changes predicted by the model are larger than blood pressure reductions observed after sodium restriction in individuals with high normal blood pressure (101). Our findings of the positive associations of plasma phospholipid 16:0, 16:1, 20:3n-6, and inverse associations of 18:2n-6 with blood pressure are supported in a large prospective study which was recently published (102). Whether the associations of plasma fatty acids with blood pressure reflect a causal relationship of dietary fats with blood pressure is not clear. If so, these findings can have public health implications. Thus, the hypothesis that blood pressure decreases on a diet low in saturated fat and high in 18:2n-6 should be further investigated in a prospective or experimental study design.

Polyunsaturated 18:2n-6 is the main constituent of corn oil, which is generally considered to be neutral in its effect on blood pressure (103). In the clinical trial (Papers I and II), as in many other trials evaluating the effects of very long-chain n-3 fatty acids on blood pressure (51), corn oil supplements were used as placebo. The supplements provided 2.2 grams of 18:2n-6 daily, corresponding to a calculated 20% increase of dietary 18:2n-6 in the corn oil group (Paper I). Serum phospholipid 18:2n-6 levels and blood pressure did not change in the corn oil group after intervention. These observations can reflect that the relative pattern of dietary fatty acid intake remained largely unchanged in the corn oil group. It is also possible that the relationship between dietary 18:2n-6 and plasma phospholipid levels of 18:2n-6 is nonlinear in this range of dietary intake. Finally, there are data suggesting that a potential blood pressure lowering effect of dietary 18:2n-6 depends on the background diet (104).

In both the clinical trial and in the observational study, there were no consistent associations of very long chain n-3 polyunsaturated fatty acids with blood pressure (Papers II and III). Both studies agree with previous findings that EPA and DHA do not influence blood pressure levels in normotensive individuals (51;52). An alternative explanation for the lack of association relates to the 'threshold hypothesis', stating that dietary supplementation with n-3 fatty acids decrease blood pressure among hypertensive individuals whose initial plasma n-3 levels are low (67;105). In the present work, the participants of both studies displayed relatively high levels of plasma phospholipid n-3 fatty acids. Thus, if there is a threshold, most individuals may have levels well over the limit at which n-3 fatty acids are associated with blood pressure.

Is research on effects of individual fatty acids useful to examine the diet-heart hypothesis? Dietary fat intake is difficult to assess, and blood levels of individual fatty acids can be used to study the effects of dietary fats on cardiovascular risk factors and disease development. If the goal is to give dietary advice to the public however, this approach has limitations. The determinants of plasma fatty acids concentrations, and how dietary changes affect plasma fatty acids are poorly understood. It is documented that blood levels of essential fatty acids reflect dietary intake, but less is known about levels of nonessential fatty acids. We need validation studies of blood levels of individual fatty acids. It would be of great interest to know the determinants of total fatty acids concentrations, which displayed strong associations with blood pressure in the present observational study.

A further complication is that most food items are composed by a mixture of fatty acids and other nutrients that may have distinct and interacting effects on cardiovascular risk, and food composition tables suffer from poor and incomplete data (15). With these limitations in mind, we should focus on the effects of food items (fish, nuts, fruit and vegetables) rather than individual fatty acids, if the goal of research is to generate dietary advice aimed to prevent cardiovascular disease. Very long chain n-3 polyunsaturated fatty acids may be an exception because they are available in a well-defined and limited number of food items. Dietary supplementation regimens for specific effects can easily be designed with DHA and EPA.

If the goal of research is to generate hypotheses and identify mechanisms by which dietary fats influence atherosclerosis and cardiovascular risk factors, then research on individual fatty acids may be rewarding. If the mechanisms of disease development are known, they may be targeted by interventions. In this context, dietary n-3 fatty acids have been most extensively studied to identify the mechanisms by which they can prevent coronary heart disease (27).

Observational and intervention studies reported cardioprotective effects of small amounts of dietary fish (19-21;24;96;97;106), or small dose of DHA and EPA (58). The hypothesis that there is a "threshold of effect" has been related to both the hypotensive and the antiarrhythmic effects of n-3 polyunsaturated fatty acids. In Norway, dietary intake of very long chain n-3 fatty acids is relatively high (0.9 grams daily in a nation-wide survey in 1993-94) (107), and has probably increased as their potential cardioprotective effects received public attention. In the Tromsø surveys, 21% of the men 30-60 years old used fish oil periodically in 1986/87 (Tromsø III, n=6 481), whereas in 1994/95 the corresponding percentage was 43% (Tromsø IV, n=6 397). Dietary DHA and EPA accumulate in human adipose tissue (108), which may serve as a reservoir with a long half-life. It may be too late to conduct studies on

cardioprotective effects of n-3 fatty acids in Norway because dietary n-3 fatty acid intake in the general population has exceed the threshold at which beneficial effects can be detected.

CONCLUSION

A randomized double blind placebo controlled trial of dietary supplementation with highly purified EPA and DHA for seven weeks showed that both fatty acids decreased serum triglyceride levels in healthy middle aged men. Blood pressure did not change in any treatment group, but left ventricular diastolic filling improved in the EPA and DHA groups. EPA and DHA had different effects on heart rate and lipoprotein metabolism. In the EPA-group, heart rate increased, and there were small decreases in serum total cholesterol and apolipoprotein A1. In the DHA-group, heart rate decreased, and serum HDL cholesterol increased slightly. Analyses of serum phospholipid fatty acids indicated that EPA is not elongated to DHA, but DHA is retroconverted to EPA.

In a cross-sectional analysis of 4033 men aged 40-42 years, blood levels of individual fatty acids within fatty acid classes displayed different associations with blood pressure, serum triglycerides and total cholesterol concentrations. Plasma phospholipid saturated and total fatty acids were positively associated, whereas levels of polyunsaturated 18:2n-6 was inversely associated with blood pressure. Linoleic (18:2n-6) was the only fatty acid inversely associated with total cholesterol. Very long chain saturated, monounsaturated and n-3 polyunsaturated fatty acids displayed inverse relations to serum triglyceride levels. Both fatty acid chain length and the degree of unsaturation were associated with serum triglycerides.

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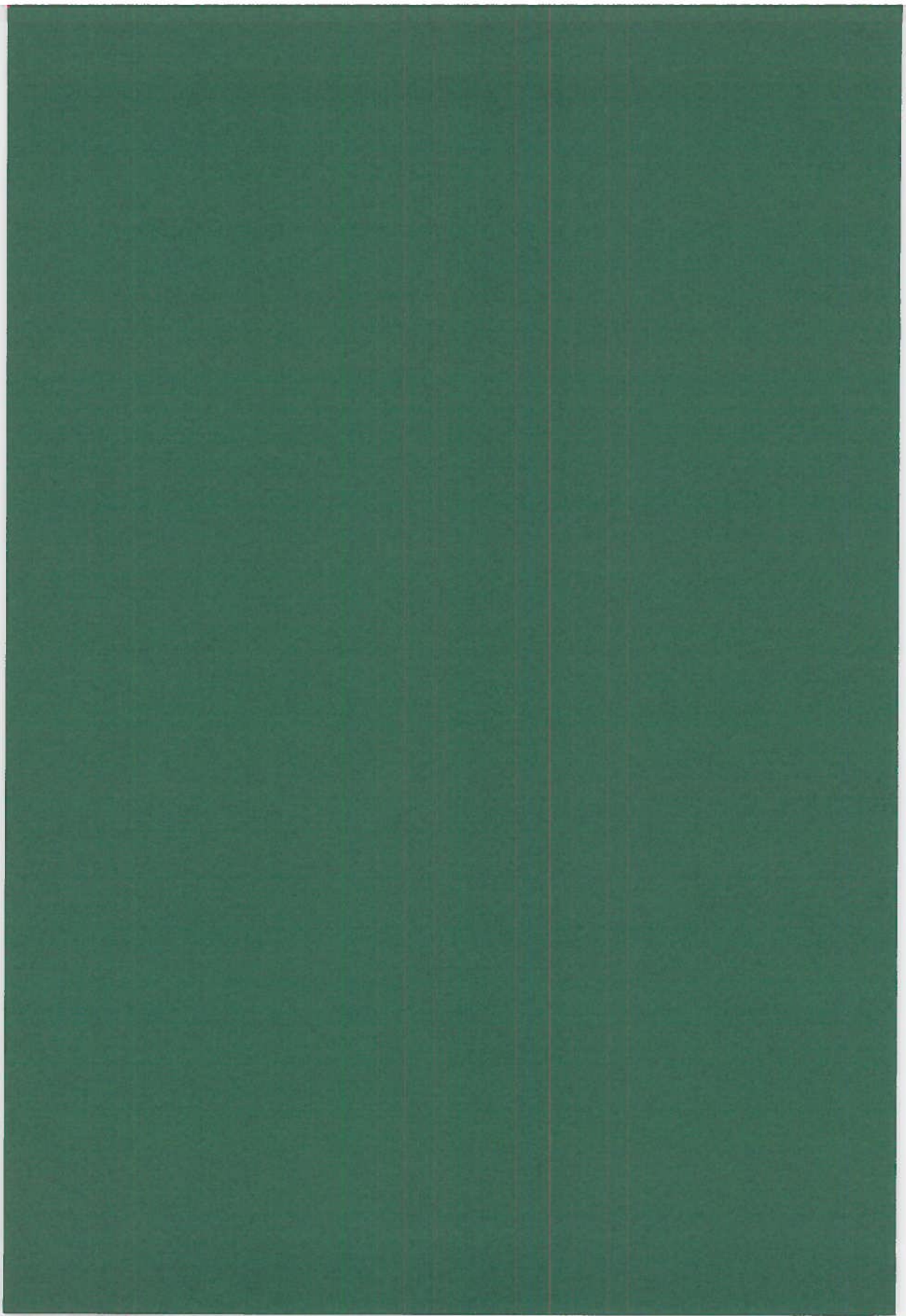
PAPER I



Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids[1–3]

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ABSTRACT To compare the effects of highly purified ethyl ester concentrates of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on serum lipids, apolipoproteins, and serum phospholipid fatty acids in humans, we conducted a double-blind, placebo-controlled, parallel design intervention study. Healthy nonsmoking men (n = 234) aged 36–56 y were randomly assigned to dietary supplementation with 3.8 g EPA/d, 3.6 g DHA/d, or 4.0 g corn oil/d (placebo) for 7 wk. Serum triacylglycerols decreased 26% ($P < 0.0001$) in the DHA group and 21% ($P = 0.0001$) in the EPA group compared with the corn oil group. Although not significant, net decreases in serum triacylglycerols were consistently greater in the DHA group across all quartiles of baseline triacylglycerol concentrations. Serum high-density-lipoprotein cholesterol increased 0.06 mmol/L ($P = 0.0002$) in the DHA group. In the EPA group, serum total cholesterol decreased 0.15 mmol/L ($P = 0.02$) and apolipoprotein A-I decreased 0.04 g/L ($P = 0.0003$). In the DHA group, serum phospholipid DHA increased by 69% and EPA increased by 29%, indicating retroconversion of DHA to EPA. In the EPA group, serum phospholipid EPA increased by 297% whereas DHA decreased by 15%, suggesting that EPA is not elongated to DHA in humans. The serum phospholipid ratio of n−3 to n−6 fatty acids increased in both groups, whereas the relative changes in n−6 fatty acids suggested possible alterations in liver desaturation activity in the DHA group. We conclude that both DHA and EPA decrease serum triacylglycerols, but have differential effects on lipoprotein and fatty acid metabolism in humans. *Am J Clin Nutr* 1997;66:649–59.

KEY WORDS Fatty acids, n−3 fatty acids, eicosapentaenoic acid, docosahexaenoic acid, triacylglycerols, phospholipids, randomized controlled trials

INTRODUCTION

Accumulating evidence indicates that fish oil, rich in eicosapentaenoic acid (EPA; 20:5n−3) and docosahexaenoic acid (DHA; 22:6n−3) of the n−3 family, can modify a variety of cellular processes associated with lipid metabolism, atherosclerosis, hypertension, thrombosis, and inflammation (1). The amount and the ratio of DHA to EPA in different marine sources vary considerably (1, 2). Earlier studies of n−3 fatty acid supplementation in humans used oils varying in dosage form, total dose of fatty acids, and relative content of DHA and EPA. Examination of these data shows that the most consistent effect of n−3 fatty acids on cardiovascular disease risk factors is a reduction in serum triacylglycerol concentration, whereas reported effects on other variables are less consistent (3–5). It is possible that the inconsistencies derive from chance findings in small-scale studies or differences in study design. However, they may also be attributed to varying metabolic effects of DHA and EPA.

Animal studies showed that EPA and DHA accumulate in different compartments in the body and thus may be subject to differences in both metabolism and effects (6–8). DHA selectively attenuated expression of proatherogenic and proinflammatory proteins in human endothelial cells, suggesting a beneficial effect of DHA on atherosclerosis (9), whereas EPA may be a more potent platelet inhibitor than DHA (10, 11). In vitro studies indicate that EPA and DHA have different effects on triacylglycerol synthesis (12), and it was suggested that EPA is primarily responsible for the hypotriacylglycerolemic effect of n−3 fatty acids both in rats (13) and humans (14). The extent to which these reports can be generalized is constrained by limitations in study design, however. Knowledge of the specific effects of EPA and DHA is needed to target n−3 supplements for specific effects. Long-term studies with adequate sample size comparing the biological effects of pure DHA and EPA in human volunteers have not been reported (10, 14–16). We therefore conducted a double-blind, randomized, placebo-controlled, parallel design intervention study to evaluate effects of dietary supplementation with highly purified EPA or DHA on serum lipids, apolipoproteins, and serum phospholipid fatty acid composition.

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SUBJECTS AND METHODS

Subjects and experimental design

In 1986–1987, 21 826 subjects, 81.3% of the men aged 20–61 y old and the women aged 20–56 y old living in the municipality of Tromsø, participated in a health survey (visit 1) (17). All subjects completed a questionnaire about previous disease, use of drugs, and diet and smoking habits, and their height, weight, blood pressure, and nonfasting serum lipid concentrations were measured. Four hundred seven men between the ages of 35 and 55 were selected according to the following criteria: they reported being healthy nonsmokers, did not use nonprescribed or prescribed drugs, and consumed less than four fish dishes per week in their usual diet. They also had serum cholesterol concentrations < 8.0 mmol/L, diastolic blood pressure < 95 mm Hg, and systolic blood pressure < 160 mm Hg. These men were then asked in 1993 to undergo a clinical examination that included a complete medical history, physical examination, and laboratory tests.

Among the 349 men who responded to the invitation, 251 subjects filled the above-mentioned criteria and were recruited into the present study. They had no cardiovascular, liver, or renal disease; bleeding disorder; diabetes mellitus; psychopathologic disease; alcoholism; or other disease that can influence blood pressure, lipid metabolism, or hemostasis. They were not consuming special diets and did not expect to change their diet or lifestyle during the study period. Their mean (± SD) age was 44 ± 5 y (range: 36–56). The study was approved by the regional board of research ethics, and each subject gave informed consent.

The study was performed according to Good Clinical Practice requirements (18). It began with a 4-mo run-in period during which subjects were asked to continue their usual diet and living habits and during which their blood pressure and fasting serum lipid concentrations were measured on two occasions (visit 2 and visit 3). Each subject's average intake of nutrients was calculated on a fourth visit. At the beginning of the run-in period and throughout the study, participants were instructed not to ingest cod liver oil or other fish-oil supplements.

For entry into the intervention phase of the study, a subject's mean serum triacylglycerol concentration during the run-in period had to be < 5.0 mmol/L and mean serum cholesterol concentration < 9.5 mmol/L. Among the 251 subjects, 2 were smokers, 2 had serum glucose or triacylglycerol concentrations above the inclusion criteria, 2 used cardiovascular drugs, 1 consumed more than three fish dishes per week, and 10 dropped out during the run-in period for personal reasons. Thus, 234 men entered the double-blind, parallel group intervention trial, which lasted for 7 wk. Computer-generated random numbers were used to assign the participants to either 4.0 g 95% ethyl ester EPA/d, 4.0 g 90% ethyl ester DHA/d, or 4.0 g corn oil/d. The dietary supplements were administered in indistinguishable soft gelatin capsules that each contained 1.0 g oil and 4–6 IU vitamin E as an antioxidant (**Table 1**). Each individual was asked to ingest two capsules in the morning and two capsules at night. The dietary supplements were manufactured by Pronova Biocare AS, Oslo.

Participants were examined after an overnight fast between 0800 and 1130 on two separate occasions separated by an interval of 3–5 d, both at baseline (visits 5 and 6) and after 7 wk of supplementation (visits 7 and 8). At each visit blood pressure was measured and blood samples were collected. Participants were asked to abstain from alcohol and strenuous exercise for 48 h before the visit. A telephone interview was performed in the middle of the intervention period to monitor study compliance, side effects, and intercurrent disease. Compliance was assessed by counting leftover capsules and was calculated as the percentage of the prescribed capsules taken. We also measured serum phospholipid fatty acid concentrations at baseline and at the end of intervention.

TABLE 1
Composition of dietary supplements[1]

Constituent	DHA	EPA	Corn oil
22:6n−3 Ethyl ester (mg)	889	12	0
20:5n−3 Ethyl ester (mg)	18	941	0
18:2n−6 (mg)	0	0	559
18:1n−9 (mg)	0	0	259
Vitamin E (IU)	4–6	4–6	3.7
p-Anisidine value	<35	<35	—
Peroxide value (mmol/g)	<0.01	<0.01	—

[1] Dietary supplements were given in indistinguishable, oblong, soft gelatin capsules of 1.4 g average weight. DHA, docosahexaenoic acid supplement; EPA, eicosapentaenoic acid supplement.

Clinical and laboratory measurements

Height was measured during the run-in period and weight was measured at baseline and after the intervention period on an electronic scale with subjects wearing light clothing and no shoes. Before the intervention each subject's habitual nutrient intake was assessed during a 1-h interview by a certified clinical nutritionist using the dietary history method. Food models and containers were used to estimate quantities. Dietary constituents were calculated from standard food tables that also cover individual fatty acids by using a specially designed computer program (19–22). Each subject completed a self-administered questionnaire at baseline and during the last week of the intervention to monitor food habits and physical activity during the intervention. Participants were asked how many times they ate fish or meat for dinner and how many units of alcohol they consumed during the past week (one unit of alcohol equals 9 g). Those who reported being physically active four or more times weekly for ≥ 20 min, leading to sweating or shortness of breath, were categorized as active; those reporting 1–3 times weekly were categorized as moderately active; and those reporting 0 times weekly were categorized as sedentary.

Blood samples were drawn from an antecubital vein into an evacuated tube system using minimal stasis. Serum was prepared by clotting whole blood in a glass tube (Becton Dickinson, Meylan Cedex, France) at room temperature for 1 h and then centrifuging the sample at 2000 × *g* for 15 min at 22 °C. One-milliliter aliquots of serum were transferred into sterile 2-mL cryovials (Corning, Park Ridge, IL), flushed with nitrogen, and stored at −70 °C. Blood for plasma preparation was collected into vacutainers (Becton Dickinson) containing 0.129 mol sodium citrate/L (blood:anticoagulant = 10:1). Plasma was prepared by centrifugation at 2000 × *g* for 15 min at 22 °C, transferred into sterile cryovials in aliquots of 1 mL,

flushed with nitrogen, and stored at −70 °C. All blood samples were analyzed after completion of the intervention period and before the randomization code was broken.

Serum lipids were analyzed on a Hitachi 737 Automatic Analyzer (Boehringer Mannheim, Mannheim, Germany) with reagents from the manufacturer. Total cholesterol was measured with an enzymatic colorimetric method (CHOD-PAP) and high-density-lipoprotein (HDL) cholesterol was assayed by the same procedure after precipitation of lower-density lipoproteins with heparin and manganese chloride. Serum triacylglycerol concentrations were determined with an enzymatic colorimetric test (GPO-PAP). Low-density-lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula (23). Apolipoprotein A-I and apolipoprotein B-1 were measured immunochemically by rate nephelometry using the Array Protein System from Beckman Instruments Inc (Brea, CA).

Fatty acids were measured by extracting total lipids from 500 μL serum according to Folch et al (24), with phosphatidylcholine diheptadecanoyl added as an internal standard (P-5014; Sigma Chemical Company, St Louis), chloroform:methanol (2:1, by vol) as a solvent, and butylated hydroxytoluene (75 mg/L) as an antioxidant. Total phospholipids were separated by solid-phase extraction with NH_2 columns (size 3 cc; Analytiche Bond Elut LRC; Varian, Harbour City, CA) (25), followed by transmethylation with boron trifluoride, extraction into hexane, and evaporation to dryness. The fatty acid methyl esters were dissolved in hexane and analyzed by gas-liquid chromatography (Shimadzu GC-14 A; Shimadzu Corporation, Kyoto, Japan) fitted with a capillary column (CP-Sil 88; length: 50 m, internal diameter: 0.25 mm) obtained from Chrompack Inc (Raritan, NJ). Retention times and response factors for each fatty acid were determined using standards obtained from Nu-Chek Prep (Elysian, MN). The results were integrated on a Shimadzu C-R4A integrator. Fatty acid concentrations are reported as μmol fatty acid/L serum.

Statistical analysis

All results are expressed as means ± SDs. On examination of the frequency distributions, all variables except serum triacylglycerol and certain lifestyle factors such as level of physical activity and fish, meat, and alcohol consumption were normally distributed at baseline and at the end of intervention. Serum lipid concentrations at baseline and at the end of the intervention were calculated as the mean of the values obtained at visits 5 and 6 and the mean of the values obtained at visits 7 and 8, respectively. Change was calculated as the value obtained after intervention minus the value obtained at baseline. Percentage change was calculated as the group-wise mean percentage change from baseline. Because of missing values, change could not be calculated for some individuals. Analysis of changes in serum lipids, serum phospholipid 16:1n−7, and sum of serum phospholipid fatty acids are therefore based on 222, 217, and 209 subjects, respectively. Two influencing outlying values were excluded from the analysis of desaturation indexes.

To evaluate within-group change, we used paired *t* tests for normally distributed variables, the Wilcoxon signed-rank test for ordinal and non–normally distributed variables, and the chi-square statistic for categorical variables. One-way analysis of variance was used to evaluate whether change differed between groups; the *F* test was used for normally distributed variables and the Kruskal-Wallis test for ordinal and non–normally distributed variables. Between-group comparisons of change were done by contrasting groups in the SAS general linear model procedure when the overall *F* test was significant at $P < 0.05$ (26). We did not adjust for multiple comparisons (27). Results were considered significant when the two-sided *P* value was < 0.05. Caution should be applied when interpreting *P* values in the present study because three contrasts were tested. When applying the Tukey multiple-comparison procedure (28), the 95% CI included the null value of no effect for those contrasts for which the unadjusted *P* value was > 0.03. Correlations were tested by computing Pearson or Spearman correlation coefficients.

RESULTS

Three of the 234 subjects who were randomly assigned to a study arm dropped out during the intervention period. One subject in the DHA group was found to have fat intolerance after cholecystectomy, one subject in the EPA group developed diarrhea, and one subject in the corn oil group experienced vertigo and vomiting that was considered unrelated to the dietary supplements. Two individuals in the DHA group, three in the EPA group, and two in the corn oil group were excluded from the analysis. The reasons for exclusions were possible renal disease ($n = 1$), poor compliance with study protocol ($n = 1$), initiation of a vasoactive drug ($n = 1$), cancer surgery ($n = 1$), and change in amount of physical activity during the intervention ($n = 3$). Thus, 224 subjects are included in the present analysis. Mean ages of the subjects were 43 ± 5, 44 ± 5, and 45 ± 6 y and mean body mass indexes (in kg/m^2) were 24.9 ± 2.6, 25.6 ± 2.9, and 24.6 ± 2.7 in the DHA, EPA, and corn oil groups, respectively.

There were no significant changes in hematology, blood chemistry (electrolytes, alanine aminotransferase, γ-glutamyl transferase, alkaline phosphatase, albumin, bilirubin, creatinine, and C-reactive protein), serum glucose, or plasma-active renin after dietary intervention with DHA, EPA, or corn oil (data not shown).

Compliance and side effects

The mean number of days in the study was 49 ± 5, 48 ± 3, and 48 ± 4 d in the DHA, EPA, and corn oil groups, respectively. Percentage compliance was slightly poorer in the DHA group (91 ± 6%) compared with the EPA and corn oil groups (both 94 ± 6%). There were no within-group correlations between compliance and change in serum DHA, EPA, or linoleic acid concentrations.

Side effects were mild and transient and for most individuals faded 1–2 wk after the start of the intervention. Fifty-eight percent of subjects in the DHA group and 57% in the EPA group experienced belching after initiation of the dietary supplements compared with 4% in the corn oil group. A taste of fish oil during the intervention was reported by 67% of subjects in the DHA group, 65% in the EPA group, and 3% in the corn oil group.

Diet, body weight, and physical activity

The DHA, EPA, and corn oil groups were well balanced at baseline. Total fat accounted for 30% of energy intake in all

TABLE 2
Composition of background diet[1]

Daily nutrient intake	DHA (n = 72)	EPA (n = 75)	Corn oil (n = 77)
Energy (kJ)	10 370 ± 2561	10 223 ± 2170	10 877 ± 2455
Protein (g)	103 ± 23	103 ± 22	107 ± 25
Carbohydrate (g)	335 ± 80	324 ± 74	349 ± 87
Fiber (g)	24.1 ± 7.3	23.2 ± 6.7	25.2 ± 7.7
Alcohol (g)	5.82 ± 6.28	6.39 ± 6.56	7.04 ± 7.00
Total fat (g)	81.2 ± 32.1	81.6 ± 23.9	85.9 ± 27.0
Cholesterol (mg)	314 ± 102	327 ± 89	334 ± 101
Saturated fat (g)	33.6 ± 12.4	34.3 ± 10.9	35.4 ± 11.4
Monounsaturated fat (g)	27.6 ± 11.3	28.0 ± 8.7	29.6 ± 9.6
Polyunsaturated fat (g)	13.5 ± 8.4	12.7 ± 4.6	14.0 ± 6.1
P:S	0.40 ± 0.15	0.39 ± 0.13	0.40 ± 0.13
18:2n−6 (g)	10.2 ± 7.0	9.50 ± 3.70	10.7 ± 5.1
20:5n−3 (g)	0.18 ± 0.20	0.19 ± 0.18	0.19 ± 0.21
22:6n−3 (g)	0.34 ± 0.32	0.35 ± 0.28	0.36 ± 0.32
β-Carotene (μg)	2651 ± 1902	2634 ± 1284	2749 ± 1905
Retinol (μg)	1003 ± 955	993 ± 717	1031 ± 697
Thiamine (mg)	1.66 ± 0.38	1.67 ± 0.35	1.76 ± 0.43
Riboflavin (mg)	2.20 ± 0.70	2.29 ± 0.67	2.31 ± 0.72
Niacin (mg)	23.6 ± 4.8	23.2 ± 4.8	23.8 ± 5.2
Vitamin C (mg)	90.0 ± 1.6	78.6 ± 35.5	88.9 ± 45.0
Vitamin D (μg)	6.08 ± 9.77	5.33 ± 4.16	5.65 ± 3.81
Vitamin E (mg)	4.87 ± 1.48	4.96 ± 1.34	5.33 ± 1.53

[1] $\bar{x}$ ± SD. DHA, docosahexaenoic acid group; EPA, eicosapentaenoic acid group; P:S, ratio of polyunsaturated to saturated fatty acids.

groups. Dietary intake of DHA and EPA at baseline accounted for 0.7% of total fat intake. Differences in nutrient intake between the DHA, EPA, and corn oil groups were minor and not significant (**Table 2**). No significantly different within- or between-group changes were found with respect to body weight, physical activity, or food habits during the intervention (**Table 3**). Body weight increased by 0.6 kg in the corn oil group and by 0.7 kg in the DHA and EPA groups. There was a nonsignificant increase in the percentage of participants who reported being sedentary after compared with before the intervention. Alcohol, meat, and fish consumption (dinner meals) increased slightly but not significantly during the intervention. None of the participants reported consuming more than three fish dishes weekly before or during the intervention. There was good agreement between measures of alcohol consumption obtained by the nutritionist during the run-in period and by the self-administered questionnaire at baseline (r = 0.73, P = 0.0001).

Serum lipids and apolipoproteins

Serum mean (95% CI in parentheses) triacylglycerol concentrations decreased 0.22 mmol/L (0.15, 0.29) in the DHA group and 0.15 mmol/L (0.06, 0.24) in the EPA group (**Table 4**). In the corn oil group serum triacylglycerols increased 0.11 mmol/L (0.03, 0.19). Compared with change for the corn oil group, serum triacylglycerols decreased 26% in the DHA group and 21% in the EPA group. The difference between the DHA and EPA groups was not significant (P = 0.14). However, net decreases in serum triacylglycerols were consistently greater in the DHA group than in the EPA group across quartiles of baseline triacylglycerol concentrations (**Table 5**). In the EPA and DHA groups there were no correlations between changes in individual n−3 fatty acids and changes in serum triacylglycerol concentrations.

Serum total cholesterol decreased 0.15 mmol/L ($P < 0.05$) in the EPA group and apolipoprotein A-I decreased 0.04 g/L ($P <$ 0.001, Table 4). These changes differed significantly from both the DHA and the corn oil groups. In the DHA group, HDL cholesterol increased 0.06 mmol/L ($P < 0.001$), differing significantly from both the EPA and corn oil groups. Hence, in both the EPA and DHA groups there was an increase in the ratio of HDL cholesterol to apolipoprotein A-I and a decrease in the ratio of total cholesterol to HDL cholesterol.

Serum phospholipid fatty acid concentrations

In the total study group (n = 224), the correlations between dietary intake and serum phospholipid concentrations of DHA and EPA at baseline were r = 0.39 and r = 0.35, respectively (both P = 0.0001). The mean of individual ratios of dietary DHA to EPA at baseline was 2.5 ± 1.2, whereas the serum phospholipid ratio of DHA to EPA was 3.8 ± 1.6 (P = 0.0001, for the difference between the ratios), indicating accumulation of DHA relative to EPA in serum phospholipids.

TABLE 3
Body weight and lifestyle factors at baseline and change after 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil[1]

	DHA (n = 72)		EPA (n = 75)		Corn oil (n = 77)	
	Baseline	Change	Baseline	Change	Baseline	Change
Body weight (kg)	80.0 ± 10.0[2]	0.7 ± 1.2	82.6 ± 10.0	0.7 ± 1.4	79.5 ± 9.4	0.6 ± 1.1
Fish consumption (dishes/wk)	2.10 ± 1.01	0.06 ± 1.13	2.16 ± 1.05	0.16 ± 1.06	2.03 ± 1.10	0.21 ± 1.29
Meat consumption (dishes/wk)	2.46 ± 1.31	0.24 ± 1.53	2.56 ± 1.39	0.16 ± 1.23	2.93 ± 1.28	0.15 ± 2.01
Teetotalers (%)	4	0	1	0	4	0
Alcohol consumption (g/wk)[3]	45.3 ± 44.3	0.3 ± 5.1	59.6 ± 63.9	−0.8 ± 6.5	55.5 ± 50.8	1.5 ± 7.0
Physical activity						
Sedentary (%)	22	3	26	1	25	6
Moderate (%)	69	−6	59	−1	54	1
Active (%)	9	3	15	0	21	−7

[1] There were no significant differences among groups.
[2] $\bar{x}$ ± SD.
[3] Teetotalers were excluded from analysis of alcohol consumption.

TABLE 4
Serum lipids and apolipoproteins at baseline and change after 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil

	DHA (n = 72)		EPA (n = 75)		Corn oil (n = 77)			Contrasts between groups: P		
	Baseline	Change	Baseline	Change	Baseline	Change	F test: P[1]	DHA vs EPA	DHA vs corn oil	EPA vs corn oil
Triacylglycerols (mmol/L)	1.24 ± 0.58[2]	−0.22 ± 0.31[3]	1.23 ± 0.57	−0.15 ± 0.40[4]	1.22 ± 0.55	0.11 ± 0.34[4]	0.0001	0.14	0.0001	0.0001
Total cholesterol (mmol/L)	6.00 ± 0.95	0.03 ± 0.49	5.98 ± 0.94	−0.15 ± 0.55[5]	6.02 ± 1.08	0.10 ± 0.55	0.01	0.04	0.4	0.004
LDL cholesterol (mmol/L)	4.06 ± 0.86	0.07 ± 0.46	4.06 ± 0.83	−0.08 ± 0.48	4.04 ± 0.98	0.06 ± 0.48	0.10	—	—	—
HDL cholesterol (mmol/L)	1.36 ± 0.30	0.06 ± 0.13[3]	1.33 ± 0.31	0.01 ± 0.12	1.41 ± 0.28	−0.01 ± 0.11	0.001	0.009	0.0005	0.4
Apolipoprotein A-I (g/L)	1.38 ± 0.21	0.02 ± 0.13	1.38 ± 0.20	−0.04 ± 0.10[3]	1.46 ± 0.23	0.00 ± 0.12	0.003	0.0008	0.3	0.02
Apolipoprotein B (g/L)	1.00 ± 0.21	−0.01 ± 0.11	1.01 ± 0.23	−0.03 ± 0.11[5]	1.02 ± 0.28	0.02 ± 0.11	0.05	—	—	—
HDL:apolipoprotein A-I	0.97 ± 0.14	0.04 ± 0.07[3]	0.96 ± 0.13	0.04 ± 0.08[3]	0.97 ± 0.12	−0.01 ± 0.06	0.0001	0.8	0.0003	0.0001
Total:HDL cholesterol	4.62 ± 1.19	−0.19 ± 0.52[4]	4.70 ± 1.24	−0.13 ± 0.47[5]	4.43 ± 1.19	0.11 ± 0.62	0.002	0.4	0.0006	0.007

[1] ANOVA for between-group comparisons of change.
[2] $\bar{x}$ ± SD.
[3–5] One-sample t test of difference between baseline and 7 wk: [3] $P < 0.001$, [4] $P < 0.01$, [5] $P < 0.05$.

TABLE 5
Change in serum triacylglycerol concentration in docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and corn oil groups according to quartiles of baseline triacylglycerol concentration

Baseline triacylglycerol concentration	Change in serum triacylglycerol concentration			Estimated n−3 fatty acid effect[1]	
	DHA (n = 72)	EPA (n = 75)	Corn oil (n = 77)	DHA	EPA
	mmol/L			*mmol/L (%)*	
1st quartile: 0.69 (0.34–0.82) mmol/L[2]	0.00 ± 0.13[3]	0.03 ± 0.21	0.10 ± 0.21	−0.10 (−14)	−0.07 (−10)
2nd quartile: 0.96 (0.83–1.09) mmol/L	−0.14 ± 0.18	−0.04 ± 0.26	0.15 ± 0.28	−0.29 (−30)	−0.19 (−20)
3rd quartile: 1.24 (1.10–1.44) mmol/L	−0.16 ± 0.23	−0.03 ± 0.34	0.14 ± 0.32	−0.30 (−24)	−0.17 (−14)
4th quartile: 2.01 (1.45–3.61) mmol/L	−0.56 ± 0.35	−0.52 ± 0.46	0.03 ± 0.50	−0.59 (−29)	−0.55 (−27)

[1] Effect attributable to DHA and EPA; ie, change in DHA group minus change in corn oil group and change in EPA group minus change in corn oil group.
[2] $\bar{x}$; range in parentheses.
[3] $\bar{x}$ ± SD.

The total amount of serum phospholipid fatty acids did not change between groups during EPA, DHA, or corn oil supplementation (**Table 6**). Likewise, there were no changes in serum phospholipid saturated fatty acids. As for the monounsaturated fatty acids, palmitoleic acid (16:1n−7) decreased significantly by 20% in the DHA group, compared with no change in the EPA or corn oil groups. Oleic acid (18:1n−9) concentrations decreased by 11% and 12% in the DHA and EPA groups, respectively.

The total serum phospholipid n−6 fatty acid concentration (sum of 18:2n−6, 20:3n−6, and 20:4n−6) decreased more in the EPA (−23%) than in the DHA (−11%) group. In the DHA group, however, the ratio between the individual n−6 fatty acids changed more than in the EPA group. The ratio of 20:4n−6 + 20:3n−6 to 18:2n−6 can be used as an index of Δ6 desaturation activity because changes in Δ5 desaturation will not influence the ratio. The Δ6 desaturation index decreased significantly in the DHA group compared with no change in the EPA or corn oil group (**Table 7**). Similarly, the ratio of 20:4n−6 to 20:3n−6 + 18:2n−6 can be used as an index of Δ5 desaturation activity. This ratio decreased significantly in the DHA group whereas it increased significantly in the EPA group. As a result, the ratio of arachidonic (20:4n−6) to linoleic (18:2n−6) acid decreased in the DHA group and increased in the EPA group.

During the intervention, the serum phospholipid concentration of n−3 fatty acids increased by 47% in the DHA group and by 68% in the EPA group. The concentration of α-linolenic acid (18:3n−3) decreased in both the DHA and EPA groups compared with the corn oil group (Table 6). In the DHA group, mean serum phospholipid DHA and EPA concentrations increased significantly by 69% (individual range: −33% to 669%) and 29% (individual range: −63% to 557%), respectively, whereas docosapentaenoic acid (DPA; 22:5n−3) decreased by 33% (individual range: −72% to 221%). In this group, the correlation between the change in serum DHA and EPA was $r = 0.30$ ($P = 0.01$, **Figure 1**). In the EPA group, serum phospholipid EPA increased by 297% (individual range: −2% to 1196%) and docosapentaenoic acid by 130% (individual range: −9% to 393%). Surprisingly, the serum phospholipid concentration of DHA decreased by 15% (individual range: −65% to 85%; $P < 0.001$) after EPA supplementation. The correlation between the change in serum DHA and EPA was $r = 0.39$ ($P = 0.0005$) during supplementation with EPA (**Figure 2**).

DISCUSSION

Numerous studies have examined the effects of marine n−3 fatty acids on lipid metabolism, but the separate effects of the two major n−3 fatty acids have remained largely unknown. The present report extends previous data by showing that both DHA and EPA lower serum triacylglycerol concentrations. DHA may be responsible for the increase in HDL cholesterol observed with some n−3 fatty acid supplements whereas EPA may produce a small decrease in serum total cholesterol. Our data further show that DHA and EPA produce different effects on the fatty acid composition of serum phospholipids. We studied a fairly large sample recruited from the general population and compliance with the study protocol was good. The generalizability of the study therefore appears sound.

It is well established that n−3 fatty acids lower serum triacylglycerols, but this is the first study in humans showing that this effect is attributable to both EPA and DHA. Surprisingly, DHA consistently had a more pronounced triacylglycerol-lowering effect than EPA across all baseline concentrations of triacylglycerol (Table 5). These observations provide strong evidence that DHA has a triacylglycerol-lowering effect of its own and is not acting solely after retroconversion to EPA, because if that were the case, DHA would not be more potent than EPA. This finding contrasts with previous studies in rats, in which dietary supplementation of highly purified EPA lowered serum triacylglycerols whereas DHA had a modest effect if any (13, 29–31). The opposing findings may be dose related: the DHA and EPA supplements in the rat studies calculated as $mg \cdot d^{-1} \cdot kg\ body\ wt^{-1}$ are 10- to 30-fold larger than in the present study in humans (13, 29). The opposing findings may also depend on species differences because rats and humans differ with respect to lipid metabolism (32). Our finding is further opposed by results from a single-blind crossover study concluding that EPA is responsible for the triacylglycerol-lowering effect in humans (14). However, the study was small with only nine individuals in the DHA group, and the wash-out period was 2 wk, which is too short in dietary intervention trials with n−3 fatty acids (33).

Previous studies suggested that serum HDL cholesterol is better maintained with oil rich in DHA than oil rich in EPA (2, 34). The present data confirm these findings. The mechanism by which DHA increases HDL is not known. Serum triacylglycerols and HDL cholesterol were inversely correlated at baseline ($r = -0.42$, $P = 0.0001$), possibly due to plasma lipid transfer protein activity (35, 36). Interestingly, there was no correlation between changes in triacylglycerols and changes in HDL concentrations during supplementation with DHA ($r = -0.04$, $P = 0.73$), suggesting that DHA affects triacylglycerol and HDL metabolism through separate mechanisms. Much evidence indicates that n−3 fatty acids decrease serum triacylglycerols mainly by reducing hepatic very-low-density-lipoprotein (VLDL) synthesis and secretion (37, 38), but n−3 fatty acids may also increase the catabolic rate of VLDL (39). Moreover, it has been reported that lipid transfer protein activity decreased after n−3 fatty supplementation in humans (40), resulting in higher HDL-cholesterol concentrations and more triacylglycerols remaining in the VLDL fraction. Whether such effects can explain the increase in HDL cholesterol and the more pronounced triacylglycerol-lowering effect after DHA supplementation in the present study remains unknown.

In both groups receiving active treatment there was an increase in the ratio of HDL to apolipoprotein A-I. The finding suggests an increased surface to core ratio of the HDL molecule and a redistribution of the HDL subclasses toward the larger and more favorable HDL_2 as previously reported (34, 40, 41). Apparently, both n−3 fatty acids produce more cholesterol-rich HDL particles, DHA by increasing the HDL-cholesterol concentration and EPA by decreasing the apolipoprotein A-I concentration. In these healthy men with moderately high serum cholesterol concentrations, no convincing effects on total cholesterol were seen in either treatment group, although a 3%

TABLE 6
Serum phospholipid fatty acid concentration at baseline and change after 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil

	DHA (n = 72)		EPA (n = 75)		Corn oil (n = 77)			Contrasts between groups: P		
Fatty acid	Baseline	Change	Baseline	Change	Baseline	Change	F test: P[1]	DHA vs EPA	DHA vs corn oil	EPA vs corn oil
	μmol/L		μmol/L		μmol/L					
16:0	1450 ± 223[2]	−8 ± 168	1448 ± 221	−32 ± 174	1502 ± 290	16 ± 211	0.3	—	—	—
18:0	531 ± 113	−4 ± 77	524 ± 96	4 ± 77	543 ± 95	11 ± 83	0.5	—	—	—
16:1n−7	23.6 ± 10.9	−4.8 ± 11.7[4]	23.9 ± 18.0	−1.1 ± 13.1	28.1 ± 40.1	−4.8 ± 31.1	0.5	—	—	—
18:1n−9	344 ± 88	−37 ± 95[4]	340 ± 88	−41 ± 74[4]	348 ± 97	6 ± 90	0.001	0.8	0.003	0.001
22:1n−9	2.03 ± 3.31	−0.42 ± 3.41	2.34 ± 3.31	0.03 ± 2.51	1.79 ± 2.86	−0.13 ± 3.15	0.7	—	—	—
24:1n−9	74.5 ± 27.1	−0.5 ± 16.0	72.3 ± 24.9	−1.9 ± 18.5	75.9 ± 27.0	0.42 ± 24.76	0.8	—	—	—
18:2n−6	845 ± 185	−74 ± 184[4]	867 ± 168	−199 ± 128[4]	902 ± 160	21 ± 159	0.0001	0.0001	0.003	0.0001
20:3n−6	102 ± 32	−24 ± 26[4]	105 ± 29	−32 ± 23[4]	104 ± 32	7 ± 28[5]	0.0001	0.046	0.0001	0.0001
20:4n−6	253 ± 73	−39 ± 49[4]	260 ± 71	−48 ± 46[4]	261 ± 65	8 ± 52	0.0001	0.3	0.0001	0.0001
18:3n−3	7.72 ± 3.78	−1.13 ± 4.23[5]	8.30 ± 4.69	−2.07 ± 5.13[4]	7.66 ± 3.71	0.32 ± 4.00	0.005	0.2	0.05	0.001
20:5n−3	59.8 ± 53.7	17.6 ± 52.9[4]	61.4 ± 41.0	182.1 ± 91.1[4]	64.0 ± 40.3	−2.0 ± 40.7	0.0001	0.0001	0.07	0.0001
22:5n−3	32.0 ± 11.8	−10.6 ± 9.9[4]	33.9 ± 10.8	44.2 ± 25.8[4]	36.2 ± 10.2	1.6 ± 10.3	0.0001	0.0001	0.0001	0.0001
22:6n−3	185 ± 88	128 ± 87[4]	184 ± 65	−28 ± 48[4]	203 ± 69	−2 ± 52	0.0001	0.0001	0.0001	0.01
Sum of fatty acids[6]	4117 ± 653	−100 ± 481	4102 ± 600	−142 ± 485[5]	4326 ± 696	55 ± 594	0.06	—	—	—
n−3:n−6[7]	0.24 ± 0.12	0.16 ± 0.16[4]	0.24 ± 0.10	0.28 ± 0.17[4]	0.25 ± 0.09	−0.01 ± 0.07	0.0001	0.0001	0.0001	0.0001

[1] ANOVA for between-group comparisons of change. [2] $\bar{x}$ ± SD. [4–5] One-sample t test of difference between baseline and 7 wk: [4] $P < 0.001$, [5] $P < 0.05$. [6] Sum of fatty acids also includes 20:0, 22:0, 24:0, 20:1, 20:2, and 22:4n−6. [7] n−3:n−6 = [(18:3n−3 + 20:5n−3 + 22:5n−3 + 22:6n−3)/(18:2n−6 + 20:3n−6 + 20:4n−6)].

TABLE 7
Desaturation indexes at baseline and change after 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil

	DHA (n = 71)		EPA (n = 75)		Corn oil (n = 77)			Contrasts between groups: P		
Index	Baseline	Change	Baseline	Change	Baseline	Change	F test: P[1]	DHA vs EPA	DHA vs corn oil	EPA vs corn oil
Δ9 Desaturation index										
18:1n−9 to 18:0	0.65 ± 0.12[2]	−0.07 ± 0.14[4]	0.66 ± 0.15	−0.08 ± 0.11[4]	0.64 ± 0.12	0.01 ± 0.12	0.0001	0.8	0.0001	0.0001
16:1n−9 to 16:0	0.016 ± 0.006	−0.003 ± 0.007[4]	0.016 ± 0.013	0.000 ± 0.01	0.016 ± 0.018	−0.001 ± 0.010	0.1	—	—	—
Δ6 Desaturation index[3]	0.42 ± 0.10	−0.04 ± 0.09[4]	0.43 ± 0.12	0.00 ± 0.10	0.41 ± 0.10	0.01 ± 0.06	0.0006	0.0009	0.0007	0.9
Δ5 Desaturation index[5]	0.27 ± 0.07	−0.02 ± 0.06[6]	0.27 ± 0.07	0.02 ± 0.06[7]	0.26 ± 0.07	0.00 ± 0.04	0.0003	0.0001	0.08	0.02
Arachidonic:linoleic acid	0.30 ± 0.08	−0.02 ± 0.07[7]	0.31 ± 0.09	0.02 ± 0.07[6]	0.29 ± 0.08	0.00 ± 0.05	0.0007	0.0001	0.03	0.09

[1] ANOVA for between-group comparisons of change. [2] $\bar{x}$ ± SD. [4,6,7] One-sample t test of difference between baseline and 7 wk: [4] $P < 0.001$, [6] $P < 0.05$, [7] $P < 0.01$. [3] Δ6 Desaturation index = [(20:4n−6 + 20:3n−6)/18:2n−6]. [5] Δ5 Desaturation index = [20:4n−6/(20:3n6 + 18:2n−6)].

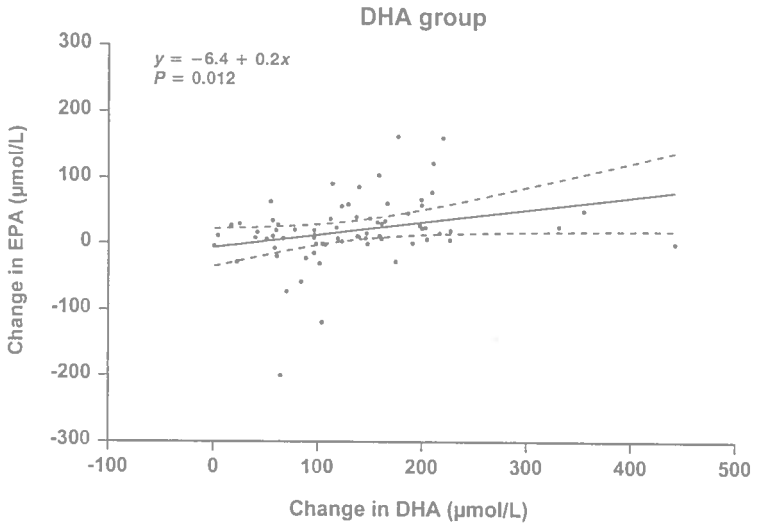

FIGURE 1. Plot of change in serum phospholipid docosahexaenoic acid (DHA) concentration versus change in serum phospholipid eicosapentaenoic acid (EPA) concentration in the DHA supplementation group (n = 72).

decrease was observed in the EPA group. This is in agreement with results obtained in previous studies concluding that n−3 fatty acids in moderate doses do not influence total cholesterol concentrations (4). There was, however, a beneficial decrease in the ratio of total cholesterol to HDL cholesterol in both the EPA and DHA groups.

Supplements with EPA or DHA produced an increase of serum phospholipid n−3 fatty acids at the expense of n−6 and monounsaturated fatty acids. In the EPA group, the percentage increase in serum phospholipid EPA (297%) was much larger than the corresponding increase in serum phospholipid DHA (69%) in the DHA group. A previous study showed that marine

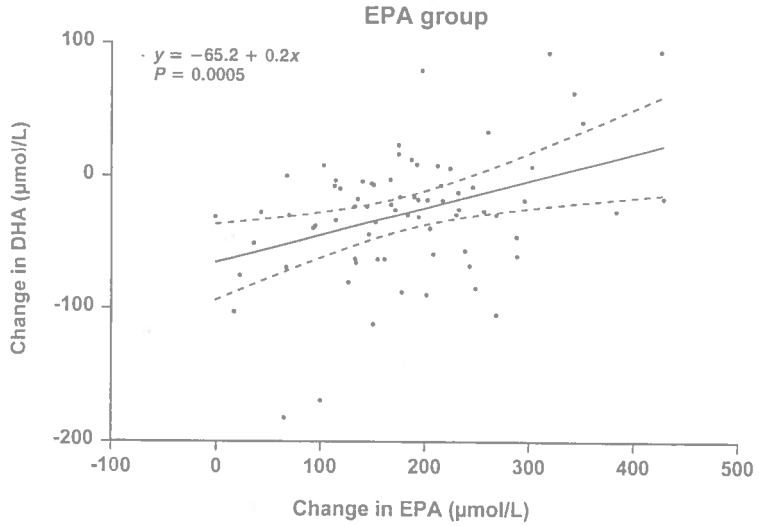

FIGURE 2. Plot of change in serum phospholipid eicosapentaenoic acid (EPA) concentration versus change in serum phospholipid docosahexaenoic acid (DHA) concentration in the EPA supplementation group (n = 75).

oil supplementation produced a rapid increase in plasma EPA that soon reached a plateau, whereas DHA concentration increased slowly and progressively (33). One might speculate that ingested EPA is more readily absorbed than DHA. Nevertheless, the observed ratio of DHA to EPA in serum phospholipids at baseline exceeded that of dietary intake (3.8 compared with 2.5, $P = 0.0001$).

In a metabolic study comparing ingestion of highly purified DHA and EPA, the two fatty acids produced identical increases in plasma chylomicron n−3 concentrations, but DHA was more rapidly cleared from plasma than was EPA (JB Hansen, personal communication, 1996). DHA is known to accumulate in the central nervous system and in cardiac tissue, and advanced atherosclerotic plaques are enriched with more DHA than EPA after dietary supplementation (42). These results indicate that the two fatty acids are distributed into different compartments of the body and have different metabolic actions. The present study supports the proposed pattern of incorporation in which DHA is selectively incorporated into extracirculatory pools whereas EPA has priority in the circulatory pool.

In the DHA group, the content of both DHA and EPA increased in serum phospholipids, indicating retroconversion of DHA to EPA. The degree of retroconversion appeared to be modest and may reach a saturation point (Figure 1). Similar findings were reported in cell and animal studies (11, 31) as well as in humans after dietary supplementation with highly purified EPA and DHA (10, 15, 16, 43). Theoretically, the increase of serum EPA in the DHA group could result from the small amount of EPA present in the DHA supplements (0.072 g/d). To examine this possibility, we did a regression analysis with dietary intake of EPA as the predictor variable and baseline serum phospholipid EPA concentration as the dependent variable (serum phospholipid EPA = 46.6 + 81.5 multiplied by dietary intake; $F = 0.0001$). The model predicted that the amount of extra EPA provided by the DHA supplement would increase serum phospholipid EPA to 67.8 mmol/L (95% CI: 60.4, 75.2) in the DHA group. The concentration of EPA after DHA supplementation (77.4 mmol/L) was higher, however, suggesting that retroconversion of DHA to EPA took place. Hence, ingested DHA may serve as a reservoir for EPA.

In the EPA group, there was a substantial increase in serum EPA and DPA whereas DHA decreased. This finding suggests that purified EPA is elongated to DPA, but not to DHA, and confirms previous studies in both rats and humans (10, 15, 16, 31). It is possible that EPA displaces DHA from the 2-position in serum phospholipids. Nevertheless, DHA concentrations increased modestly (20%) in 14 participants in the EPA group (Figure 2). This subgroup was characterized by large increases in both EPA (360%) and DPA (189%) during the intervention. Although not detectable from their dietary habit records, it may be that these individuals increased their fish consumption and consequently their DHA concentrations increased during intervention. An alternative explanation is that some DHA production takes place when EPA and DPA concentrations increase substantially.

A recent report established that DHA is biosynthesized from α-linolenic acid in human infants, although the conversion rate may be inadequate to support infant needs (44). Thus, α-linolenic acid and EPA may have different metabolic pathways because it seems that humans are not capable of synthesizing DHA from EPA unless the EPA concentration is high. This may have clinical importance if the purpose of n−3 fatty acid supplementation is to increase DHA concentration.

The change in the ratio of n−3 to n−6 serum phospholipid fatty acids was larger in the EPA group than in the DHA group. The proposed beneficial effects of n−3 fatty acids on cardiovascular disease have been partly attributed to an increase in the ratio of n−3 to n−6 fatty acids in cell membranes, resulting in a shift in eicosanoid synthesis toward a more vasodilatory and antiaggregatory state (5). In this study, the arachidonic acid concentration decreased similarly in the EPA and DHA groups. In the DHA group, however, the ratios (ie, desaturation indexes) of the individual n−6 fatty acids changed more than in the EPA group. Bearing in mind that serum phospholipid fatty acids are influenced by several factors, one might speculate that EPA and DHA have different effects on liver desaturation enzymes. Apparently, EPA exerts no effects on liver desaturation activity, suggesting that arachidonic acid decreased as a result of displacement from serum phospholipids. In the DHA group however, the decrease in arachidonic acid concentration may have resulted from decreased Δ6 and Δ5 desaturation. These enzymes are influenced by several factors including diet (45). n−3 Fatty acids decrease Δ6 and Δ5 desaturation in rats (46–48) and DHA in particular has been held responsible for reducing Δ6 desaturation, possibly by a feedback mechanism (31). The present data suggest that n−3 fatty acids may influence eicosanoid synthesis both by reducing arachidonic acid synthesis (DHA) and by displacing arachidonic acid from serum phospholipids (EPA).

In both the EPA and DHA groups there was a reduction in monoenes. This may represent a reduction in Δ9 desaturation as judged by the ratios of 18:0 to 18:1n−9 and of 16:0 to 16:1n−7. Similar observations were made in rats after supplementation with n−3 fatty acids (48, 49). Hence, n−3 fatty acids may affect membrane structure and function by altering activity in Δ9, Δ6, and Δ5 desaturation enzymes.

There were no important clinical or biochemical side effects during 257 man-months (number of men taking the supplements times the number of months the supplements were consumed) of dietary supplementation with highly purified ethyl esters of EPA or DHA, although transient belching with a taste of fish oil was experienced frequently. Consequently, participants knew they were ingesting an n−3 fatty acid but not whether it was EPA or DHA. This points to a blinding problem in controlled studies with n−3 fatty acids. Compliance was slightly poorer and side effects a little more frequent in the DHA group.

We conclude that both DHA and EPA lower serum triacylglycerol concentration, but have differential effects on lipoprotein and fatty acid metabolism in humans. The present data suggest that effects of individual n−3 fatty acids should be taken into consideration when interpreting the effects of n−3 fatty acid supplementation.

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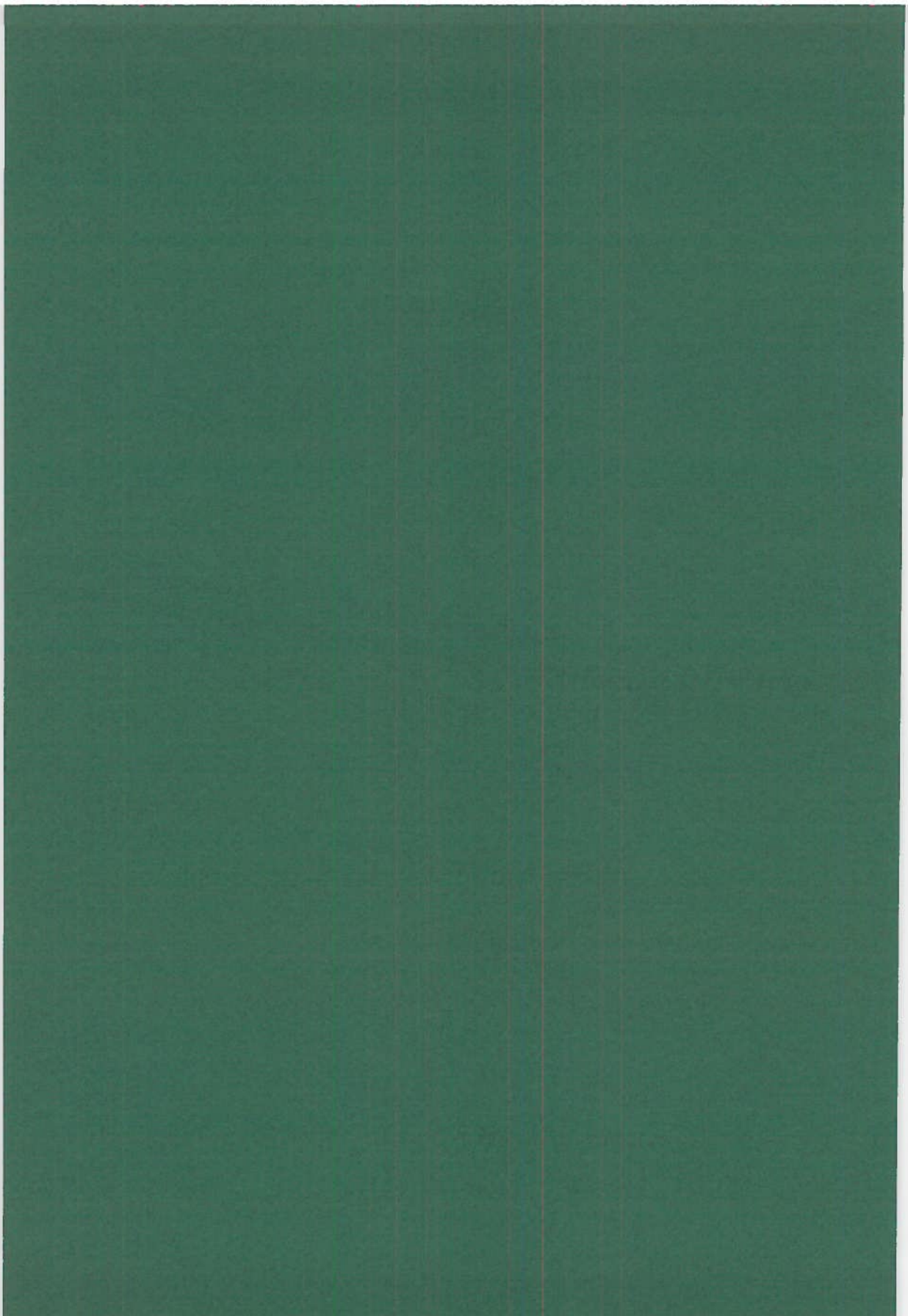
PAPER II



Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans[1–3]

Sameline Grimsgaard, Kaare H Bønaa, John-Bjarne Hansen, and Eivind SP Myhre

ABSTRACT The hemodynamic effects of highly purified eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3) have not been evaluated in humans. We therefore conducted a randomized, double-blind, parallel-design intervention study to assess possible separate effects of EPA and DHA on blood pressure, heart rate, and cardiac mechanics. Healthy, nonsmoking men aged 36–56 y (n = 224) were randomly assigned to dietary supplementation with 4 g/d of ethyl ester concentrates of DHA or EPA or 4 g corn oil/d (control). Mean blood pressure at baseline was 122/77 mm Hg and was positively associated with concentrations of serum phospholipid saturated fatty acids. Blood pressure did not change during the intervention. Mean heart rate at baseline was 63.4 beats/min; it decreased 2.2 beats/min in the DHA group (P = 0.006 compared with control), increased 1.9 beats/min in the EPA group (P = 0.04 compared with control), and remained practically unchanged in the control group. In a pooled analysis, changes in heart rate were independent of baseline heart rate and were associated with changes in concentrations of serum phospholipid DHA and docosapentaenoic acid (22:5n−3). Echocardiography in a subsample of 52 men showed improved left ventricular diastolic filling in the marine oil groups compared with the corn oil group (P = 0.02). In contrast, an increase in plasma concentrations of saturated fatty acids was associated with delayed diastolic filling. We conclude that dietary DHA and EPA influence heart rate and that the fatty acid composition of plasma phospholipids may affect cardiac mechanics in humans. *Am J Clin Nutr* 1998;68:52–9.

KEY WORDS Eicosapentaenoic acid, EPA, docosahexaenoic acid, DHA, phospholipids, heart rate, blood pressure, echocardiography, clinical trials, humans, saturated fatty acids, n−3 fatty acids, marine oil

INTRODUCTION

Dietary supplementation with n−3 polyunsaturated fatty acids of marine origin is reported to exert a wide range of biological effects of relevance to cardiovascular disease. The protective effects of these fatty acids include the ability to lower serum triacylglycerols and decrease the ability of platelets to aggregate (1, 2). The effects of marine oils on hemodynamics are less clear. Several reports indicate that marine oils may lower blood pressure in subjects with hypercholesterolemia and in patients with essential hypertension (3); however, these findings are not corroborated by other investigators (4). Marine oils have been reported to lower resting heart rate (5), to reduce ventricular extrasystoles in humans (6), and to possibly prevent ventricular fibrillation (7). Incorporation of dietary fat into cell membranes may alter the physiochemical properties of the membrane and thereby influence vascular tone (8) and myocardial relaxation (9). Dietary saturated fat lowered the left ventricular ejection fraction in nonhuman primates, whereas marine oils increased the left ventricular ejection fraction by enhancing ventricular filling (9). Whether n−3 fatty acids modify cardiac function and diastolic filling in healthy humans has to our knowledge not been examined.

Docosahexaenoic acid (DHA, 22:6n−3) and eicosapentaenoic acid (EPA, 20:5n−3) are considered to be the biologically active fatty acids in marine oils. The amount and proportion of DHA and EPA in different marine sources vary considerably. Earlier studies of hemodynamic effects of n−3 fatty acids in humans used oils differing both in total dose and in relative content of DHA and EPA. Animal studies have shown that DHA and EPA accumulate in different compartments in the body and may be metabolized differently and have different functions (10). Studies in rats indicate that DHA, which in contrast with EPA normally accumulates in mammalian heart cells, prevents ischemia-induced arrhythmias more effectively than EPA (11). It is unknown whether some of the inconsistent findings on hemodynamic effects of marine oils in humans are due to separate effects of DHA and EPA.

We therefore designed a randomized, controlled intervention trial to extend previous findings by comparing the effects of dietary supplements containing highly purified DHA and EPA on cardiovascular function. We also examined the relations between blood pressure, heart rate, left ventricular mechanics, and concentrations of serum phospholipid fatty acids.

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SUBJECTS AND METHODS

Subjects and study design

The subjects and experimental design were described in detail previously (12). In 1993 we recruited 234 nonsmoking, healthy men aged 36–56 y from a population study. The men met the following criteria at 2 visits during a 4-mo observation period: mean systolic blood pressure < 170 mm Hg, mean diastolic blood pressure < 100 mm Hg, and the difference between the diastolic blood pressures measured on the 2 visits < 15 mm Hg. The study was approved by the regional board of research ethics and each subject gave his written, informed consent.

The study was a double-blind, placebo-controlled intervention trial performed according to *Good Clinical Trial Practice* requirements (13). At baseline, computer-generated random numbers were used to assign subjects to dietary supplementation with 4 g 90% pure ethyl ester DHA/d, 4 g 95% pure ethyl ester EPA/d, or 4 g corn oil/d (control) for 7 wk. The supplements were administered in indistinguishable soft gelatin capsules that each contained 1 g oil and 4–6 IU vitamin E as the antioxidant (Pronova Biocare A/S, Oslo). Each subject was asked to ingest 4 capsules daily. In comparison, 500 g fatty fish contains ≈3.4 g EPA and 5 g DHA (14).

Blood pressure and echocardiographic measurements

Participants were examined after an overnight fast on 2 separate occasions within an interval of 3–5 d both at baseline and after 7 wk of supplementation. A trained assistant measured blood pressure and heart rate with an automatic instrument (Dinamap; Critikon, Tampa, FL) as described previously (15). The instrument measured pressure by the oscillometric method and calculated the mean arterial pressure automatically as the area under the pressure wave form divided by the time during which the area was measured (16). Heart rate was derived from the median pulse-to-pulse interval during the time that blood pressure was measured (17). After the subjects had been seated for 5 min, 3 recordings were made at 2-min intervals. The mean of the 2 final recordings was used in the analyses. Calibration of the instrument before and after the study showed that there was no drift during the study period.

M-mode echocardiography was used as a noninvasive tool to measure left ventricular function at baseline and at the end of intervention. One blinded investigator (ESPM) studied 20 randomly selected subjects in each intervention group. With the subject in a supine, left lateral position, a transverse section of the left ventricle was obtained at the level of the tips of the mitral valves (**Figure 1**). End diastole was defined at the instant of the peak R wave in a simultaneously obtained electrocardiogram. End systole was defined at the instant of the maximum posterior position of the interventricular septum. End of early diastolic filling was defined at the instant of the end of early fast posterior movement of the posterior wall. The following variables were obtained as the mean of 10 consecutive recordings: left ventricular transverse end-diastolic diameter (LVEDD); left ventricular transverse end-systolic diameter (LVESD); fractional shortening (FS), defined as [(LVEDD − LVESD)/LVEDD] × 100; left ventricular transverse diameter at the end of fast expansion (LVEFED); and fractional early expansion (FEE), defined as [(LVEFED − LVESD)/(LVEDD − LVESD)] × 100. Left ventricular mass was calculated from a spheroid model by using $4/3\pi r^3$.

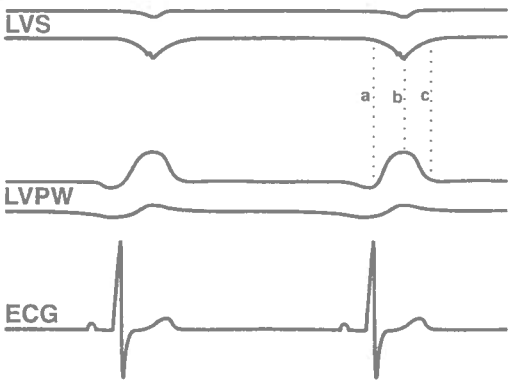

FIGURE 1. An ultrasonic M-mode recording of the left ventricle at the level of the mitral valve tips, showing the movements of the left ventricular septum (LVS) and left ventricular posterior wall (LVPW) during the cardiac cycle. ECG, electrocardiogram; a, left ventricular end-diastolic diameter (LVEDD); b, left ventricular end-systolic diameter (LVESD); c, left ventricular diameter at the end of fast expansion (LVEFED).

Clinical and laboratory measurements

Diets were assessed by a certified clinical nutritionist using the diet history method as described previously (12). Body mass index (BMI) was calculated as body weight divided by the square of the height (kg/m^2). We assessed physical activity (sedentary, moderate, or active) with a questionnaire at baseline and at the end of the intervention.

The collection and preparation of blood samples have been described in detail (12). Blood samples were analyzed after the completion of the intervention and before the randomization code was broken. Fatty acids in serum phospholipids were measured by gas-liquid chromatography as described previously (12). Serum total cholesterol and triacylglycerol were analyzed by enzymatic colorimetric methods with commercial kits (CHOD-PAP for cholesterol and GPO-PAP for triacylglycerols; Boehringer Mannheim, Mannheim, Germany). HDL cholesterol was measured after precipitation of the LDLs with heparin and manganese chloride. Serum lipids and serum creatinine, sodium, and potassium were analyzed on a Hitachi 737 Automatic Analyzer (Boehringer Mannheim) with reagents from the manufacturer. Plasma active renin was measured quantitatively by immunoradiometric assay (Nichols Institute BV, Wijchen, Netherlands) with reagents from the manufacturer.

Statistical analysis

The primary endpoint was a change in diastolic blood pressure between the beginning (baseline) and the end of the 7-wk intervention trial. The study was designed to detect a difference of 3 mm Hg in diastolic blood pressure at a two-sided level of significance of 0.05, with a power of 0.90. Blood pressure and heart rate at baseline and at the end of the intervention were calculated as the average of the values obtained on 2 separate occasions within an interval of 3–5 d. All variables were normally distributed except baseline serum phospholipid EPA. Log transformation normalized the distribution of EPA but did not influence the analyses; hence, untransformed data were used. Because of missing values, analyses of plasma active renin; all

fatty acids; and serum creatinine, sodium, and potassium were based on 216, 219, and 222 subjects, respectively. Change was calculated as the value obtained after the intervention minus the value obtained at baseline. Percentage change was calculated as the groupwise percentage change from baseline. One-sample *t* tests were used to assess within-group change. Results were considered statistically significant when the two-sided *P* value was <0.05. One-way analysis of variance was used for between-group comparisons of change by contrasting group in the SAS general linear models procedure when the overall *F* test was significant at $P < 0.05$ (18).

We did not adjust for multiple comparisons (19) and thus caution should be applied when interpreting *P* values in the present study because 3 contrasts were tested. When Tukey's multiple comparison procedure was used (20), the 95% CI included the null value of no effect for those contrasts for which the unadjusted *P* value was >0.03. In subgroup analyses, two-sample *t* tests were used to compare change in the marine oil groups (pooled) with change in the corn oil group. Linear relations were examined by computing crude and adjusted Pearson correlation coefficients and by developing multiple linear regression models.

RESULTS

Three of the 234 men who were randomly assigned to a study group did not complete the study. Seven men were excluded because of either renal disease ($n = 1$), poor compliance with the study protocol ($n = 1$), initiation of a vasoactive drug ($n = 1$), cancer surgery ($n = 1$), or change in level of physical activity during the intervention ($n = 3$), thus leaving 224 men for the present analysis. The DHA, EPA, and corn oil groups were well balanced at baseline (**Table 1**) (12). Compliance was satisfactory: the subjects in each of the 3 groups took > 90% of the prescribed number of capsules. There were no important side effects.

In the DHA group, mean serum phospholipid DHA and EPA concentrations increased by 69% and 29%, respectively, whereas docosapentaenoic acid (DPA, 22:5n−3) decreased by 33%. In the EPA group, mean serum EPA and DPA concentrations increased by 297% and 130%, respectively, whereas serum DHA decreased by 15%. We reported previously that in both the DHA and EPA groups, serum n−3 fatty acid concentrations increased at the expense of saturated, monounsaturated, and n−6 fatty acid concentrations (12).

There were minor changes in concentrations of serum sodium, whereas creatinine, potassium, and plasma active renin concentrations did not change during supplementation with DHA, EPA, or corn oil (**Table 2**). Baseline plasma active renin concentrations were inversely correlated with baseline systolic ($r = -0.24$, $P = 0.0004$) and diastolic ($r = -0.18$, $P = 0.006$) blood pressure, whereas serum total cholesterol concentrations were positively correlated with baseline systolic ($r = 0.22$, $P = 0.001$) and diastolic ($r = 0.34$, $P = 0.0001$) blood pressure (pooled analysis of all participants).

Effect of marine oil supplementation on blood pressure and heart rate

Systolic and diastolic blood pressure did not change in any treatment group during the intervention (Table 2). Neither were there changes in blood pressure in subgroup analyses when the participants were stratified according to baseline values for systolic blood pressure, diastolic blood pressure, plasma active renin, serum cholesterol, serum saturated fatty acids, serum n−3 fatty acids, dietary intake of saturated fat, or dietary intake of salt (data not shown).

TABLE 1
Baseline characteristics of subjects randomly assigned to dietary supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil[1]

Characteristic	DHA ($n = 72$)	EPA ($n = 75$)	Corn oil ($n = 77$)
Age (y)	43.2 ± 5.1	44.3 ± 5.2	45.1 ± 5.6
BMI (kg/m²)	24.9 ± 2.6	25.6 ± 2.9	24.6 ± 2.7
Serum lipids (mmol/L)			
Total cholesterol	6.00 ± 0.95	5.98 ± 0.94	6.02 ± 1.08
HDL cholesterol	1.36 ± 0.30	1.33 ± 0.31	1.41 ± 0.28
Triacylglycerols	1.24 ± 0.58	1.23 ± 0.57	1.22 ± 0.55
Diet (daily intake)			
Energy (kJ)	10370 ± 2561	10223 ± 2170	10877 ± 2455
Fat (% of energy)	29.0 ± 5.7	30.0 ± 4.6	29.7 ± 5.6
Saturated fat (g)	33.6 ± 12.4	34.3 ± 10.9	35.4 ± 11.4
P:S	0.40 ± 0.15	0.39 ± 0.13	0.40 ± 0.13
EPA (g)	0.18 ± 0.20	0.19 ± 0.18	0.19 ± 0.21
DHA (g)	0.34 ± 0.32	0.35 ± 0.28	0.36 ± 0.32
Sodium (mg)	3497 ± 913	3501 ± 772	3679 ± 968
Serum phospholipid fatty acids (μmol/L)			
Sum of fatty acids	4090 ± 676	4106 ± 592	4257 ± 708
Saturated fatty acids	2131 ± 310	2119 ± 300	2192 ± 380
EPA	59.8 ± 53.7	61.4 ± 41.0	64.0 ± 40.3
DHA	185 ± 88	184 ± 65	203 ± 69

[1] $\bar{x}$ ± SD. P:S, ratio of polyunsaturated to saturated fatty acids.

Heart rate decreased by 2.2 ± 4.6 beats/min in the DHA group, increased by 1.9 ± 5.1 beats/min in the EPA group, and remained practically unchanged in the corn oil group (Table 2). The change in the DHA group was significantly different from that in the EPA and corn oil groups (DHA compared with EPA, $P = 0.0001$; DHA compared with corn oil, $P = 0.006$; EPA compared with corn oil, $P = 0.04$). The predictors of baseline heart rate were analyzed in a multiple regression model that included baseline blood pressure and baseline concentrations of phospholipid fatty acids. Baseline heart rate was associated with baseline diastolic blood pressure ($\beta = 0.38$, $P = 0.0001$) and baseline DHA ($\beta = -0.02$, $P = 0.002$; adjusted model $R^2 = 0.12$, $P = 0.0001$). The model predicted a reduction in heart rate of 2.6 beats/min (95% CI: −4.4, −0.8 beats/min) if diastolic blood pressure increased by 0.74 mm Hg and serum phospholipid DHA increased by 128 μmol/L (the mean changes in diastolic blood pressure and serum phospholipid DHA in the DHA group). This predicted value was similar to the observed reduction in heart rate during supplementation with DHA (Table 2). Compared with that in the control group, heart rate in the DHA group decreased and that in the EPA group increased across all values of baseline heart rate (**Figure 2**). The slopes of the changes in the DHA and EPA groups were parallel and noncoincident when tested in multiple regression analysis (20), indicating that EPA and DHA have different effects on change in heart rate independent of baseline heart rate. In pooled analyses of all subjects, change in heart rate was inversely correlated with change in serum phospholipid DHA ($r = -0.25$, $P = 0.0002$) and positively correlated with DPA ($r = 0.26$, $P = 0.0001$), but was not correlated with change in EPA.

TABLE 2
Blood pressure; heart rate; serum concentrations of creatinine, sodium and potassium; and concentrations of plasma active renin at baseline and change after 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil[1]

Variable	DHA (n = 72)		EPA (n = 75)		Corn oil (n = 77)		F test:
	Baseline	Change	Baseline	Change	Baseline	Change	P[2]
Systolic BP (mm Hg)	121.3 ± 9.3	0.5 ± 5.4	123.2 ± 9.8	−0.5 ± 5.7	122.2 ± 10.8	0.7 ± 4.8	0.4
Diastolic BP (mm Hg)	76.1 ± 6.9	0.7 ± 4.6	78.1 ± 7.3	0.5 ± 4.2	76.9 ± 8.0	1.1 ± 3.9[3]	0.7
Mean arterial BP (mm Hg)	90.6 ± 7.3	1.2 ± 5.9	2.9 ± 8.0	0.4 ± 4.8	91.8 ± 9.1	0.8 ± 4.3	0.6
Heart rate (beats/min)	63.1 ± 8.3	−2.2 ± 4.6[4–6]	63.7 ± 7.6	1.9 ± 5.1[7,8]	63.5 ± 8.8	0.2 ± 6.1	0.0001
Creatinine (μmol/L)	90.4 ± 10.5	1.1 ± 5.4	89.4 ± 10.4	0.0 ± 5.0	86.6 ± 9.7	1.8 ± 5.3[4]	0.10
Sodium (mmol/L)	142.3 ± 1.7	−0.4 ± 1.4[3,5]	141.9 ± 1.5	0.5 ± 1.7[7,9]	141.6 ± 1.6	−0.1 ± 1.3	0.0003
Potassium (mmol/L)	4.5 ± 0.3	0.0 ± 0.4	4.5 ± 0.3	0.0 ± 0.3	4.5 ± 0.3	0.0 ± 0.3	0.9
Plasma active renin (mU/L)	27.4 ± 16.8	1.6 ± 9.3	24.7 ± 9.8	0.3 ± 8.2	25.3 ± 11.1	−0.3 ± 8.0	0.4

[1] $\bar{x}$ ± SD. BP, blood pressure.
[2] ANOVA for between-group comparisons of change.
[3,4,7] Significantly different from baseline (one-sample t test): [3] $P < 0.05$, [4] $P < 0.001$, [7] $P < 0.01$.
[5] Significantly different from EPA, $P = 0.0001$.
[6,8,9] Significantly different from corn oil: [6] $P = 0.006$, [8] $P = 0.04$, [9] $P = 0.009$.

Effect of marine oil supplementation on left ventricular function

Echocardiography was performed in a random subsample and the analysis suffered from lack of statistical power. Two of 60 men undergoing echocardiography dropped out of the study during the intervention. Six men were excluded because high-quality echocardiographic measurements could not be obtained (n = 4) or because they changed their level of physical activity during the intervention (n = 2), leaving 52 men in the analysis. Left ventricular dimensions as measured by LVEDD, LVESD, LVEFED, and FEE tended to increase after supplementation with DHA and EPA (**Table 3**). Because the effects of DHA and EPA were similar, we pooled the data from the DHA and EPA groups. Changes in LVEFED (**Figure 3**) and FEE (**Figure 4**) were significantly different from changes in the corn oil group (P = 0.02 and P = 0.03, respectively), indicating improved early left ventricular filling after marine oil supplementation.

Serum phospholipid fatty acids and left ventricular function

In a pooled analysis of all men undergoing echocardiography, there was a strong inverse association between change in LVEDD, LVESD, and LVEFED and change in serum saturated fatty acids (r = −0.36, r = −0.38, and r = −0.41, respectively; all $P < 0.01$; **Table 4**). The association was strongest for change in serum phospholipid palmitic acid (16:0) (r = −0.45, P = 0.0008, **Figure 5**). The relation between plasma fatty acids and left ventricular function was independent of changes in blood pressure, heart rate, blood lipids, serum n−3 fatty acids, and the ratio of polyunsaturated to saturated fatty acids (data not shown).

Serum phospholipid fatty acids and blood pressure at baseline

Mean baseline fatty acid concentrations were reported previously (12). Fatty acids of all main classes were positively correlated with blood pressure in univariate and age-adjusted analyses (**Table 5**). The associations between saturated and monounsaturated fatty acids and systolic blood pressure were not significant after adjustment for BMI. Adjustment for serum total cholesterol weakened the association between saturated fatty acids and diastolic blood pressure (data not shown). In multiple regression analysis, the statistically significant predictors of systolic blood pressure were BMI, physical activity, plasma active renin, α-linolenic acid (18:3n−3), and DPA (adjusted model R^2 = 0.33, P = 0.0001), whereas diastolic blood pressure was predicted by BMI, physical activity, serum total cholesterol, and DPA (adjusted model R^2 = 0.28, P = 0.0001). There were no statistically significant associations between serum fatty acids and heart rate in univariate or age-adjusted analyses.

DISCUSSION

This study showed that the fatty acid composition of serum phospholipids can affect heart rate and possibly left ventricular diastolic function in humans. Several lines of evidence support a causal relation between increased dietary intake of DHA and decreased heart rate. First, we compared the effects of purified DHA and EPA in a randomized, double-blind intervention trial. Although sufficient blinding is a problem in dietary supplementation trials with n−3 fatty acids, the participants in the present study were unable to discriminate between the DHA and EPA

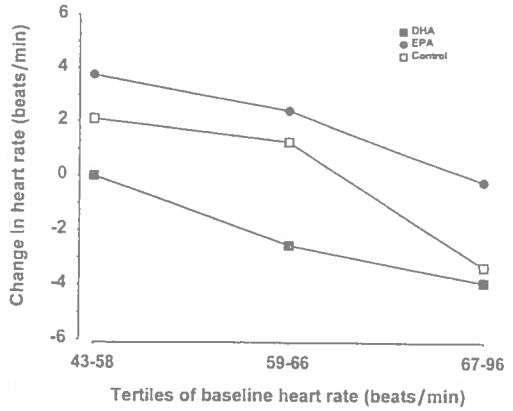

FIGURE 2. Change in heart rate after 7 wk of dietary supplementation with docosahexaenoic acid (DHA; n = 72), eicosapentaenoic acid (EPA; n = 75), or corn oil (control; n = 77), according to baseline heart rate (tertiles).

TABLE 3
Left ventricular dimensions at baseline and change after 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil[1]

Variable	DHA (n = 16)		EPA (n = 18)		Corn oil (n = 18)		F test: P[2]
	Baseline	Change	Baseline	Change	Baseline	Change	
LVEDD (cm)	5.67 ± 0.51	0.13 ± 0.68	5.34 ± 0.74	0.24 ± 0.86	5.81 ± 0.81	−0.19 ± 1.01	0.3
LVESD (cm)	3.32 ± 0.29	0.11 ± 0.35	3.16 ± 0.57	0.10 ± 0.51	3.51 ± 0.56	−0.19 ± 0.68	0.2
FS (%)	41.3 ± 4.4	−0.5 ± 4.6	40.6 ± 5.7	1.1 ± 4.8	39.7 ± 4.3	1.1 ± 4.1	0.5
LVEFED (cm)	4.98 ± 0.37	0.26 ± 0.56	4.75 ± 0.73	0.31 ± 0.76	5.31 ± 0.64	−0.22 ± 0.81	0.06
FEE (%)	71.3 ± 9.7	5.3 ± 10.8	72.5 ± 10.6	4.8 ± 10.5	78.9 ± 9.5	−2.9 ± 13.9	0.08
LV mass (g)	238 ± 56	20 ± 55	205 ± 94	43 ± 109	266 ± 91	−15 ± 117	0.2

[1] $\bar{x}$ ± SD. LVEDD, left ventricular transverse end-diastolic diameter; LVESD, left ventricular transverse end-systolic diameter; FS, fractional shortening; LVEFED, left ventricular transverse diameter at the end of fast expansion; FEE, fractional early expansion; LV mass, left ventricular mass.
[2] ANOVA for between-group comparisons of change.

supplements. Next, the difference between change in the DHA group and that in the EPA and corn oil groups was highly significant (Table 2), which reduces the possibility of a chance finding. Third, the effect was consistent across baseline heart rate values (Figure 2) and, finally, the change in heart rate was correlated with change in serum phospholipid DHA in a pooled analysis of all participants. Whether the increase in heart rate in the EPA group was due to EPA, DPA, or a relative deficit of DHA remains unknown.

A decrease in heart rate after marine oil supplementation has been observed both in animals (7, 9, 21) and in humans (5, 22). Free DHA and EPA reduced both contraction rate and fibrillation after arrhythmogenic stimuli in neonatal rat cardiac myocytes (23). Reduced electrical excitability by inhibition of myocyte voltage-gated sodium channels has been suggested as the primary underlying mechanism. Marine oils reduced the susceptibility to ischemia-induced and reperfusion-induced arrhythmias in animal feeding studies (7, 24, 25). In humans, dietary marine oils reduced the incidence of ventricular premature complexes (6) and increased electrocardiogram R-R variability (26), indicating possible antiarrhythmic effects. Dietary intake of DHA and EPA from seafood was associated with a reduced risk of primary cardiac arrest in a population-based case-control study (27). Finally, 2 secondary prevention trials of myocardial infarction survivors hypothesized that high intakes of fish (28) and α-linolenic acid (18:3n−3) (29) reduce the incidence of fatal cardiac arrhythmias. DHA may be more effective than EPA in preventing ischemia-induced arrhythmia in rats (11). On the basis of our data, we can speculate that DHA is responsible for the postulated antiarrhythmic effect of n−3 fatty acids.

Early left ventricular diastolic filling is mainly due to the myocardial relaxation rate and elastic recoil, and a delayed fall of intraventricular pressure in the diastole is an early sign of left ventricular ischemia and hypertrophy (30). The present study suggests that highly unsaturated n−3 fatty acids improve early left ventricular diastolic filling. In contrast, an increase in serum saturated fatty acids was associated with delayed diastolic filling. Our findings are supported by results obtained in human cardiac transplant patients showing that dietary marine oils improved left ventricular diastolic function, as measured by reduced deceleration time (31).

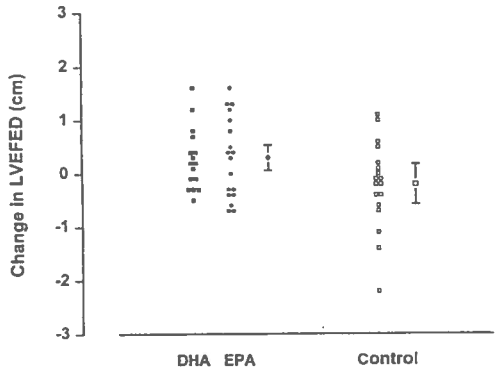

FIGURE 3. Change in left ventricular transverse diameter at the end of fast expansion (LVEFED) after 7 wk of dietary supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil (control). Bar with solid circle denotes the mean value and 95% CI for pooled analysis of the DHA and EPA groups. Bar with open square denotes the mean value and 95% CI for the corn oil group. DHA and EPA groups (pooled mean) were significantly different from the corn oil group, P = 0.02.

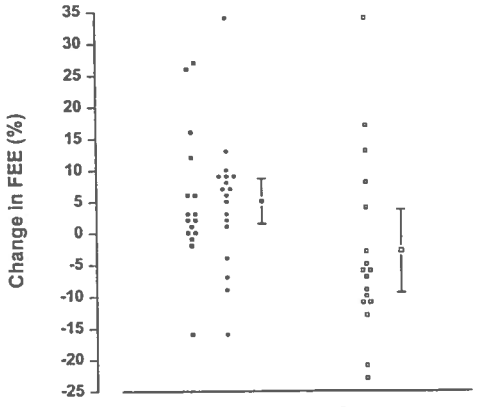

FIGURE 4. Change in left ventricular fractional early expansion (FEE) after 7 wk of dietary supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil (control). Bar with solid circle denotes the mean value and 95% CI for pooled analysis of the DHA and EPA groups. Bar with open square denotes the mean value and 95% CI for the corn oil group. DHA and EPA groups (pooled mean) were significantly different from the corn oil group, P = 0.03.

TABLE 4
Mean change in phospholipid fatty acid concentrations and correlations between change in fatty acid concentrations and change in left ventricular dimensions after dietary supplementation with docosahexaenoic acid, eicosapentaenoic acid, or corn oil[1]

Fatty acid	Change in fatty acid concentration	Pearson correlation coefficients					
		LVEDD	LVESD	FS	LVEFED	FEE	LV mass
	μmol/L						
Saturated fatty acids[2]	−8.43 ± 215[3]	−0.36[4]	−0.38[4]	0.09	−0.41[4]	−0.09	−0.31[5]
16:0	−4.21 ± 144	−0.38[4]	−0.42[4]	0.14	−0.45[6]	−0.13	−0.33[5]
Monounsaturated fatty acids[7]	−30.1 ± 89.9	−0.24	−0.21	−0.09	−0.30[5]	−0.18	−0.19
Polyunsaturated fatty acids[8]	−23.0 ± 191	−0.07	−0.11	0.08	−0.16	−0.24	−0.10
n−6 fatty acids[9]	−129 ± 219	−0.16	−0.21	0.08	−0.27	−0.28[5]	−0.23
n−3 fatty acids[10]	108 ± 128	0.18	0.20	−0.02	0.24	0.13	0.24
All fatty acids	−54.8 ± 432	−0.26	−0.29[4]	0.08	−0.33[5]	−0.18	−0.24

[1] $n = 52$, except saturated fatty acids and all fatty acids, for which $n = 51$. LVEDD, left ventricular transverse end-diastolic diameter; LVESD, left ventricular transverse end-systolic diameter; FS, fractional shortening; LVEFED, left ventricular transverse diameter at the end of fast expansion; FEE, fractional early expansion; LV mass, left ventricular mass.
[2] Saturated fatty acids include 16:0, 18:0, 20:0, 22:0, and 24:0.
[3] $\bar{x} \pm$ SD.
[4] $P < 0.01$.
[5] $P < 0.05$.
[6] $P < 0.001$.
[7] Monounsaturated fatty acids include 16:1, 18:1, 20:1, 22:1, and 24:1.
[8] Polyunsaturated fatty acids include 20:2, 18:2, 20:3, 20:4, 22:4, 18:3, 20:5, 22:5, and 22:6.
[9] n−6 fatty acids include 18:2, 20:3, 20:4, and 22:4.
[10] n−3 fatty acids include 18:3, 20:5, 22:5, and 22:6.

Similarly, marmosets fed diets enriched in n−3 and n−6 fatty acids had increased left ventricular ejection fractions due to enhanced diastolic filling compared with marmosets fed a saturated fat diet (9). Taken together, these data support the hypothesis that dietary patterns can affect left ventricular diastolic filling by modifying the fatty acid composition of phospholipids in plasma and cell membranes. We observed that saturated fatty acids were associated with changes in left ventricular filling independent of changes in heart rate. Hence, the underlying mechanism or mechanisms may be other than those responsible for the changes in heart rate. The fatty acid composition of cell membranes may affect heart function by modifying membrane fluidity and elastic properties of myocardial cells. It is also possible that saturated fatty acids and n−3 fatty acids affect diastolic function through separate mechanisms. Nitrogen monoxide has been found to influence left ventricular relaxation (32), and n−3 fatty acids may influence nitrogen monoxide release (33).

Neither DHA nor EPA lowered blood pressure in this group of normotensive men. This is in accordance with recent meta-analyses that concluded that marine oils in relatively high doses decrease blood pressure in persons with hypertension or cardiovascular disease (3, 34). Salt restriction augmented the decrease in blood pressure observed in hypertensive humans after marine oil supplementation (35) and it has been suggested that marine oils affect hypertension when it is mediated through increased activity in the renin-angiotensin system (36). It is also possible that long-term effects of n−3 fatty acids depend on both the amount and type of other fats consumed. However, in the present 7-wk study, we found no effect modification by values for blood pressure, serum cholesterol, plasma active renin, serum concentrations of saturated fatty acids, serum concentrations of n−3 fatty acids, or dietary intake of salt or fat. The study participants were a homogenous group, which may limit the possibility of detecting effect modification.

At baseline, both systolic and diastolic blood pressure were positively associated with concentrations of serum phospholipid DPA, whereas there was no association between EPA or DHA and blood pressure. A previous study in mildly hypertensive humans found a negative association between plasma EPA and blood pressure (37). Absorption or metabolism of n−3 fatty acids may differ between normotensive and hypertensive persons. A potential source of bias in cross-sectional studies is a change in behavior and dietary habits caused by the awareness of high blood pressure and the recent focus on the potential cardioprotective effects of marine oils.

We observed a strong positive association between baseline concentrations of saturated fatty acids in serum and blood pressure. A positive relation between dietary intake of saturated fat

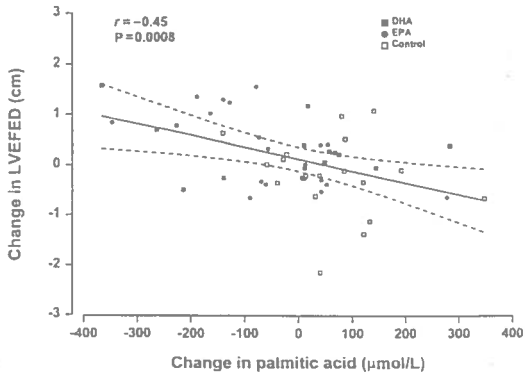

FIGURE 5. Relation between change in left ventricular transverse diameter at the end of fast expansion (LVEFED) and change in serum phospholipid palmitic acid (16:0) concentrations during 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil (control). The lines represent the regression line (solid) with 95% CI (broken lines) for the pooled analysis of all subjects.

TABLE 5
Correlations between serum phospholipid fatty acid concentrations (μmol/L) and blood pressure at baseline[1]

	Systolic blood pressure			Diastolic blood pressure		
Fatty acid	I[2]	II[3]	III[4]	I[2]	II[3]	III[4]
Saturated fatty acids	0.22[5]	0.20[5]	0.07	0.33[6]	0.33[6]	0.24[6]
Monounsaturated fatty acids	0.19[5]	0.20[5]	0.08	0.29[6]	0.31[6]	0.23[6]
Polyunsaturated fatty acids	0.15[7]	0.16[7]	0.15[7]	0.26[6]	0.28[6]	0.28[6]
n−6 fatty acids	0.13	0.15[7]	0.13	0.21[5]	0.23[6]	0.22[5]
n−3 fatty acids	0.10	0.10	0.10	0.22[5]	0.23[6]	0.24[6]
α-Linolenic acid	0.20[5]	0.22[5]	0.19[5]	0.17[5]	0.20[5]	0.16[7]
Eicosapentaenoic acid	0.05	0.04	0.04	0.14[7]	0.12	0.13
Docosapentaenoic acid	0.20[5]	0.21[5]	0.20[5]	0.31[6]	0.34[6]	0.33[6]
Docosahexaenoic acid	0.09	0.10	0.10	0.22[5]	0.23[6]	0.24[6]
All fatty acids	0.21[5]	0.21[5]	0.11	0.33[6]	0.34[6]	0.28[6]

[1] Values are Pearson correlation coefficients. See footnotes to Table 4 for individual fatty acids within fatty acid families.
[2] Age adjusted correlation coefficients (n = 219–224).
[3] Correlation coefficients adjusted for age and level of physical activity (n = 213–218).
[4] Correlation coefficients adjusted for age, level of physical activity, and body mass index (n = 213–218).
[5] $P < 0.01$.
[6] $P < 0.001$.
[7] $P < 0.05$.

and blood pressure has been shown in population studies (38–40), whereas the relation between saturated fatty acids in blood and blood pressure is less clear (41, 42). Saturated fatty acids may influence blood pressure through direct effects on the vascular system or through the actions of cholesterol as an intermediate promoting atherosclerosis and vascular stiffness. In a dietary intervention trial, blood pressure decreased significantly after an 8-wk diet low in both total fat and saturated fat, thereby suggesting a direct effect of saturated fat on blood pressure (43).

The present study provides evidence for a relation between concentrations of serum fatty acids and hemodynamics in humans. EPA and DHA supplementation had differential effects on resting heart rate, and further studies should examine whether the two n−3 fatty acids influence the susceptibility to cardiac arrhythmias differently. A short-term increase in dietary EPA or DHA does not affect blood pressure in normotensive subjects, but may improve left ventricular function and thereby contribute to the postulated cardioprotective effects of marine oils.

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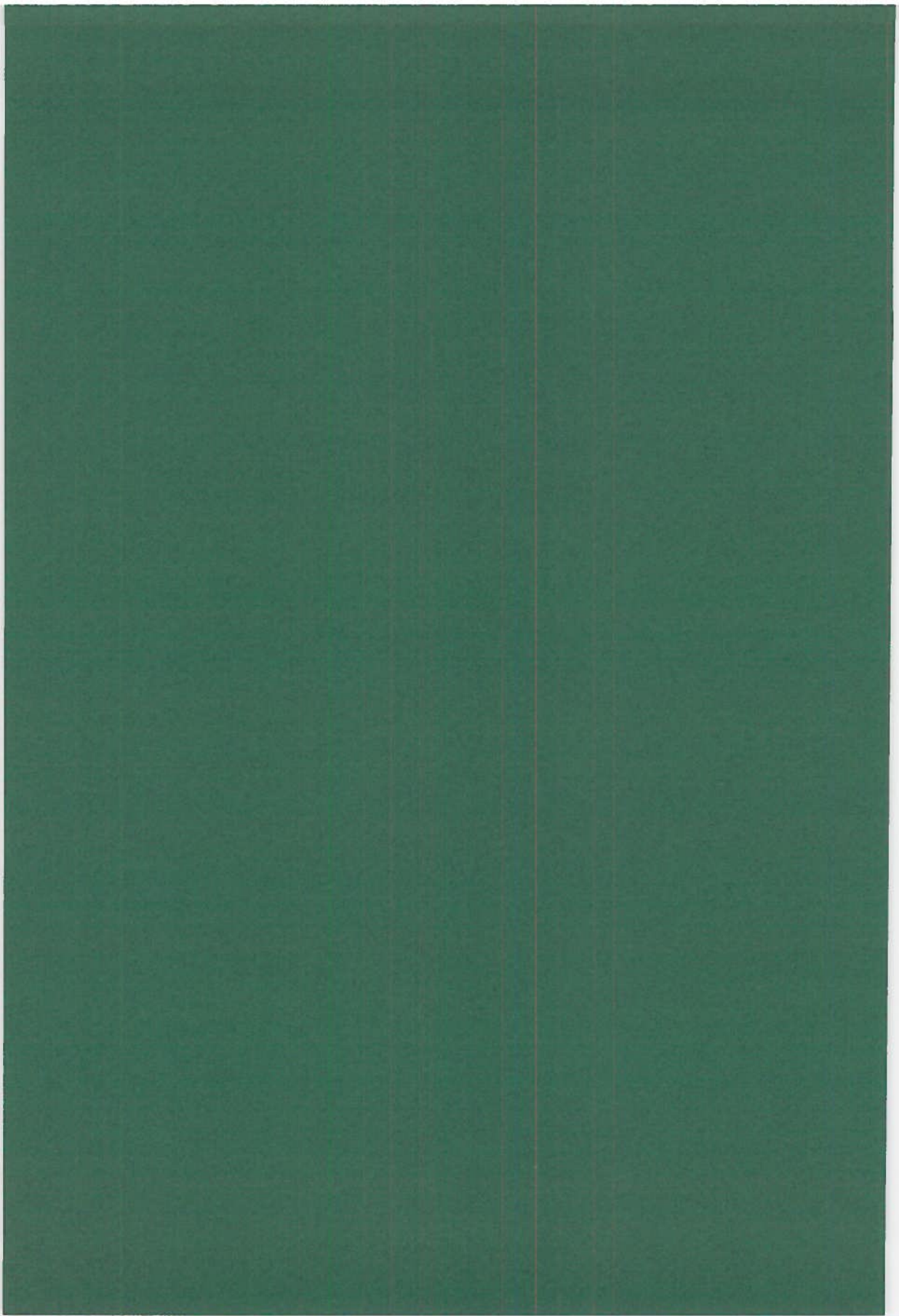
PAPER III



Plasma Saturated and Linoleic Fatty Acids Are Independently Associated With Blood Pressure

Sameline Grimsgaard, Kaare H. Bønaa, Bjarne K. Jacobsen, Kristian S. Bjerve

Abstract—The role of individual fatty acids in blood pressure regulation is unclear. We studied the cross-sectional relationship of blood pressure, total plasma phospholipid fatty acid concentrations, and proportions of individual fatty acids among participants in a population study. Blood pressure was measured automatically, and plasma phospholipid fatty acids were determined by gas-liquid chromatography in 4033 healthy men 40 to 42 years old. Significant positive linear associations existed between total fatty acids and saturated fatty acids and blood pressure, whereas polyunsaturated linoleic acid was inversely associated with blood pressure. In multiple regression analyses, a 2-SD increase in total fatty acids was associated with an increase of 6.0 (95% CI, 5.1 to 6.8) mm Hg systolic blood pressure. A 2-SD increase in saturated palmitic acid was associated with 1.4 (95% CI, 0.5 to 2.3) mm Hg increase in systolic blood pressure. In contrast, a 2-SD increase in polyunsaturated linoleic acid was associated with a 1.9 (95% CI, 1.0 to 2.8) mm Hg decrease in systolic blood pressure. We conclude that plasma levels of total fatty acids, saturated fatty acids, and polyunsaturated linoleic acid are independently associated with blood pressure. The present study supports the hypothesis that the composition of dietary fat influences blood pressure. (***Hypertension*. 1999;34:478-483.**)

Key Words: fatty acids ■ blood pressure ■ human

The relationship between dietary fats and blood pressure is controversial. Systolic blood pressure was positively correlated with dietary saturated fat in ecological data.[1] Observational studies that indicate that dietary saturated fat is positively associated with blood pressure[2–4] and polyunsaturated fat and the polyunsaturated/saturated fat (P/S) ratio are inversely associated with blood pressure[2,3,5] contradict reports that found no such associations.[6,7]

Blood levels of fatty acids may be used to examine the relationship between individual fatty acids and blood pressure. There are reports of positive associations between blood pressure and blood levels of saturated and monounsaturated fatty acids,[8–10] whereas polyunsaturated fatty acids have been both positively and inversely associated with blood pressure.[8,10] Most previous studies included selected study groups[9,10] or few subjects.[8,10] On the basis of experimental data in animals and humans, 1 review found that n-6 polyunsaturated fatty acids decrease blood pressure in hypertensive individuals.[11] However, 2 reviews of observational data and clinical trials concluded that dietary fats do not influence blood pressure levels.[12,13]

Plasma levels of essential polyunsaturated fatty acids reflect dietary intake,[14,15] whereas plasma levels of nonessential fatty acids are less reliable indicators of dietary fat. Nevertheless, plasma levels of palmitic acid (16:0) and stearic acid (18:0) and monounsaturated fatty acids correlated with dietary saturated fat.[14,15] In addition, high levels of dihomo-γ-linolenic acid (20:3n-6) may reflect a diet rich in saturated fat.[16]

We analyzed the association between plasma levels of phospholipid fatty acids and blood pressure in 4033 men 40 to 42 years old. The large sample size provided enough information to evaluate the independent associations of total fatty acids and individual fatty acids with blood pressure.

Methods

Subjects and Measurements

All men and women 40 to 42 years old who lived in Nordland County, Norway, were invited in 1988–1989 to a health screening organized by the National Health Screening Service, the University of Tromsø (Norway), and the local health authorities.[17] Plasma phospholipid fatty acids were quantified in a substudy of men, of whom 5492 were invited and 4302 (78%) participated. In our analysis, we excluded men who reported previous myocardial infarction (n=19), use of antihypertensive drugs (n=104), or both (n=7) and men whose blood pressure (n=1) or plasma fatty acid (n=138) measurements were unavailable.

The health screening invitation included a questionnaire on cardiovascular disease, smoking habits, and leisure time physical activity (3 levels).[18] A second questionnaire, which included questions on alcohol consumption,[19] was distributed at the screening and returned via mail by 3483 men. Data on alcohol consumption were available for 3396 men. The study was approved by the Norwegian Data Inspectorate, which considered the legal and ethical issues of the study, and the subjects gave informed consent.

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Body weight was measured on an electronic scale with subjects dressed in lightweight clothing. Height was measured in centimeters. Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2). We measured blood pressure by the oscillometric method[20] with an automatic device (Dinamap, Critikon).[17] After the subject had rested for 2 minutes, 3 recordings were made at 1-minute intervals with the individual sitting. The lowest blood pressure value was used in the analysis.

A nonfasting blood sample was analyzed for serum cholesterol at the Central Laboratory, Ullevål Hospital, Oslo.[21] Plasma phospholipid fatty acids (myristic acid [14:0], 16:0, 18:0, arachidic acid [20:0], behenic acid [22:0], lignoceric acid [24:0], palmitoleic acid [16:1], oleic acid [18:1], gondoic acid [20:1], erucic acid [22:1], nervonic acid [24:1], eicosatrienoic acid [20:3n-9], linoleic acid [18:2n-6], eicosadienoic acid [20:2n-6], 20:3n-6, arachidonic acid [20:4n-6], adrenic acid [22:4n-6], linolenic acid [18:3n-3], eicosapentaenoic acid [20:5n-3], docosapentaenoic acid [22:5n-3, 22:5n-6], and docosahexaenoic acid [22:6n-3]) were quantified by gas-liquid chromatography as described previously.[22] The coefficients of variation for individual fatty acids estimated from replicate analyses (n=55) ranged from 3.3% to 6.6%. Fatty acids were measured as μmol/L and relative concentrations, mol%. Trans-fatty acids were not measured.

Statistical Analysis

All variables were normally distributed except 20:5n-3, which was log-transformed. Pearson and Spearman correlation coefficients were computed to evaluate unadjusted relationships between fatty acids and blood pressure, BMI, total cholesterol, daily smoking, physical activity, and alcohol consumption. Total fatty acids and individual fatty acids that showed significant univariate associations with blood pressure were included in multiple regression analyses. We included fatty acids associated with dietary saturated fat (16:0, 16:1, and 20:3n-6), dietary n-6 (18:2n-6), and dietary n-3 polyunsaturated fat (20:5n-3) and also examined possible contributions of other fatty acids. Finally, we adjusted for BMI, daily smoking, physical activity, and alcohol consumption. Residual analyses confirmed the model assumptions. Logistic regression was used to estimate the odds ratio for hypertension (defined as systolic blood pressure ≥160 mm Hg and/or diastolic blood pressure ≥95 mm Hg) by a 2-SD change in fatty acid concentrations. Two-sided $P<0.05$ was considered statistically significant. The SAS software package was used (SAS Corp).[23]

Results

Ten percent of the study participants (Table 1) were hypertensive. Saturated, monounsaturated, n-6, and n-3 fatty acids accounted for 44%, 11%, 35%, and 10% of total fatty acids, respectively. The most abundant saturated, monounsaturated, and n-6 fatty acids were 16:0, 18:1, and 18:2n-6, respectively (Table 2). Monounsaturated and polyunsaturated fatty acids displayed larger interindividual variability than did levels of saturated fatty acids. The correlations between individual and total fatty acids were generally weak (data not shown). Levels of 16:0, 16:1, 18:1, 20:3n-9, and 20:3n-6 acids were positively intercorrelated (r=0.10 to 0.53) and were inversely correlated with 18:2n-6 (r=−0.08 to −0.36). Very-long-chain n-3 fatty acids were inversely correlated with levels of n-6 fatty acids (r=−0.18 to −0.65). Total fatty acids were highly correlated with serum total cholesterol (r=0.72).

Blood pressure was positively associated with total fatty acids (Table 2). Mean systolic blood pressure increased by 10 mm Hg from the bottom to the top decile of total fatty acid concentration, without any evidence of a threshold level below which or a plateau above which there was no association (Figure 1, top left). Levels of 18:2n-6 were inversely associated with blood pressure: mean systolic blood pressure decreased by 6 mm Hg from the bottom to the top decile of the 18:2n-6 concentration (Figure 1, bottom left). Systolic blood pressure was also positively associated with levels of 16:0, 16:1, 18:1, 20:3n-9, 20:3n-6, and 20:5n-3 (Table 2 and Figure 1, right).

TABLE 1. Participants in the Nordland Health Study: 4033 Men 40 to 42 Years Old

Characteristic	Mean±SD or %
Systolic blood pressure, mm Hg	133±14.0
Diastolic blood pressure, mm Hg	80.4±10.0
BMI, kg/m^2*	25.2±3.18
Serum total cholesterol, mmol/L	6.11±1.16
Daily smoking, %	47
Leisure time physical activity, %†	
1. Sedentary	23
2. Moderate	51
3. Regular/hard training	26
Alcohol consumption, %‡	
Teetotaler	4
Consumption ≥5 units of alcohol	
<Once yearly	20
A few times yearly	45
1 to 2 times monthly	24
1 to 2 times weekly or more	7

*n=4031, †n=4032, and ‡n=3396.

Total fatty acids and relative concentrations of 16:0 and 18:2n-6 were independently associated with blood pressure in multiple regression analysis (Table 3, model 1). Models 2 to 4 show the relationship between systolic blood pressure and fatty acids when we substituted 16:0 with 16:1 and 20:3n-6, which also are considered to reflect dietary saturated fat. All 3 fatty acids showed highly significant independent positive relationships with systolic blood pressure. When fatty acids were added one at a time, no fatty acids other than 16:0, 16:1, and 20:3n-6 were significantly associated with systolic blood pressure when total fatty acids and 18:2n-6 were already included in the regression models. In addition, total fatty acids, 16:0, and 18:2n-6 remained significantly associated with blood pressure when we controlled for BMI, physical activity, smoking, and alcohol consumption (Table 3, model 5). Fatty acids were similarly associated with systolic and diastolic blood pressure (data not shown). The regression model, which included total fatty acids, 16:0, and 18:2n-6, explained 6% and 9% of the variability in systolic and diastolic blood pressure, respectively. The association between levels of 18:2n-6 and diastolic blood pressure was stronger than that of systolic blood pressure and remained significant after adjustment for BMI.

Figure 2 (top) illustrates the independent association of total fatty acids and BMI with blood pressure. The prevalence of hypertension was 23% among the 605 men in the top tertile of total fatty acids and BMI. In contrast, 3% of the 576 men in the bottom tertile of total fatty acids and BMI were

TABLE 2. Levels of Plasma Phospholipid Fatty Acids and Correlations With Systolic Blood Pressure, BMI, Smoking, Physical Activity, and Alcohol Consumption in 4033 Men

Fatty Acid	Mean±SD	CV*	Systolic Blood Pressure†	BMI†	Daily Smoking‡	Physical Activity‡§	Alcohol Consumption‡§
Total fatty acids, μmol/L‖	4523±682	15	0.23	0.23	−0.09	−0.09	0.18
Mol%							
16:0	26.6±1.54	6	0.09	0.11	−0.02	−0.03	0.28
18:0	13.9±0.93	7	−0.03	0.11	0.02	0.00	−0.21
16:1	0.37±0.18	49	0.17	0.17	−0.01	−0.06	0.23
18:1	8.52±1.24	15	0.06	−0.05	−0.12	−0.04	0.10
20:3n-9	0.11±0.07	64	0.10	0.11	−0.03	−0.05	0.13
18:2n-6	23.5±3.62	15	−0.12	−0.25	−0.10	0.04	−0.17
20:3n-6	2.45±0.67	27	0.09	0.30	0.04	−0.09	0.00
20:4n-6	7.87±1.40	18	0.02	0.13	0.06	−0.04	0.10
18:3n-3	0.18±0.09	50	0.02	−0.06	−0.04	0.03	−0.01
20:5n-3	2.37±1.73	73	0.07	0.11	0.08	0.04	0.10
22:6n-3	6.52±1.66	26	0.03	0.09	0.17	0.04	0.04

*Coefficients of variation.

†Pearson and ‡Spearman coefficients: absolute values >0.03, 0.04, and 0.05 are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

§See Table 1 for categories of physical activity and alcohol consumption.

‖Includes 14:0, 16:0, 18:0, 20:0 22:0, 24:0, 16:1, 18:1 20:1, 22:1, 24:1, 20:3n-9, 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

hypertensive. Total fatty acids and 18:2n-6 were independently associated with blood pressure (Figure 2, bottom). The prevalence of hypertension was 21% among 532 men in the top tertile of total fatty acids and bottom tertile of 18:2n-6. In contrast, 4% were hypertensive among the 528 men in the bottom tertile of total fatty acids and top tertile of 18:2n-6. By multiple logistic regression in which we controlled for BMI, daily smoking, alcohol consumption, and physical activity,

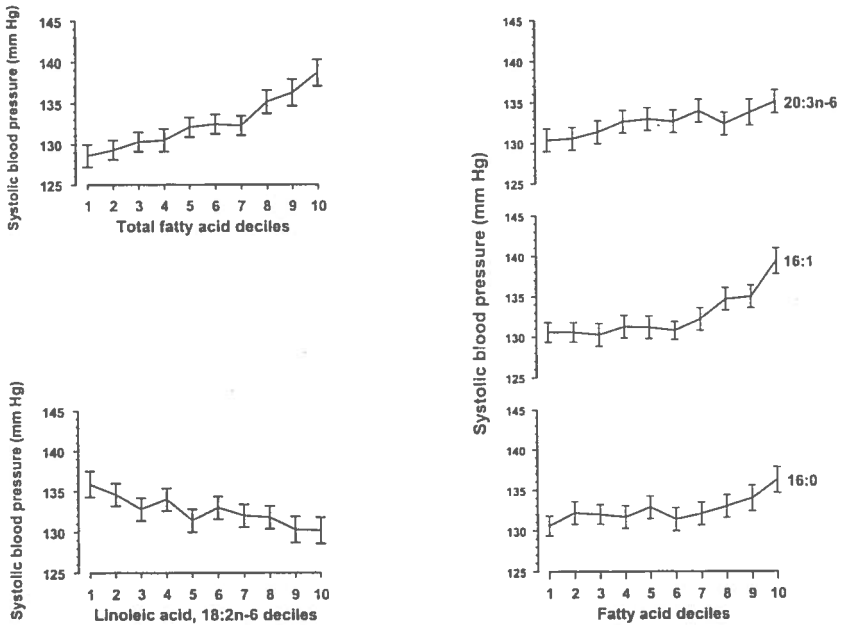

Figure 1. Top left, Mean systolic blood pressure by deciles of total fatty acids in plasma phospholipids (μmol/L). Bottom left, Mean systolic blood pressure by deciles of 18:2n-6 in plasma phospholipids (mol%). Right, Mean systolic blood pressure by deciles of 16:0, 16:1, and 20:3n-6 acids in plasma phospholipids (mol%). Error bars denote 95% CI.

TABLE 3. Multiple Linear Regression Analysis of Systolic Blood Pressure in 4033 Men

Predictor Variable	Model 1	Model 2	Model 3	Model 4	Model 5*
Total fatty acids, μmol/L†	6.0 (5.1, 6.8)	5.3 (4.4, 6.2)	5.8 (5.0, 6.7)	5.8 (4.9, 6.6)	4.9 (3.9, 5.9)
16:0, mol%	1.4 (0.5, 2.3)				1.1 (0.1, 2.1)
16:1, mol%		2.5 (1.6, 3.4)			
18:2n-6, mol%	−1.9 (−2.8, −1.0)	−1.6 (−2.5, −0.7)	−2.3 (−3.1, −1.4)	−1.7 (−2.6, −0.8)	−1.0 (−2.0, −0.1)
20:3n-6, mol%			1.5 (0.6, 2.3)		
Sum of 16:0, 16:1, and 20:3n-6, mol%				2.0 (1.1, 2.9)	
BMI, kg/m^2					4.8 (3.9, 5.8)
Physical activity 1 to 3‡					0.6 (0.0, 1.3)
Alcohol consumption‡					0.0 (−0.5, 0.6)
Daily smoking, no/yes					0.1 (−1.0, 1.0)
Adjusted R^2	0.06	0.06	0.06	0.06	0.08

Values are estimated mean change (95% CI) in systolic blood pressure (mm Hg) by 2-SD or 1-category increase in predictor variable.
*n=3394.
†See Table 2 for individual fatty acids included in total fatty acids.
‡See Table 1 for categories of physical activity and alcohol consumption.

the odds ratio for hypertension was 2.2 (95% CI, 1.7 to 2.7) for a 2-SD increase in total fatty acids and 0.6 (95% CI, 0.5 to 0.8) for a 2-SD increase in 18:2n-6.

Discussion

We found a strong positive and linear relationship between the total amount of plasma phospholipid fatty acids and blood pressure. To the best of our knowledge, this association has not been examined in previous reports. Concentrations of fatty acids associated with dietary saturated fat (16:0, 16:1, and 20:3n-6) were positively associated with blood pressure, and there was an inverse relationship between the concentration of polyunsaturated 18:2n-6 and blood pressure. These associations were independent in multivariate analyses. Our findings are strengthened by the population-based study design, the relatively high participation rate, and the large sample size. However, because we studied men 40 to 42 years old who consumed a Western diet, the results need confirmation in other age groups and in women.

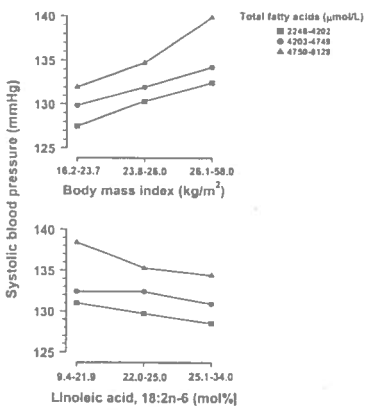

Figure 2. Top, Mean systolic blood pressure by levels of BMI (tertiles), stratified by levels of total fatty acids in plasma phospholipids (tertiles). Bottom, Mean systolic blood pressure by levels of 18:2n-6 in plasma phospholipids (tertiles), stratified by levels of total fatty acids in plasma phospholipids (tertiles).

The association between plasma phospholipid fatty acids and blood pressure was independent of BMI. A positive relationship between dietary saturated fat and blood pressure independent of BMI has been found in some[2–4] but not all[6,7] population studies. Earlier clinical trials were small and had methodological problems.[12] However, a recent controlled trial [the Dietary Approaches to Stop Hypertension (DASH) study][24] reported a modest reduction in blood pressure independent of BMI in subjects who were fed a diet formulated to reduce saturated fat (although intake of cholesterol, calcium, and protein were slightly altered versus the other experimental diet). BMI is a strong determinant of blood pressure.[1] Part of the association between BMI and blood pressure may depend on dietary fat. Therefore, it can be questioned whether adjustment for BMI is appropriate when assessing the strength of the relationship between dietary fat, as reflected in plasma fatty acids, and blood pressure.

We found that plasma concentrations of 16:0, 16:1, and 20:3n-6 were positively associated with blood pressure. Observational studies from Finland,[25] France,[9] and US[10] also found positive associations between blood levels of 16:0 and 16:1 and blood pressure, and the ratio of 20:3n-6 to 18:2n-6 was increased in erythrocyte membranes of hypertensive subjects.[26] Both 16:0 and 16:1 may be obtained from a diet high in saturated fat.[27] Although 20:3n-6 is a polyunsaturated fatty acid, blood levels may increase on a saturated fat diet,[16] possibly because dietary fat can influence enzymes involved in the metabolism of 20:3n-6.[28]

Blood pressure was inversely associated with 18:2n-6. Other investigators have found dietary[5] and plasma[8,10,25] levels of 18:2n-6 to be inversely associated with blood pressure, but the results are inconsistent.[2,9,29] The possibility of detecting a relationship between 18:2n-6 and blood pressure may be limited by low interindividual variation, imprecise measures of dietary 18:2n-6, small sample size, and the degree of statistical control for potential confounders.

We found that plasma levels of n-3 fatty acids were positively associated with blood pressure in crude analysis, but no consistent relationship existed in the multivariate analyses. Blood pressure was positively associated with plasma 20:5n-3 concentrations in a small study of Finnish men.[8] In contrast, blood pressure was inversely associated with plasma 20:5n-3 in mildly hypertensive individuals.[30] A meta-analysis concluded that marine n-3 fatty acids in pharmacological doses have a hypotensive effect, which is restricted to hypertensive subjects and individuals with atherosclerosis.[31]

Plasma levels of 18:2n-6 and n-3 polyunsaturated fatty acids reflect dietary intake.[14,15,32] However, levels of saturated and monounsaturated fatty acids may reflect both dietary fat and endogenous fat synthesis. The concentrations of saturated fatty acids displayed little variation among the men in our study and indicated that levels of saturated fat in plasma phospholipids are actively regulated. Nevertheless, in populations that consume diets high in saturated fat, blood levels of saturated and monounsaturated fatty acids were associated with dietary saturated fat,[14,15] probably because of their common sources in milk products and animal fat.[27] Blood levels of 20:3n-6 increased on a diet high in saturated fat and low in 18:2n-6.[16] These data suggest that high levels of 16:0, 16:1, and 20:3n-6 reflect a diet high in saturated fat relative to polyunsaturated fat.

A limitation when interpreting relative concentrations of fatty acids is that if the dietary intake of 1 fatty acid increases, the relative concentrations of some other fatty acids may decrease. However, in the present analysis, the associations of saturated and polyunsaturated fatty acids with blood pressure remained significant in multivariate analyses, which suggests that we observed true independent associations. Given the lack of dietary data and the crude measures of physical activity and alcohol used in the present study, we cannot exclude the possibility of residual confounding by lifestyle variables. However, the question of physical activity segregated groups according to physical fitness,[18] and the measure of alcohol use was strongly associated with levels of γ-glutamyltransferase[19] and usual alcohol consumption[34] in a population study conducted in the same geographical area as the present study.

The extent to which total plasma phospholipid fatty acids reflect fat metabolism or dietary fat is unknown. There was a strong positive association between the concentration of total fatty acids and total cholesterol, and dietary saturated fat is the main lifestyle determinant of total cholesterol levels.[1] We hypothesize that total fatty acids partly reflect dietary total fat and saturated fat intake.

The mechanisms by which fatty acids may influence blood pressure remain unknown. In humans, blood pressure and cardiac β-adrenergic receptor responsiveness decreased on a low-fat diet with a high P/S ratio.[34] A high fat meal reduced brachial artery reactivity, which suggested that fatty acids influence blood pressure by modulating endothelial function.[35] Dietary saturated fat may also promote atherosclerosis and arterial stiffening and thereby increase blood pressure. Carotid intima thickness was positively associated with blood levels of saturated fat.[36] Animal studies suggest that 18:2n-6 may reduce blood pressure by serving as a substrate for vasoactive prostaglandins[11] and promote relaxation of vascular smooth muscle cells.[37]

This study showed that plasma phospholipid total fatty acids and the proportions of saturated fatty acids and 18:2n-6 were independently associated with blood pressure and suggested that fatty acids are involved in blood pressure regulation. Additional studies are needed to determine whether these associations reflect cause-and-effect relations and whether blood pressure can decrease on a diet low in total and saturated fat and high in polyunsaturated 18:2n-6.

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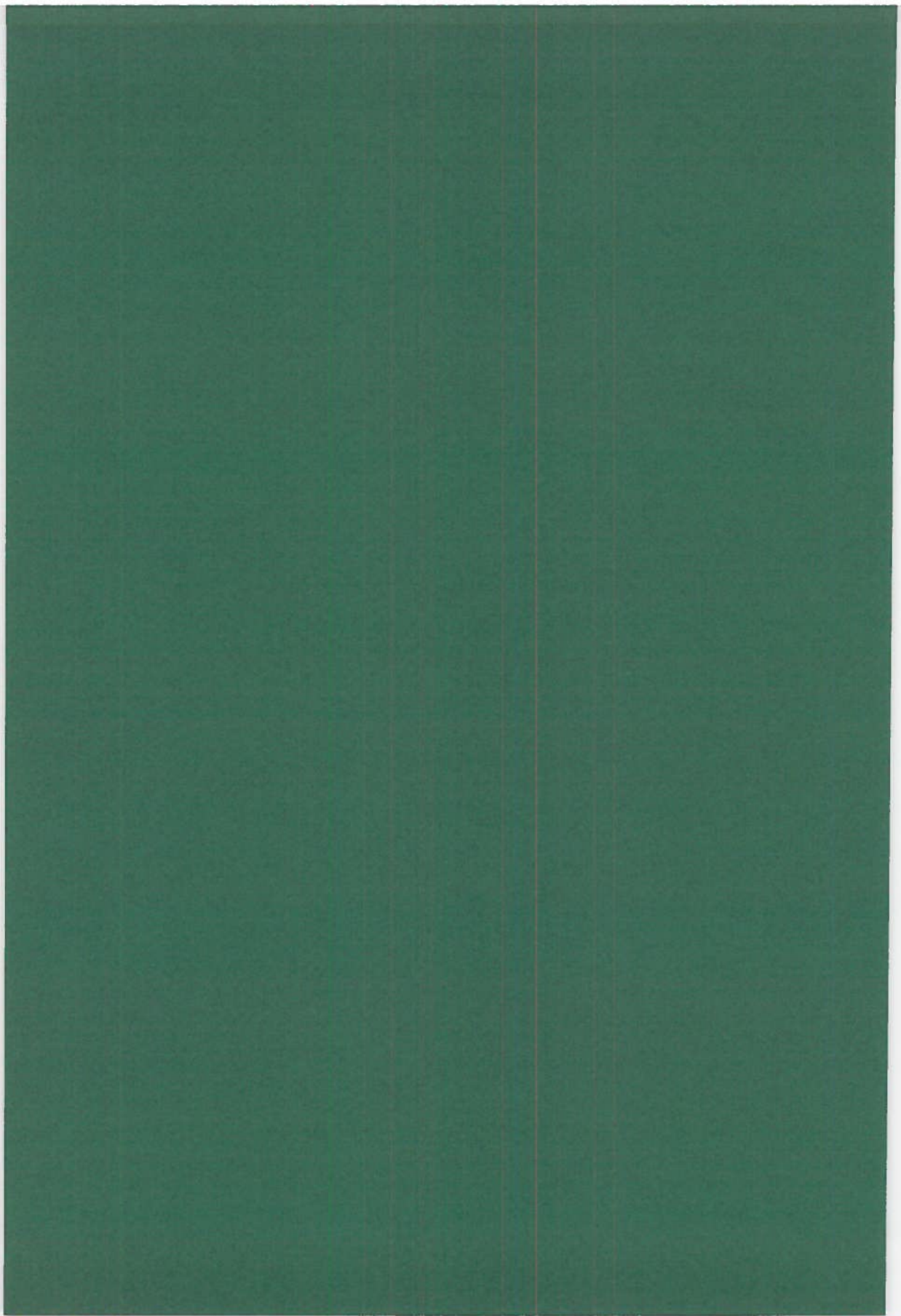
PAPER IV



Fatty Acid Chain Length and Degree of Unsaturation are Inversely Associated with Serum Triglycerides

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FOOTNOTES

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ABSTRACT

Little is known about the association between dietary fatty acids and serum triglyceride concentrations. Plasma fatty acids may reflect dietary intake and can be used to study the relationship between concentrations of individual fatty acids and serum lipids. We examined the cross-sectional relationship of plasma fatty acids with serum non-fasting triglyceride and total cholesterol concentrations. Relative concentrations of individual plasma phospholipid fatty acids were determined by gas liquid chromatography among 4158 men aged 40-42 years, who participated in a population study.

The pattern of associations between individual fatty acids and cholesterol was different than that between individual fatty acids and triglyceride concentrations. All fatty acids displayed positive associations with total cholesterol concentration, except linoleic acid, which was inversely related to cholesterol. In contrast, associations between individual fatty acids and triglyceride concentrations differed in strength and direction depending on both carbon chain length and the degree of unsaturation. Concentrations of very long chain (20 carbon atoms or longer) saturated, monounsaturated, and n-3 polyunsaturated fatty acids showed significant inverse associations with triglycerides, whereas shorter fatty acids within these classes were positively associated with triglyceride concentrations.

The present data suggest that the associations between concentrations of serum triglycerides and plasma phospholipid fatty acids depend on both fatty acids chain length and the degree of unsaturation.

INTRODUCTION

Serum triglyceride concentration is probably an independent risk factor for coronary heart disease (1), and the increased risk seems to start at relatively low concentrations of triglycerides (2). Dietary supplementation with n-3 polyunsaturated fatty acids decreases serum triglyceride concentrations (3). Little is known about the effects of other dietary fats (4), although a meta-analysis indicated that dietary polyunsaturated fat lowered triglycerides compared with monounsaturated fat (5).

There is an increasing awareness that individual fatty acids within the saturated, monounsaturated, n-6 and n-3 polyunsaturated fatty acid classes may have different effects on serum lipid and lipoprotein concentrations (6). Dietary fat intake is difficult to measure, and blood concentrations of fatty acids may be used to examine the relationship between individual fatty acids and serum lipids. Plasma concentrations of essential n-6 and n-3 polyunsaturated fatty acids are highly correlated with dietary intake (7-9). Concentrations of nonessential fatty acids are less reliable indicators of diet because they reflect dietary consumption as well as fatty acid synthesis and metabolism. Nevertheless, plasma concentrations of palmitic, stearic and monounsaturated fatty acids correlated with intake of saturated fat in individuals consuming a Western diet (7;8), probably because of their common sources in milk products and animal fat (10). In addition, high concentrations of eicosatrienoic (20:3n-9) and dihomo-γ-linolenic (20:3n-6) acids may reflect a diet relatively rich in saturated fatty acids (11;12).

Studies examining the relationship of blood concentrations of individual fatty acids with serum triglycerides and total cholesterol are not consistent (13-18). Most studies included few subjects (13-15), or measured a limited number of fatty acids (13;15;17;18).

We analyzed the association of individual plasma phospholipid fatty acids with serum concentrations of total cholesterol and triglycerides among 4158 men aged 40-42 years. This

large data set allowed a detailed investigation of the relationship between plasma fatty acids and serum triglycerides and total cholesterol in a population consuming a Western diet.

METHODS

Subjects

In 1988/89, all men and women aged 40-42 years living in Nordland county, Norway were invited to a health screening organized by the National Health Screening Service, the University of Tromsø and the local health authorities (19). Plasma phospholipid fatty acids were quantitated in men, of whom 5492 were invited and 4302 participated (78%). Measurements of plasma phospholipid fatty acids were missing in 144 men, leaving 4158 for the analysis. A second questionnaire was completed by 3590 men; thus the analysis on certain variables is restricted. The Norwegian Data Inspectorate considered legal and ethical issues and approved of the study. The subjects gave informed consent.

Questionnaires

The letter of invitation gave information about the survey and included a questionnaire about previous cardiovascular disease and smoking habits (yes/no). Leisure time physical activity was graded from I to IV according to which of the following categories that would best describe the participant's usual level of physical activity. I: reading or watching television or other sedentary activity; II: walking, cycling or other forms of exercise at least 4 hours a week; III: participation in recreational sports, heavy gardening etc. for at least 4 hours a week; IV: participation in hard training or sports competitions regularly several times a week. Categories III and IV were merged in our analysis. A nurse checked the questionnaire for logical inconsistencies and incomplete items at the examination, and obtained information about time since last meal. A second questionnaire, covering alcohol consumption and dietary habits, was handed out at the screening site and returned by mail. Alcohol consumption was categorized from I to VI. I: teetotaller; consumption of alcohol equivalent to 5 units of alcohol (1 unit

equals 9 grams of alcohol) II: no times last year; III: a few times last year; IV: 1-2 times per month; V: 1-2 times per week; VI: 3 times per week or more. We merged categories V and VI in the analysis. Two questions about habitual consumption of lean and fat fish for dinner meals were categorized from I to V. I: less than once weekly; II: once weekly; III: twice weekly; IV: three times weekly; V: four times weekly or more. We merged the two questions into four categories covering total weekly consumption of lean and fat fish for dinner. The questionnaire also assessed present use of fish oil supplements (yes/no).

Measurements

Body weight was measured on an electronic scale with subjects wearing light inner clothing and no shoes, and height was measured to the nearest centimeter. Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2). Systolic and diastolic blood pressure were measured by an automatic device (Dinamap, Critikon, Tampa) (19). A non-fasting blood sample was collected and analyzed in fresh serum for total cholesterol and triglycerides at the Central Laboratory, Ullevål Hospital, Oslo. The analyses were performed by an enzymatic colorimetric method (20;21), on a Hitachi 737 Automatic Analyzer (Boehringer Mannheim, Mannheim Germany) with reagents from the manufacturer. Measurement precision expressed as the coefficient of variation, was 3%, for both serum total cholesterol and triglycerides respectively.

EDTA plasma was stored at -80°C for a maximum of two years before phospholipid fatty acids (14:0, 16:0, 18:0, 20:0, 22:0, 24:0, 16:1, 18:1, 20:1, 22:1, 24:1, 20:3n-9, 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3) were quantitated by gas liquid chromatography as described previously (22). The coefficients of variation for individual fatty acids estimated from replicate analyses (n=55) of a serum sample ranged from 3.3% to 6.6%. Fatty acids were measured as relative concentrations (mol%). Trans-fatty acids were not measured.

Statistical analysis

All variables were normally distributed except serum triglycerides and plasma 20:5n-3, which were right skewed. Logtransformation had minor impact on the results and we therefore used untransformed data in the analysis. Pearson correlation coefficients were computed to evaluate the relationships between concentrations of individual plasma phospholipid fatty acids. We used simple linear regression analysis to predict change and 95% confidence interval (CI) in serum triglyceride and total cholesterol concentrations by 1 standard deviation (SD) increase in fatty acid concentration. Next, we developed multiple linear regression models to examine independent associations between individual fatty acids and serum lipids. Fatty acids significantly associated with serum triglycerides and total cholesterol in univariate analysis were considered for inclusion in the regression models. We included up to two fatty acids from each fatty acid class. Within each fatty acid class we chose the fatty acid which had the strongest positive and inverse relationship with triglycerides (18:0, 22:0 16:1, 24:1, 20:3n-6, 22:6n-3) and total cholesterol (22:0, 22:1, 20:3n-9, 18:2n-6, 22:4n-6), and where intercorrelations between fatty acids were less than r=0.60. Fatty acids significantly associated with triglycerides and total cholesterol respectively, were retained in the models. Finally we adjusted for body mass index and evaluated the effects of potential confounding variables, such as physical activity, alcohol consumption, daily smoking and time since last meal. Residual analyses confirmed the model assumptions (23). Differences between means were tested by two sample t-tests. Two sided p-values < 0.05 were considered statistically significant. The SAS software package was used (24).

RESULTS

Characteristics of study participants are shown in table 1. Among the 4158 men, 34% had serum total cholesterol concentrations ≥ 6.50 mmol/L, and 24% had non-fasting triglycerides > 2.50 mmol/L. A history of myocardial infarction, angina pectoris, stroke, diabetes mellitus,

or use of antihypertensive drugs was reported by 176 men. Fish consumption was relatively high, and 26% were present users of fish oil supplements. Non-fasting serum triglyceride level decreased with time since last meal (Table 2). Plasma phospholipid palmitic (16:0), palmitoleic (16:1), 20:3n-9, 20:3n-6, eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids increased, whereas linoleic (18:2n-6) acid concentrations decreased slightly when time since last meal increased (data not shown).

Inter-relationships between concentrations of plasma phospholipid fatty acids

Concentrations of very long chain (20-24 carbon atoms) fatty acids within the saturated and n-3 polyunsaturated fatty acid classes were highly intercorrelated (Table 3). Very long chain polyunsaturated fatty acids of the n-3 class were positively correlated with very long chain monounsaturated fatty acids and inversely correlated with fatty acids of the n-6 class.

Univariate relationship of plasma phospholipid fatty acids with serum non-fasting triglycerides and total cholesterol

Concentrations of stearic (18:0) acid, 16:1 and 20:3n-6 showed strong positive associations with triglyceride concentrations (Table 4 and figures 1-2). Very long chain saturated, monounsaturated and n-3 polyunsaturated fatty acids, particularly lignoceric (24:0) acid, nervonic (24:1) acid and 22:6n-3 were inversely associated with triglycerides. Both fatty acid chain length and the degree of unsaturation were related to triglyceride concentrations (Table 5). The association between triglycerides and chain length appeared to be stronger than the association between triglycerides and degree of unsaturation. Very long chain saturated and monounsaturated fatty acids were positively associated with total cholesterol and inversely associated with triglyceride concentrations (Table 4 and figure 1). The associations of saturated and monounsaturated fatty acids with triglycerides differed depending on carbon chain length, whereas all saturated and monounsaturated fatty acids were positively associated with total cholesterol. The associations of fatty acids with triglyceride concentrations were

generally stronger than the associations with total cholesterol. 18:2n-6 was the only fatty acid showing a significant inverse association with cholesterol concentrations.

Multivariate relationship of plasma phospholipid fatty acids with serum non-fasting triglycerides and total cholesterol

Fatty acids explained 19% of the variance in triglyceride concentration (Table 6, model I). Concentrations of 18:0, 16:1, 24:1 and 20:3n-6 were independently associated with triglycerides. The associations remained significant when controlling for BMI, physical activity and alcohol consumption (Table 6, model II). n-3 fatty acids were not significantly associated with triglycerides when 24:1 was included in the multiple regression model. There was interaction between alcohol consumption and 16: 1 in prediction of triglycerides. Concentrations of 16:1 increased significantly with increasing alcohol consumption (p=0.0001, data not shown). The association between 16:1 and triglyceride concentrations weakened with increasing alcohol consumption, and 16:1 was not significantly associated with triglycerides among men in the highest category of alcohol consumption. The strongest association between concentrations of 16:1 and triglycerides was observed among non-drinkers (data not shown).

Fatty acids explained 7% of the variance in total cholesterol concentration (Table 6 model I). Concentration of 18:2n-6 was not significantly associated with cholesterol after adjustment for body mass index. (Table 6 model II). Adjustment for daily smoking did not affect the results of the multiple regression analyses. Both stratified and adjusted analyses showed that time since last meal did not influence the relationship of plasma phospholipid fatty acids with serum triglycerides and total cholesterol.

Consumption of fish products, plasma phospholipid fatty acids and serum triglycerides

Use of fish oil supplements and the level of habitual fish consumption and were positively associated with concentrations of gondoic (20:1) acid, erucic (22:1) acid, 24:1 and n-3 fatty

acids (all $p<0.001$) (data not shown). Present-users of fish oil had 10% lower mean triglyceride concentration compared with non-users ($p=0.0001$). In multiple regression analysis, fish consumption was inversely associated with serum triglyceride concentrations after adjustment for body mass index ($p=0.047$). Fish consumption was not significantly associated with serum triglyceride concentrations when 24:1 or 22:6n-3 were added to the regression model.

DISCUSSION

This study shows that fatty acid chain length, as well as the degree of unsaturation, is associated with serum non-fasting triglyceride concentrations. Very long chain fatty acids of the saturated, monounsaturated and n-3 classes were inversely associated with triglycerides, and the associations increased in magnitude with increasing fatty acid chain length. Interestingly, individual very long chain saturated and monounsaturated fatty acids were positively associated with total cholesterol, and inversely associated with triglyceride concentrations. This observation has to our knowledge not been reported previously.

Associations of fatty acids with non-fasting triglycerides were not influenced by time since last meal. The analysis can reflect that plasma phospholipid fatty acids explain a component of the inter-individual variability in serum triglyceride levels, which is not determined by time since last meal.

Dietary supplementation with very long chain n-3 fatty acids decreases liver VLDL synthesis (25) and serum triglyceride concentrations (3). Studies in rats showed that 20:5n-3 increased mitochondrial beta-oxidation, and reduced fatty acid substrate for VLDL synthesis (26). Mitochondria, the major sites for fatty acid oxidation, are poor oxidizers of very long chain fatty acids (27), but proliferated during dietary supplementation with very long chain n-3 fatty acids (28). Based on these observations we may speculate that very long chain saturated and monounsaturated fatty acids stimulate mitochondrial proliferation, whereby fatty acid

oxidation increase and VLDL triglycerides decrease. In contrast to fatty acids of the saturated, monounsaturated and n-3 classes, n-6 fatty acids displayed no distinct pattern of association with triglycerides.

We found that concentrations of very long chain monounsaturated and n-3 fatty acids were positively associated with habitual fish consumption and inversely associated with serum triglycerides. Others have reported that level of fish consumption and triglyceride concentrations were inversely related (14;29). In previous cross-sectional studies however, concentrations of 20:5n-3 were both positively (13), and inversely (14) associated with serum triglycerides.

Cross-sectional studies indicate that plasma phospholipid levels of essential polyunsaturated fatty acids reflect dietary intake (7-9). Dietary supplementation studies confirm that plasma phospholipid concentrations of 18:2n-6, 18:1, 20:5n-3 and 22:6n-3 are sensitive to changes in dietary intake of these fatty acids (30;31). The origins of very long chain saturated and monounsaturated fatty acids are less clear. They may be metabolic products of shorter fatty acids. Fish and fish oils contain 20:1 and 22:1 as well as n-3 fatty acids (10), and one cross-sectional study showed that plasma phospholipid concentrations of 24:1, 20:5n-3 and 22:6n-3 were positively associated with fish consumption (14). It is possible that concentrations of very long chain monounsaturated fatty acids reflect dietary fish and fish oil intake.

In the present study, the inverse associations of 22:1 and 24:1 with triglycerides were stronger than the association of 22:6n-3 with triglyceride concentrations. When 22:1 or 24:1 was added to a regression model including only 22:6n-3, the explained variability in triglycerides increased significantly. This indicates that very long chain monounsaturated fatty acids are independently associated with triglyceride concentrations, and are not merely indicators of dietary n-3 fatty acids.

Plasma phospholipid concentrations of 18:0, 16:1 and 20:3n-6 displayed independent positive associations with triglyceride concentrations. The observation finds support in the Paris Prospective Study, where plasma concentrations of 16:1 and 20:3n-6 were positively associated with triglycerides (16). A dietary intervention trial showed that serum triglyceride concentration increased significantly on a 18:0 enriched diet, compared with a diet rich in 18:2n-6 (32). Plasma concentrations of 18:0 and 16:1 were positively associated with dietary saturated fat in cross-sectional studies (7;8) and 20:3n-6 increased on a saturated fat diet (11). Thus plasma concentrations of 18:0, 16:1 and 20:3n-6 appear to reflect dietary saturated fat to some extent.

Alcohol consumption may be a potential confounder in the relationship between 16:1 and triglycerides, since alcohol intake is associated with high levels of 16:1 (16;33;34) and serum triglycerides (35). In the present study the association between 16:1 and triglycerides remained significant when controlling for alcohol intake, but residual confounding may remain since we used a rather crude estimate of alcohol intake. However, stratified analyses showed that the association between 16:1 and triglycerides was particularly strong among teetotallers and those who consumed alcohol less than, or a few times yearly. This suggests that the association between 16:1 and triglycerides does not depend on alcohol intake.

Serum non-fasting triglycerides are subject to larger intraindividual variation than are total cholesterol concentrations (36), and this will tend to dilute any association between triglycerides and concentrations of fatty acids. We found that fatty acids explained 19% of the variability in triglyceride, and 7% of the variability in total cholesterol concentrations. Therefore, the biological association of plasma phospholipid fatty acids with serum triglycerides appears to be stronger than the association with total cholesterol.

In the present study, plasma phospholipid concentrations of 22:0, 22:1 and 20:3n-9 were independently and positively associated with serum total cholesterol concentrations. It is not

clear which factors determine plasma concentrations of 22:0, 22:1 and 20:3n-9. They may be metabolic products of shorter saturated fatty acids. Fish products can be a source of 22:1, as noted above. When dietary intake of polyunsaturated fat is low, the metabolic conversion of 18:1 to 20:3n-9 increases (12). High levels of plasma 20:3n-9 may thus reflect a diet relatively rich in saturated fat.

Plasma concentrations of 18:2n-6 are correlated with dietary intake (7;8). We found that 18:2n-6 concentrations were inversely associated with total cholesterol. Small observational studies failed to detect any association between 18:2n-6 and total cholesterol (13;15). Larger studies however, found that plasma (16;18) and adipose tissue (37;38) concentrations of 18:2n-6 were inversely associated with total cholesterol. These data are supported by feeding experiments showing that dietary 18:2n-6 decrease total and LDL cholesterol concentrations (6), supposedly by increasing liver LDL receptor activity (39).

Our findings are strengthened by the population based study design, the relatively high participation rate and large sample size. The study was conducted among men in a narrow age range consuming a Western diet, and the results need confirmation among women and other age groups. We did not measure trans-fatty acids and it is not known how they may influence the results. Relative concentration (proportions) of fatty acids were used in the analysis. The use of proportions is problematic since an increase in one proportion is associated with a decrease in some other proportion(s). However, as seen in table 2, most correlations between fatty acids included in the present analyses were not particularly strong. We therefore believe the findings reflect biologic differences between individual fatty acids and not simply the interrelationship between proportions of fatty acids. Many associations of individual fatty acids with serum triglyceride and total cholesterol concentrations weakened after adjustment for body mass index. It is however questionable whether adjustment for body mass index is

appropriate, since plasma phospholipid fatty acids and body mass index may be intermediate variables in the relationship of dietary fat with serum cholesterol and triglycerides.

In summary, the present data show that there are major differences with regard to both the strength and the direction of the association between serum triglyceride concentration and individual fatty acids within the same fatty acid class. These results suggest that the association between triglycerides and fatty acid depends on both fatty acid chain length and the degree of unsaturation.

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FIGURE LEGENDS

Figure 1

Mean serum total cholesterol (upper panels) and triglyceride (lower panels) concentrations by deciles of plasma phospholipid saturated (left panels) and monounsaturated (right panels) fatty acids

Figure 2

Mean serum total cholesterol (upper panels) and triglyceride (lower panels) concentrations by deciles of plasma phospholipid n-6 and n-9 polyunsaturated (left panels) and n-3 polyunsaturated (right panels) fatty acids

TABLE 1
Characteristics of Study Participants: 4158 Men 40-42 Years Old

Characteristic	Mean ± SD or %
Serum total cholesterol (mmol/L)	6.12 ± 1.16
Serum non-fasting triglycerides (mmol/L)	1.98 ± 1.22
Systolic blood pressure (mmHg)	133 ± 14
Diastolic blood pressure (mmHg)	81 ± 10
Body mass index (kg/m^2)	25.3 ± 3.2
Daily smoking	46
Leisure time physical activity	
Sedentary	23
Moderate	52
Regular/hard training	25
Alcohol consumption [a]	
Teetotaller	4
≥ 5 units of alcohol	
< once yearly	20
A few times yearly	45
1-2 times monthly	24
1-2 times weekly or more	7
Lean or fat fish for dinner [b]	
< twice weekly	34
Twice weekly	30
Three times weekly	22
Four times weekly or more	14

[a] n=3501
[b] n=3530

TABLE 2
Non-fasting Serum Triglycerides According to Time Since Last Meal in 4158 Men

Time since last meal	n	Serum triglycerides Mean ± SD
hours		*mmol/L*
0	850	2.11 ± 1.23
1	1204	2.08 ± 1.20
2	792	2.04 ± 1.30
3	509	1.88 ± 1.18
4	375	1.75 ± 1.16
5	178	1.60 ± 0.87
6	75	1.40 ± 0.98
7	22	1.26 ± 0.74
8	11	1.82 ± 1.02
9	128	1.66 ± 1.28
missing	14	2.30 ± 2.10

TABLE 3
Correlations Between Levels of Plasma Phospholipid Fatty Acids (mol%) in 4158 Men [a]

Fatty acid	Fatty acid																				
	16:0	18:0	20:0	22:0	24:0	16:1	18:1	20:1	22:1	24:1	20:3n-9	18:2n-6	20:2n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6	18:3n-3	20:5n-3	22:5n-3	22:6n-3
14:0	0.11	-0.04	0.01	0.08	-0.03	0.20	0.14	0.06	0.07	-0.03	0.14	-0.13	0.09	0.14	-0.11	0.08	0.07	0.07	0.03	0.03	-0.05
16:0		-0.60	-0.14	-0.14	-0.05	0.46	0.23	-0.19	-0.20	0.06	0.19	-0.35	-0.04	0.09	0.05	-0.07	-0.01	-0.12	-0.01	-0.02	-0.02
18:0			-0.07	-0.12	-0.14	-0.32	-0.16	0.00	-0.06	-0.18	-0.10	0.14	-0.01	0.12	-0.03	-0.21	-0.03	0.03	-0.02	0.02	0.01
20:0				0.80	0.65	-0.16	-0.29	-0.21	0.25	0.56	-0.16	-0.05	0.19	-0.11	-0.04	0.37	-0.12	0.09	-0.07	-0.07	-0.03
22:0					0.62	-0.27	-0.31	-0.22	0.31	0.41	-0.17	0.04	0.15	-0.15	-0.04	0.53	-0.13	-0.12	-0.13	-0.18	-0.12
24:0						-0.23	-0.27	-0.36	-0.01	0.71	-0.15	-0.09	0.21	0.00	0.05	0.24	-0.07	-0.13	-0.07	-0.05	-0.08
16:1							0.60	0.06	-0.08	-0.17	0.53	-0.35	0.21	0.32	0.05	0.08	0.29	0.30	-0.02	0.08	-0.08
18:1								-0.01	-0.22	-0.30	0.59	-0.09	0.20	0.24	-0.03	-0.02	0.27	0.18	-0.26	-0.04	-0.32
20:1									0.52	-0.04	-0.02	-0.29	-0.13	-0.26	-0.19	-0.08	-0.01	0.08	0.65	0.27	0.41
22:1										0.25	-0.07	-0.23	-0.07	-0.32	-0.21	0.34	0.02	-0.06	0.46	0.16	0.32
24:1											-0.20	-0.46	0.08	-0.24	-0.05	0.16	-0.18	-0.19	0.37	0.25	0.36
20:3n-9												-0.26	0.23	0.45	0.31	0.21	0.51	0.06	-0.18	0.00	-0.26
18:2n-6													0.02	-0.08	-0.26	-0.07	-0.11	0.17	-0.64	-0.53	-0.62
20:2n-6														0.37	-0.07	0.26	0.21	0.14	-0.27	-0.04	-0.21
20:3n-6															0.37	0.07	0.47	0.00	-0.40	-0.13	-0.34
20:4n-6																0.15	0.39	-0.20	-0.19	-0.09	-0.18
22:4n-6																	0.31	-0.05	-0.14	-0.12	-0.17
22:5n-6																		-0.01	-0.23	-0.02	-0.22
18:3n-3																			-0.08	-0.01	-0.14
20:5n-3																				0.47	0.72
22:5n-3																					0.65

[a] Pearson correlation coefficients between relative concentrations (mol%) of fatty acids. Values > |0.03|, |0.04| and |0.05| are significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

TABLE 4
Concentrations of Plasma Phospholipid Fatty Acids, and Change in Serum Non-Fasting Triglycerides and Total Cholesterol by 1 SD Increase in Fatty Acid Concentration in 4158 Men

		Predicted mean change (95% CI)	
Fatty acid	Mean ± SD	Triglycerides	Total cholesterol
	mol%	*mmol/L*	
Saturated fatty acids [a]	44.10 ± 1.34	0.02 (-0.02, 0.06)	0.16 (0.13, 0.19)
Myristic, 14:0	0.36 ± 0.13	0.10 (0.07, 0.14)	0.08 (0.05, 0.11)
Palmitic, 16:0	26.64 ± 1.54	0.00 (-0.03, 0.04)	0.01 (-0.03, 0.04)
Stearic, 18:0	13.89 ± 0.94	0.27 (0.24, 0.31)	0.07 (0.04, 0.11)
Arachidic, 20:0	0.50 ± 0.13	-0.26 (-0.29, -0.22)	0.10 (0.06, 0.13)
Behenic, 22:0	1.87 ± 0.58	-0.23 (-0.27, -0.19)	0.17 (0.14, 0.21)
Lignoceric, 24:0	0.85 ± 0.28	-0.28 (-0.32, -0.24)	0.05 (0.01, 0.08)
Monounsaturated fatty acids [b]	10.71 ± 1.32	0.06 (0.03, 0.10)	0.06 (0.02, 0.09)
Palmitoleic, 16:1	0.37 ± 0.19	0.25 (0.22, 0.29)	0.11 (0.08, 0.15)
Oleic, 18:1	8.52 ± 1.25	0.19 (0.15, 0.22)	0.00 (-0.03, 0.04)
Gondoic, 20:1	0.27 ± 0.13	-0.06 (-0.10, -0.02)	0.10 (0.06, 0.13)
Erucic, 22:1	0.15 ± 0.13	-0.13 (-0.17, -0.09)	0.19 (0.15, 0.22)
Nervonic, 24:1	1.39 ± 0.54	-0.33 (-0.36, -0.29)	0.03 (-0.01, 0.06)
Eicosatrienoic acid, 20:3n-9	0.11 ± 0.07	0.20 (0.16, 0.24)	0.18 (0.15, 0.22)
n-6 fatty acids [c]	34.74 ± 3.67	0.12 (0.08, 0.15)	-0.12 (-0.15, -0.09)
Linoleic, 18:2n-6	23.44 ± 3.65	0.05 (0.02, 0.09)	-0.15 (-0.18, -0.11)
Eicosadienoic, 20:2n-6	0.38 ± 0.07	0.13 (0.09, 0.17)	0.15 (0.12, 0.18)
Dihomo-γ-linolenic, 20:3n-6	2.46 ± 0.67	0.39 (0.35, 0.43)	0.10 (0.06, 0.14)
Arachidonic, 20:4n-6	7.87 ± 1.39	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.00)
Adrenic, 22:4n-6	0.50 ± 0.27	-0.03 (-0.07, 0.01)	0.20 (0.17, 0.24)
Docosapentaenoic, 22:5n-6	0.09 ± 0.05	0.20 (0.17, 0.24)	0.11 (0.07, 0.15)
n-3 fatty acids [d]	10.34 ± 3.31	-0.17 (-0.20, -0.13)	0.04 (0.01, 0.08)
α-Linolenic, 18:3n-3	0.18 ± 0.09	0.10 (0.06, 0.13)	0.01 (-0.03, 0.04)
Eicosapentaenoic, 20:5n-3	2.39 ± 1.75	-0.14 (-0.18, -0.10)	0.05 (0.01, 0.08)
Docosapentaenoic, 22:5n-3	1.22 ± 0.24	-0.15 (-0.18, -0.11)	0.00 (-0.03, 0.04)
Docosahexaenoic, 22:6n-3	6.54 ± 1.67	-0.17 (-0.20, -0.13)	0.04 (0.00, 0.07)

[a] Saturated fatty acids include 14:0, 16:0, 18:0, 20:0, 22:0, 24:0
[b] Monounsaturated fatty acids include 16:1, 18:1, 20:1, 22:1, 24:1.
[c] n-6 fatty acids include 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.
[d] n-3 fatty acids include 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.

TABLE 5
Concentrations of Plasma Phospholipid Fatty Acids and Change in Serum Non-Fasting Triglyceride Concentrations According to Fatty Acid Chain Length and Number of Double Bonds in 4158 Men

Fatty acid group	Mean ± SD	Change in triglycerides [a]
Carbon chain length [b]	*mol%*	*mmol/L*
C20	13.99 ± 2.11	-0.02 (-0.06, 0.02)
C22	10.37 ± 1.93	-0.24 (-0.28, -0.20)
C24	2.24 ± 0.76	-0.33 (-0.37, -0.30)
Number of double bonds [c]		
4	8.37 ± 1.46	-0.03 (-0.07, 0.01)
5	3.70 ± 1.87	-0.15 (-0.18, -0.11)
6	6.54 ± 1.67	-0.17 (-0.20 ,-0.13)

[a] Values are predicted mean change (95%CI) by 1 SD increase in fatty acid concentration
[b] C20 = 20:0+20:1+20:3n-9+20:2n-6+20:3n-6+20:4n-6+20:5n-3
C22 = 22:0+22:1+22:4n-6+22:5n-6+22:5n-3+22:6n-3
C24 = 24:0+24:1
[c] 4 = 20:4n-6+22:4n-6
5 = 22:5n-6+20:5n-3+22:5n-3
6 = 22:6n-3

TABLE 6
Multiple Linear Regression Analysis of Serum Non-Fasting Triglycerides and Total Cholesterol in 4158 Men [a]

Predictor variable	Model I	Model II
	Triglycerides (mmol/L)	
Fatty acid (mol%)		
18:0	0.29 (0.25, 0.33)	0.25 (0.21, 0.29)
16:1	0.24 (0.20, 0.28)	0.18 (0.14, 0.23)
24:1	-0.18 (-0.22, -0.14)	-0.22 (-0.26, -0.19)
20:3n-6	0.24 (0.20, 0.28)	0.15 (0.11, 0.19)
Body mass index (kg/m^2)		0.28 (0.24, 0.31)
Physical activity (1-3) [2]		-0.16 (-0.21, -0.11)
Alcohol consumption (0-4) [b]		0.05 (0.01, 0.09)
Adjusted R^2 (%)	19	25 [c]
	Total cholesterol (mmol/L)	
Fatty acid (mol%)		
22:0	0.17 (0.13, 0.21)	0.17 (0.13, 0.20)
22:1	0.13 (0.09, 0.17)	0.15 (0.11, 0.19)
20:3n-9	0.20 (0.17, 0.24)	0.19 (0.16, 0.23)
18:2n-6	-0.07 (-0.11, -0.04)	-0.02 (-0.05. 0.02)
Body mass index (kg/m^2)		0.20 (0.17, 0.23)
Physical activity (1-3) [b]		-0.10 (-0.14, -0.05)
Adjusted R^2 (%)	7	11 [d]

[a] Values are predicted mean change (95% CI) by 1 SD increase in predictor variable and by one category increase in physical activity and alcohol consumption
[b] See table 1 for categories of physical activity and alcohol consumption
[c] n=3499
[d] n=4155

Figure 1

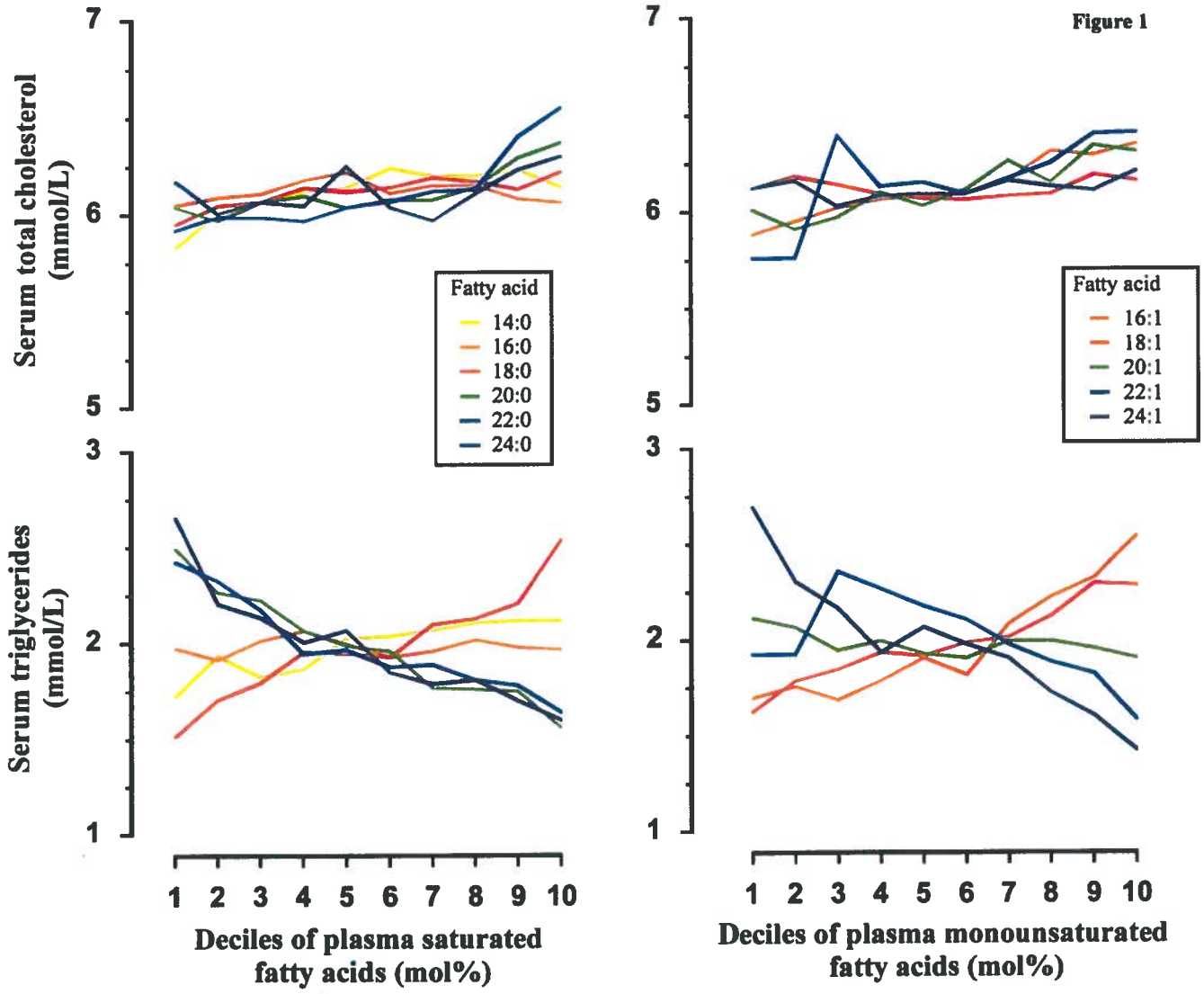
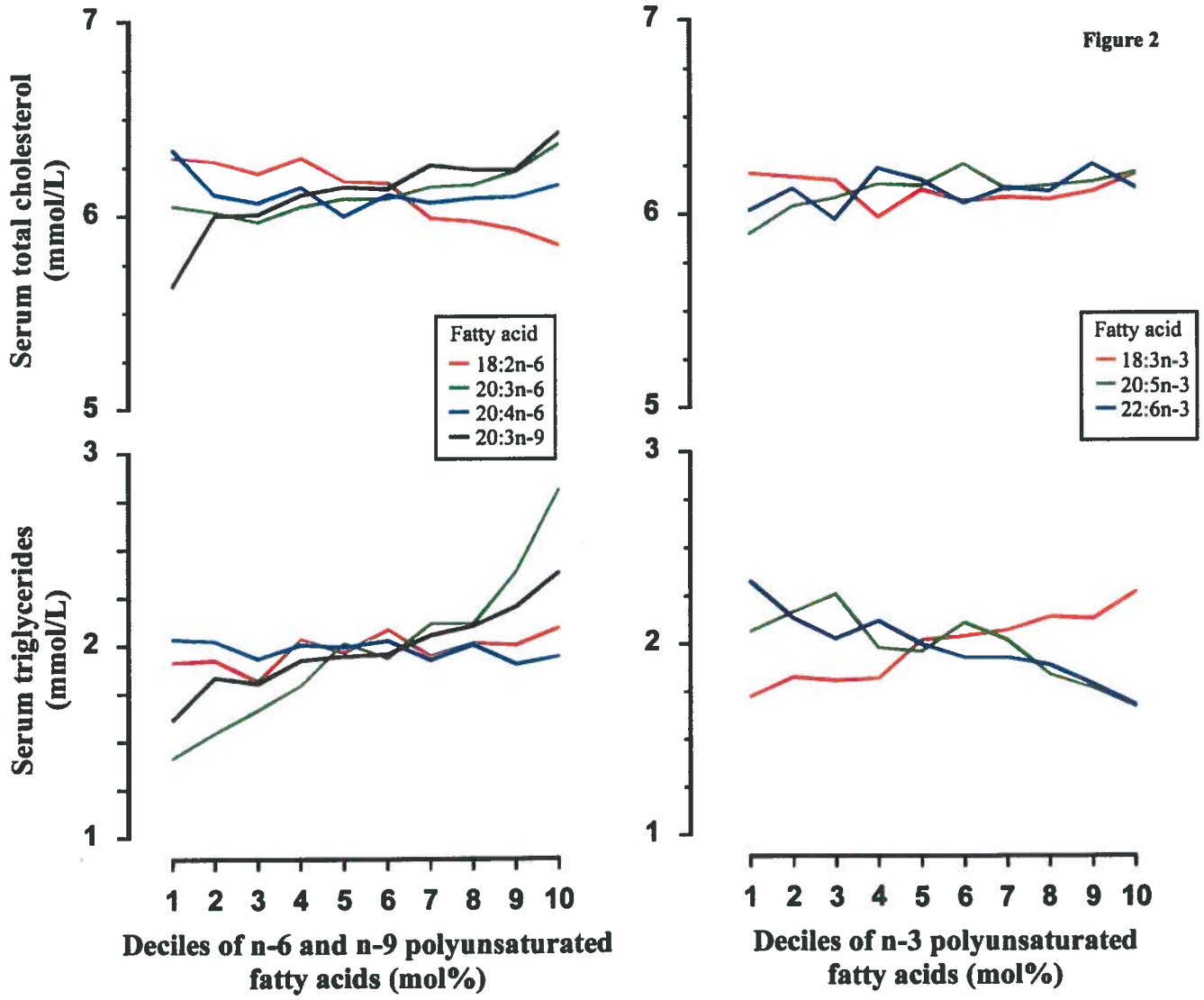

Serum total cholesterol (mmol/L)
7
6
5
Fatty acid
14:0
16:0
18:0
20:0
22:0
24:0
16:1
18:1
20:1
22:1
24:1
Serum triglycerides (mmol/L)
3
2
1
1 2 3 4 5 6 7 8 9 10
Deciles of plasma saturated fatty acids (mol%)
Deciles of plasma monounsaturated fatty acids (mol%)

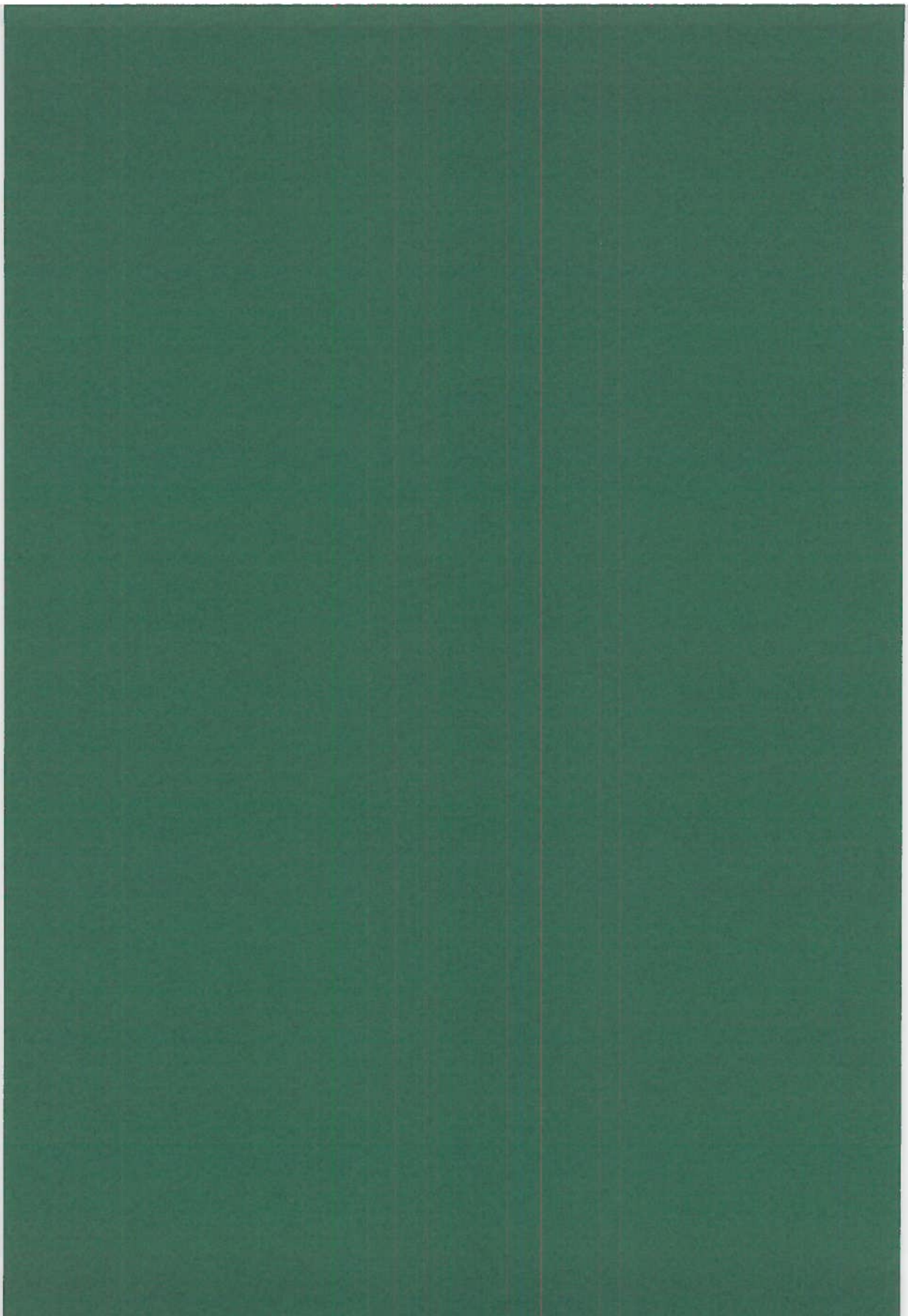
Figure 2


Serum total cholesterol (mmol/L)
Serum triglycerides (mmol/L)
Fatty acid
18:2n-6
20:3n-6
20:4n-6
20:3n-9
Deciles of n-6 and n-9 polyunsaturated fatty acids (mol%)
Fatty acid
18:3n-3
20:5n-3
22:6n-3
Deciles of n-3 polyunsaturated fatty acids (mol%)

APPENDIX 1

Questionnaire 1 The Tromsø Study 1986-1987

Original version and English translation



HELSEUNDERSØKELSEN I TROMSØ

(Gjelder bare den person som brevet er adressert til.)

Helseundersøkelsen kommer nå til Deres distrikt.

Tid og sted for frammøte vil De finne nedenfor.

De finner en orientering om undersøkelsen i den vedlagte brosjyren.

Vi ber Dem vennligst fylle ut spørreskjemaet på baksiden og ta med dette til undersøkelsen.

Vi ber Dem eventuelt melde fra om fravær på den vedlagte fraværsmeldingen.

Med hilsen

KOMMUNEHELSETJENESTEN I TROMSØ
FYLKESLEGEN I TROMS UNIVERSITETET I TROMSØ
STATENS HELSEUNDERSØKELSER

Født dato | Personnr. | Kommune | Kretsnr.

Møtested | Kjønn | Første bokstav i etternavn | Dag og dato | Klokkeslett

HØYDE VEKT ANM 70	M P Ø KODE 75	AVVIK ARM MAN APP.NR. TSM 82
MÅLING 1	**MÅLING 2**	**MÅLING 3**
MAR 85 S 88	MAR 91 S 94	MAR 97 S 100
HR 103 D 106	HR 109 D 112	HR 115 D 118

A FAMILIE

Har en eller flere av foreldre eller søsken hatt hjerteinfarkt (sår på hjertet) eller angina pectoris (hjertekrampe)? 12

JA | NEI | VET IKKE

B EGEN SYKDOM

Har De, eller har De hatt:

Hjerteinfarkt? 13
Angina pectoris (hjertekrampe)? 14
Hjerneslag? 15
Sukkersyke? 16

Er De under behandling for:

Høyt blodtrykk? 17

Bruker De:

Nitroglycerin? 18

C SYMPTOMER

Får De smerter eller ubehag i brystet når De:

Går i bakker, trapper eller fort på flat mark? 19
Går i vanlig takt på flat mark? 20

Dersom De får smerter eller vondt i brystet ved gange, pleier De da:

Stoppe? 21
Saktne farten? .....
Fortsette i samme takt? .....

Dersom De stopper eller saktner farten, går da smertene bort:

Etter mindre enn 10 minutter? 22
Etter mer enn 10 minutter? .....

Har De vanligvis:

Hoste om morgenen? 23
Oppspytt fra brystet om morgenen? 24

D MOSJON

Bevegelse og kroppslig aktivitet i Deres fritid. Dersom aktiviteten varierer mye, f.eks. mellom sommer og vinter, så ta ett gjennomsnitt. Spørsmålet gjelder bare det siste året.

Sett kryss i den ruten som passer best.

Leser, ser på fjernsyn eller annen stillesittende beskjeftigelse? ..... 25 — 1

Spaserer, sykler eller beveger Dem på annen måte minst 4 timer i uken? ..... (Her skal De også regne med gang eller sykling til arbeidsstedet, søndagsturer m.m.) — 2

Driver mosjonsidrett, tyngre hagearbeid e.l.? .. (Merk at aktiviteten skal være i minst 4 timer i uken.) — 3

Trener hardt eller driver konkurranseidrett regelmessig og flere ganger i uken? ..... — 4

E SALT/FETT

Hvor ofte bruker De salt kjøtt eller salt fisk til middag?

Sett kryss i den ruten som passer best.

Aldri eller sjeldnere enn en gang i måneden ..... 26 — 1
Inntil en gang i uken ..... — 2
Inntil to ganger i uken ..... — 3
Mer enn to ganger i uken ..... — 4

Hvor ofte pleier De å strø ekstra salt på middagsmaten?

Sett kryss i den ruten som passer best.

Sjelden eller aldri ..... 27 — 1
Av og til eller ofte ..... — 2
Alltid eller nesten alltid ..... — 3

Hva slags margarin eller smør bruker De vanligvis på brødet?

Sett kryss i den ruten som passer best.

Bruker ikke smør eller margarin på brød ..... 28 — 1
Smør ..... — 2
Hard margarin ..... — 3
Myk (Soft) margarin ..... — 4
Smør/margarin blanding ..... — 5

Hva slags fett blir vanligvis brukt til matlaging i husholdningen Deres?

Sett kryss i den ruten som passer best.

Smør eller hard margarin ..... 29 — 1
Myk (Soft) margarin eller olje ..... — 2
Smør/margarin blanding ..... — 3

F RØYKING

Røyker De daglig for tida? 30

Dersom svaret er «JA», svar da på dette:

Røyker De sigaretter daglig? 31
(håndrullede eller fabrikkfremstilte)

Dersom De ikke røyker sigaretter nå, svar da på dette:

Har De røykt sigaretter daglig tidligere? 32

Dersom De svarte «JA», hvor lenge er det da siden De sluttet?

Mindre enn 3 måneder? ..... 33 — 1
3 måneder – 1 år? ..... — 2
1–5 år? ..... — 3
Mer enn 5 år? ..... — 4

Skal besvares av de som røyker nå eller som har røykt tidligere:

Hvor mange år til sammen har De røykt daglig? ..... 34 — År

Hvor mange sigaretter røyker eller røykte De daglig?
Gi opp antallet sigaretter daglig ..... 36 — Sigaretter
(håndrullede + fabrikkfremstilte)

Røyker De noe annet enn sigaretter daglig?

Sigarer eller sørutter/sigarillos? 40
Pipe? 41

Dersom De røyker pipe, hvor mange pakker tobakk (50 gram) bruker De i pipen på en uke?

Gi opp gjennomsnittlig tall på pakker i uken ..... 42 — Tobakkspk

G KAFFE

Hvor mange kopper kaffe drikker De vanligvis hver dag?

Sett kryss i den ruten som passer best.

Drikker ikke kaffe, eller mindre enn en kopp ..... 45 — 1
1 – 4 kopper ..... — 2
5 – 8 kopper ..... — 3
9 eller flere kopper ..... — 4

Hva slags kaffe drikker De vanligvis hver dag?

Kokekaffe 46
Filterkaffe 47
Pulverkaffe 48
Koffeinfri kaffe 49
Drikker ikke kaffe 50

H ARBEID

JA | NEI

Har De i de siste 12 månedene fått arbeidsledighetstrygd? 51

Er De for tiden sykemeldt, eller får De attføringspenger? 52

Har De full eller delvis uførepensjon? 53

Har De vanligvis skiftarbeid eller nattarbeid 54

Har De i det siste året hatt:

Sett kryss i den ruten som passer best.

For det meste stillesittende arbeid? ..... 55 — 1
(f.eks. skrivebordsarb., urmakerarb., montering)
Arbeide som krever at De går mye? ..... — 2
(f.eks. ekspeditørarb., lett industrarb., undervisn.)
Arbeide der De går og løfter mye? ..... — 3
(f.eks. postbud, tyngre industrarb., bygningsarb.)
Tungt kroppsarbeid? ..... — 4
(f.eks. skogsarb., tungt jordbruksarb., tungt bygningarb.)

JA | NEI

Er husmorarbeid hovedyrket Deres? 56

I ETTERUNDERSØKELSE

Har noen i husstanden Deres (utenom Dem selv) vært innkalt til nærmere undersøkelse hos lege etter den siste hjertekarundersøkelsen? 57

Dersom denne helseundersøkelsen viser at De bør undersøkes nærmere: Hvilken almenpraktiserende lege ønsker De da å bli henvist til?

Skriv navnet på legen her →

..... 58

Ingen spesiell lege .....

61

Ikke skriv her

Ikke skriv her

QUESTIONNAIRE I, TROMSØ SURVEY 1986-87

English translation; Mrs. Anne Clancy and Mr. Kevin McCafferty

A FAMILY

Have one or both of your parents, or any of your siblings (brothers and sisters) had a heart attack or angina pectoris (heart cramp)?

Yes	*No*	*Don't know*
☐	☐	☐

B OWN ILLNESSES

Have you, or have you had:	*Yes*	*No*
A heart attack?	☐	☐
Angina pectoris (heart cramp) ?	☐	☐
A cerebral stroke?	☐	☐
Diabetes?	☐	☐

Are you receiving treatment for:	*Yes*	*No*
High blood pressure?	☐	☐
Do you use nitroglycerine?	☐	☐

C SYMPTOMS

Do you get pain or discomfort in the chest, when:	*Yes*	*No*
Walking up hills, stairs or walking fast on level ground?	☐	☐
Walking at ordinary pace on level ground?	☐	☐

If you get pain or discomfort in your chest when walking, do you usually :

	Yes
Stop	☐
Slow down	☐
Carry on at the same pace	☐

If you stop or slow down, does the pain disappear:

	Yes
After less than 10 minutes?	☐
After more than 10 minutes?	☐

D EXERCISE

Exercise and physical exertion in leisure time. If your activity varies much, for example between summer and winter, then give an average. The questions refer only to the last twelve months.

Tick "yes" in the most appropriate box:

	Yes
- Reading , watching TV or other sedentary activity?	☐
- Walking, cycling or other forms of exercise at least 4 hours a week? (including walking or cycling to place of work, Sunday walking ,etc.)	☐
- Participation in recreational sports, heavy gardening, etc.? (Note: duration of activity at least 4 hours a week)	☐
- Participation in hard training or sports competitions regularly several times a week?	☐

E SALT/ FAT

How often do you use salted meat or salted fish for dinner?

Tick the appropriate box	*Yes*
Never or less than once a month	☐
Once a week or less	☐
Twice a week or less	☐
More than twice a week	☐

How often do you add extra salt to your dinner ?

Tick the appropriate box	*Yes*
Rarely or never	☐
Sometimes or often	☐
Always or nearly always	☐

What type of margarine or butter do you usually use on your bread?

Tick the most appropriate box	*Yes*
Do not use margarine or butter on bread	☐
Butter	☐
Margarine	☐
Soft (soya) margarine spread	☐
Butter/ margarine mixtures	☐

What type of cooking fat do you normally use in your household ?

Tick the appropriate box.	*Yes*
Butter or hard margarine	☐
Soft (soya) margarine or oil	☐
Butter/ margarine mixtures	☐

F SMOKING

Do you smoke daily at present?	*Yes*	*No*

If "*Yes* ": ☐ ☐
Do you smoke cigarettes daily? ☐ ☐
(hand-rolled or factory made)
If you do not smoke cigarettes at present:

Have you previously smoked cigarettes on a daily basis?	*Yes* ☐	*No* ☐

If "*Yes*", how long is it since you gave up smoking ?

	Yes
More than 3 months?	☐
3 months to 1 year?	☐
1 - 5 years?	☐
More than 5 years?	☐

The following questions are to be answered by those who smoke at present or who have smoked previously.
How many years altogether have you smoked on a daily basis: .....

How many cigarettes do you smoke or did you smoke daily: .....
(hand-rolled + factory made)

Do you smoke anything else other than cigarettes daily?

	Yes
Cigars, cigarillos, cheroots ?	☐
Pipe?	☐

If you smoke a pipe, how many packets of tobacco (50 gr.) do you smoke in a week?
Give the average number of packets a week: .....

G COFFEE

How many cups of coffee do you usually drink daily?

Tick the most appropriate box	*Yes*
Do not drink coffee, or less than one cup	☐
1 - 4 cups	☐
5 - 8 cups	☐
9 or more cups	☐

What type of coffee do you usually drink daily?

Coarse ground coffee for brewing (boiled)	☐
Finely ground filter coffee	☐
Instant coffee	☐
Caffeine free coffee	☐
Do not drink coffee	☐

H EMPLOYMENT

Have you received unemployment benefit within the past 12 months?	*Yes* ☐	*No* ☐

Are you at present on sick leave, or receiving rehabilitation allowance? ☐ ☐

Are you on a full time or partial disability pension?	*Yes* ☐	*No* ☐
Do you usually work shifts or do night work?	☐	☐

During the past year have you had :

Tick the most appropriate box.	*Yes*
- Mostly sedentary work? (office work, watchmaker, light manual work)	☐
- Work requiring a lot of walking? (shop assistant, light industrial work, teaching)	☐
- Work requiring a lot of walking and lifting? (postman, heavy industrial work, construction)	☐
- Heavy manual labour? (forestry, heavy farmwork, heavy construction)	☐

Is house-keeping your main occupation?	*Yes* ☐	*No* ☐

I FOLLOW - UP EXAMINATION

Has any one in your household (other than yourself) been called in to a doctor for further medical examination after the previous cardiovascular disease survey?	*Yes* ☐	*No* ☐

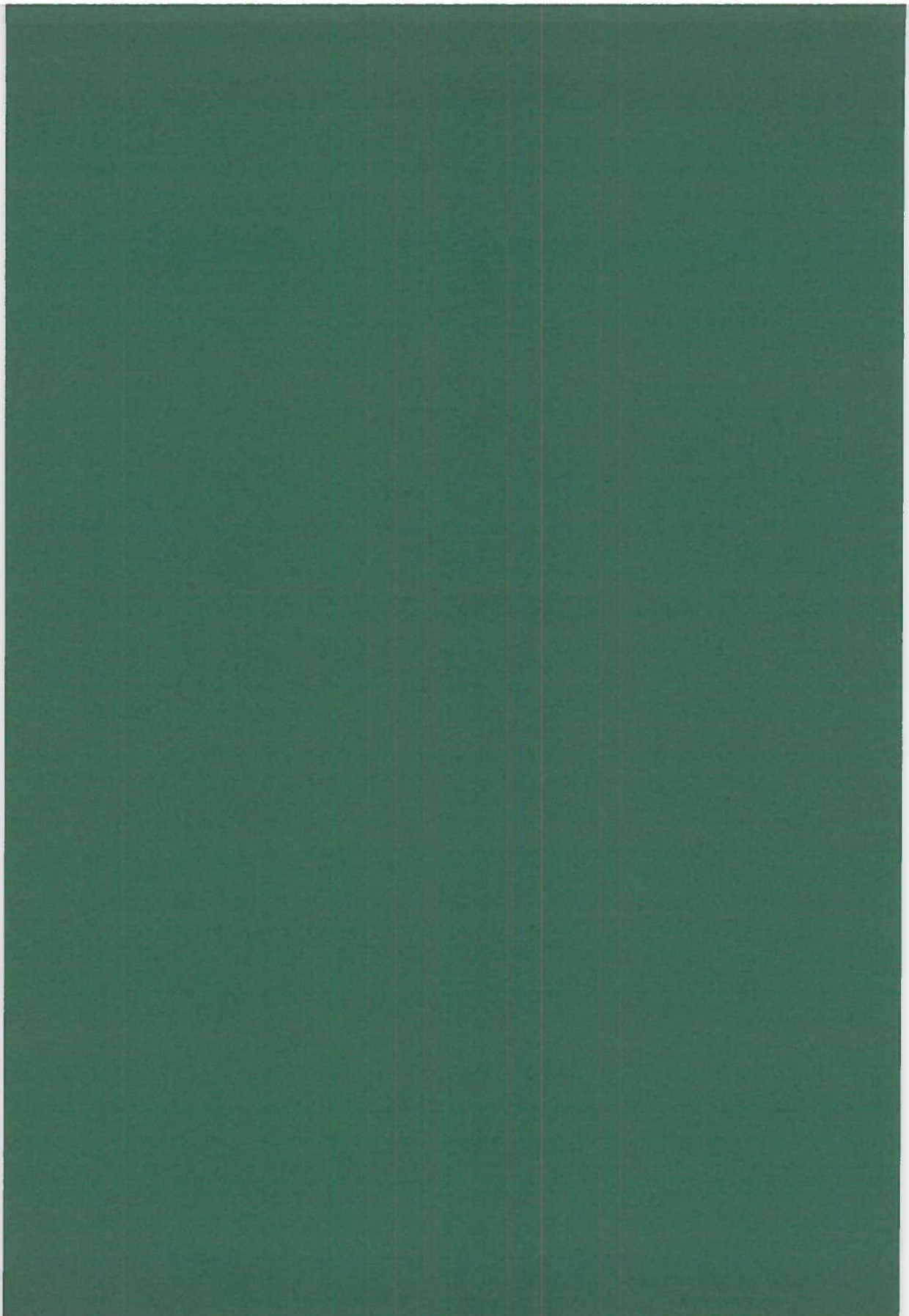
If as a result of this survey you need further medical examination, which general practitioner do you wish to be referred to ?
Write the doctor's name here:
.....

No particular doctor .....

APPENDIX II

Questionnaire 2 The Tromsø Study 1986-1987

Original version and English translation



Tilleggsspørsmål til Helseundersøkelsen i Tromsø 1986-87.

Hjerte-karsykdommene, som Hjerte-karundersøkelsene i 1974 og 1979–80 spesielt tok opp, er en mangeartet sykdomsgruppe med tildels dårlig kjente årsaksforhold. I Tromsø vil vi derfor forsøke å få en mer fullstendig kartlegging av forhold som kan være av betydning for sykdommens forløp, f.eks. kosthold, psykisk press «*stress*», sosiale forhold og sykdomsforekomst blant slektninger. En slik kartlegging er også viktig for å finne fram til sykdomsskapende forhold for kreftsykdommene, som er en sykdomsgruppe vi også vil prøve å bekjempe i årene som kommer.

Sammen med innkallingen fikk De et spørreskjema som De leverte ved undersøkelsen. Dette spørreskjema kartlegger helseforholdene bedre og inkluderer spørsmål om noen forskjellige sykdommer og fysiske/psykiske plager. Spesielt er det tatt med spørsmål vedrørende svangerskap, fødsel og menstruasjon.

Dessuten er vi interessert i å få oversikt over hvordan folk bruker helsetjenesten, for å få kunnskap om hvordan helsetjenesten kan bedres.

Vi håper De vil være brydd med å fylle ut også dette skjemaet, og sende det tilbake til Tromsø Helseråd i den utleverte konvolutt. Alle opplysninger i forbindelse med Helseundersøkelsen vil bli behandlet strengt konfidensielt. Har De noen kommentarer til undersøkelsen kan De skrive dem i kommentarfeltet på siste side.

Med hilsen

Tromsø Helseråd Fagområdet medisin

HELSETILSTAND

Hvordan er Deres helsetilstand?
Sett kryss i den ruten der «Ja» passer best.

		Ja
Meget dårlig	12	☐ 1
Dårlig		☐ 2
Hverken god eller dårlig, middels		☐ 3
Bra		☐ 4
Utmerket		☐ 5

SYKDOM

Har De, eller har De hatt:
Kryss av «Ja» eller «Nei» for hvert spørsmål.

		Ja	Nei
Hudsykdommen psoriasis	13	☐	☐
Astma	14	☐	☐
Allergisk eksem	15	☐	☐
Høysnue	16	☐	☐
Kronisk bronkitt	17	☐	☐
Sår på magesekken	18	☐	☐
Sår på tolvfingertarmen	19	☐	☐
Blindtarms-operasjon	20	☐	☐
Magesårs-operasjon	21	☐	☐
Leddgikt (kronisk revmatoid artritt)	22	☐	☐
Kreftsykdom	23	☐	☐
Epilepsi (fallesyke)	24	☐	☐
Migrene	25	☐	☐

INFEKSJON

		Antall	Ja	Nei
Hvor mange ganger har De hatt infeksjon slik som forkjølelse, influensa, «ræksjuka» og lignende siste halvår?	26	☐		
Har De hatt slik infeksjon siste 14 dager?	27		☐	☐

SYKDOM HOS FORELDRE OG SØSKEN

Kryss av for de slektningene som har eller har hatt noen av sykdommene:

		Mor	Far	Bror	Søster
Hjerneslag eller hjerneblødning	28	☐	☐	☐	☐
Sukkersyke	32	☐	☐	☐	☐
Leddgikt (revmatoid artritt)	36	☐	☐	☐	☐
Kreft	40	☐	☐	☐	☐
Psoriasis	44	☐	☐	☐	☐
Magesår eller tolvfingertarmsår	48	☐	☐	☐	☐
Astma	52	☐	☐	☐	☐

		Ja	Nei
Kryss av dersom slektningene ikke har eller har hatt noen av disse sykdommene	56	☐	☐

MEDISINER

		Ja	Nei
Har De siste år brukt tabletter, sprøyter eller astmaspray mot astma eller allergi	60	☐	☐

Har De brukt følgende medisiner siste 14 dager?

		Ja	Nei
Smertestillende	61	☐	☐
Febersenkende	62	☐	☐
Eksemsalve	63	☐	☐
Blodtrykksmedisin	64	☐	☐
Hjertemedisin	65	☐	☐
Sovemedisin	66	☐	☐
Nervemedisin	67	☐	☐
Migrenemedisin	68	☐	☐
Medisin mot epilepsi (fallesyke)	69	☐	☐
Annen medisin	70	☐	☐

KONTAKT PGA. EGEN HELSE ELLER SYKDOM

Hvor mange besøk har De hatt siste år på grunn av egen helse eller sykdom?

		Antall besøk
Hos vanlig lege	71	☐
Hos spesialist utenfor sykehuset	72	☐
På legevakta	85	☐
Hos bedriftslege	87	☐
Hos fysioterapeut	89	☐
Hos kiropraktor	81	☐
Hos naturmedisiner (homeopat, soneterapeut o.l.)	83	☐
På sykehusets poliklinikk	85	☐
Antall innleggelser på sykehus siste år	87	☐

KOSTHOLD

Hvor mange brødskiver spiser De vanligvis daglig?
Sett kryss i den ruten der «Ja» passer best

		Ja
Mindre enn 2 skiver	88	☐ 1
2 – 4 skiver		☐ 2
5 – 6 skiver		☐ 3
7 – 12 skiver		☐ 4
13 eller flere skiver		☐ 5

Hva slags melk drikker de vanligvis?
Sett kryss i den ruten der «Ja» passer best.

		Ja
Drikker ikke melk	89	☐ 1
Melk (helmelk), søt, sur		☐ 2
Lettmelk		☐ 3
Skummet melk, søt, sur		☐ 4

Hvor mange glass/kopper melk drikker De vanligvis daglig?

		Ja
Mindre enn ett glass/kopp	90	☐ 1
1 – 2 glass/kopper		☐ 2
3 – 4 glass/kopper		☐ 3
5 eller flere glass/kopper		☐ 4

FISKEMAT

Hvor ofte spiser De torsk/sei eller annen mager fisk til middag eller som pålegg?
Sett kryss i den ruten der «Ja» passer best.

		Ja
Sjeldnere enn en gang i uken	91	☐ 1
1 gang i uken		☐ 2
2 ganger i uken		☐ 3
3 eller flere ganger i uken		☐ 4

Hvor ofte spiser De fet fisk som sild, kveite, uer, makrell, laks, ørret til middag eller som pålegg?
Sett kryss i ruten der «Ja» passer best.

		Ja
Sjeldnere enn en gang i uken	92	☐ 1
1 gang i uken		☐ 2
2 ganger i uken		☐ 3
3 eller flere ganger i uken		☐ 4

→ Bruker De tran regelmessig?
Sett kryss i ruten der «Ja» passer best.

		Ja
Nei	93	☐ 1
I mørketida		☐ 2
Hele året		☐ 3

FROKOST

		Ja	Nei
Spiser De vanligvis frokost daglig?	94	☐	☐

MIDDAGSMAT

Hvor ofte spiser De vanligvis kjøtt til middagen?
Sett kryss i ruten der «Ja» passer best.

		Ja
Sjeldnere enn en gang i uken	95	☐ 1
1 – 2 ganger i uken		☐ 2
3 – 4 ganger i uken		☐ 3
5 eller flere ganger i uken		☐ 4

Hvor ofte bruker De fett (smør, margarin, remulade, majones og lignende) til eller på middagsmaten?
Sett kryss i ruten der «Ja» passer best.

		Ja
Sjeldnere enn en gang i uken	96	☐ 1
1 – 2 ganger i uken		☐ 2
3 – 4 ganger i uken		☐ 3
5 eller flere ganger i uken		☐ 4

		Ja	Nei
Bruker De vanligvis grønnsaker som del av middagsmaten?	97	☐	☐

FRUKT

Hvor ofte spiser De vanligvis frukt?
Sett kryss i ruten der «Ja» passer best.

		Ja
Sjeldnere enn en gang i uken	98	☐ 1
Omtrent en gang i uken		☐ 2
2 – 3 ganger i uken		☐ 3
4 – 5 eller flere ganger i uken		☐ 4
Omtrent daglig		☐ 5

ALKOHOL

	Ja	Nei
Er De total avholdsmann/-kvinne	☐	☐

Hvis nei,
– Hvor ofte pleier De å drikke øl?
Sett kryss i ruten der «Ja» passer best.

		Ja
Aldri, eller noen få ganger i året	100	☐ 1
1 – 2 ganger i måneden		☐ 2
Omtrent 1 gang i uken		☐ 3
2 – 3 ganger i uken		☐ 4
Omtrent hver dag		☐ 5

Hvor ofte pleier De å drikke vin?
Sett kryss i ruten der «Ja» passer best.

		Ja
Aldri, eller noen få ganger i året	101	☐ 1
1 – 2 ganger i måneden		☐ 2
Omtrent 1 gang i uken		☐ 3
2 – 3 ganger i uken		☐ 4
Omtrent hver dag		☐ 5

– Hvor ofte pleier De å drikke brennevin?
Sett kryss i ruten der «Ja» passer best.

		Ja
Aldri, eller noen få ganger i året	102	☐ 1
1 – 2 ganger i måneden		☐ 2
Omtrent 1 gang i uken		☐ 3
2 – 3 ganger i uken		☐ 4
Omtrent hver dag		☐ 5

Omtrent hvor ofte har De i løpet av siste år drukket alkohol tilsvarende minst 5 halvflasker øl, en helflaske vin eller ¼ flaske brennevin?
Sett kryss i ruten der «Ja» passer best.

		Ja
Ikke siste år	103	☐ 1
Noen få ganger		☐ 2
1 – 2 ganger i måneden		☐ 3
3 eller flere ganger i uken		☐ 4

FYSISK AKTIVITET

Hvor ofte utfører De fysisk aktivitet av minst 20 minutters varighet og som fører til at De blir svett eller andpusten?
Sett kryss i ruten der «Ja» passer best.

		Ja
Sjelden eller aldri	104	☐ 1
Ukentlig		☐ 2
Flere ganger i uka		☐ 3
Daglig		☐ 4

Dersom De vanligvis utfører slik aktivitet minst en gang i uka, hvor mye tid bruker De ukentlig til slik aktivitet?
Sett kryss i ruten der «Ja» passer best.

		Ja
Mindre enn 30 minutter i uka	105	☐ 1
Mellom 30 minutter og 1 time i uka		☐ 2
Mellom 1 og 2 timer i uka		☐ 3
Mer enn 2 timer i uka		☐ 4

VANE- OG KOSTENDRINGER

Har De endret Deres vaner/kosthold i løpet av de siste 5 år når det gjelder:(Sett kryss for hvert spørsmål)

		Bruker nå mer	som før	mindre
Fett i kosten	106	☐	☐	☐
Soyamargarin eller matoljer	107	☐	☐	☐
Skummet melk eller lettmelk	108	☐	☐	☐
Kaffe-forbruk	109	☐	☐	☐
Alkohol-forbruk	110	☐	☐	☐
Fysisk aktivitet	111	☐	☐	☐

EKTESKAPS-/SAMBO-FORHOLD

		Ja	Nei
Er De gift eller samboende	112	☐	☐

Hvor gammel var De da De første gang giftet Dem eller innledet et samboerforhold? ... 113 ☐ år

HUSSTAND

		Antall	Ja	Nei
Hvor mange personer bor det i deres husstand?	115	☐		
Er noen i Deres hussland 10 år eller yngre?	117		☐	☐
Trenger noen i Deres hussland spesiell tilsyn/pleie – *utenom barna?*	118		☐	☐

SKOLEGANG

Hvor mange års skolegang har De (ta også med folkeskole og ungdomsskole)? ... 119 ☐ år

ARBEID

Har De hatt lønnet arbeid hele siste år?
Sett kryss i ruten der «Ja» passer best.

		Ja
Fulltidsarbeid	121	☐ 1
Deltidsarbeid		☐ 2
Ikke lønnet arbeid		☐ 3

Hvor stor del av det daglige arbeid i hjemmet gjør De vanligvis selv?
Sett kryss i ruten der «Ja» passer best.

Alt eller nesten alt	122	☐ 1
Minst halvparten		☐ 2
Mer enn en fjerdedel		☐ 3
Mindre enn en fjerdedel		☐ 4

RYGG- OG LEDDPLAGER

		Ja	Nei
Har De i løpet av siste år vært plaget av smerter i ryggen som har vart lenger enn 4 uker?	123	☐	☐
Hvis ja, bedrer ryggsmertene seg dersom De beveger Dem?	124	☐	☐
Har De vært plaget av stivhet i ryggen om morgenen som varte lenger enn 30 minutter?	125	☐	☐

Har De i løpet av siste 3 år vært plaget av smerter i noen av de følgende ledd i mer enn 3 måneder?

		Ja	Nei
Kneleddene	126	☐	☐
Albueleddene	127	☐	☐
De innerste fingerleddene	128	☐	☐
Andre ledd	129	☐	☐

Hvis ja, merket De stivhet i leddene om morgenen av mer enn 30 minutters varighet	130	☐	☐

PLAGER I HODE, NAKKE OG SKULDRE

Hvor ofte er De plaget av hodepine?
Sett kryss i ruten der «Ja» passer best.

		Ja
Sjelden eller aldri	131	☐ 1
En eller flere ganger i måneden		☐ 2
En eller flere ganger i uken		☐ 3
Daglig		☐ 4

Hvor ofte er De plaget av smerter i nakke eller skuldre?
Sett kryss i ruten der «Ja» passer best.

		Ja
Sjelden eller aldri	132	☐ 1
En eller flere ganger i måneden		☐ 2
En eller flere ganger i uken		☐ 3
Daglig		☐ 4

Reduserer plagene i hodet, nakken eller skuldrene Deres arbeidsevne?
Sett kryss i ruten der «Ja» passer best.

		Ja
Aldri, eller i ubetydelig grad	133	☐ 1
I noen grad		☐ 2
I betydelig grad		☐ 3
Klarer ikke vanlig arbeid		☐ 4

		Ja	Nei
Har De noen gang fått røntgenundersøkt ryggen, nakken og/eller skuldre	134	☐	☐

SØVNLØSHET/BEVISSTLØSHET

		Ja	Nei
Hender det at De er plaget av søvnløshet	135	☐	☐

Hvis ja, når på året er De mest plaget?
Sett kryss i ruten der «Ja» passer best.

		Ja
Ingen spesiell tid	136	☐ 1
Særlig mørketiden		☐ 2
Særlig i midnattsoltiden		☐ 3
Særlig høst og vår		☐ 4

		Ja	Nei
Har De gjennom hele siste år vært plaget av søvnighet slik at det går ut over arbeidsevnen?	137	☐	☐
Har De siste år hatt anfall med plutselig tap av bevissthet?	138	☐	☐
Har De merket anfall med plutselig endring i pulsen eller hjerterytmen siste år	139	☐	☐

REAKSJONER PÅ PROBLEMER

Hvis De får store personlige problemer, regner De da med å få hjelp og støtte fra ektefelle, samboer eller familie? ..... 140 — Ja ☐ Nei ☐

Har De i lengere tid følt behov for å oppsøke noen på grunn av personlige problem siste år, uten at De har tatt slik kontakt? ..... 141 — Ja ☐ Nei ☐

Har De i de siste 14 dager følt Dem ute av stand til å takle Deres vanskeligheter?
Sett kryss i ruten der «Ja» passer best. 142
- Aldri eller sjelden ..... ☐ 1
- Av og til ..... ☐ 2
- Ofte ..... ☐ 3
- Nesten hele tida ..... ☐ 4

Har De i de siste 14 dager følt Dem ulykkelig og nedtrykt (deprimert)?
Sett kryss i ruten der «Ja» passer best. 143
- Aldri eller sjelden ..... ☐ 1
- Av og til ..... ☐ 2
- Ofte ..... ☐ 3
- Nesten hele tida ..... ☐ 4

Hender det ofte at De føler Dem ensom?
Sett kryss i ruten der «Ja» passer best. 144
- Meget ofte ..... ☐ 1
- Av og til ..... ☐ 2
- Aldri eller nesten aldri ..... ☐ 3

RESTEN AV SKJEMAET BESVARES BARE AV KVINNER

MENSTRUASJON

Hvor gammel var De da De fikk menstruasjon første gang? ..... 145 — ☐ år

Når begynte Deres siste menstruasjon? .. 147 — dag mnd. år / /

Hvor mange dager er/var det vanligvis fra menstruasjonens 1. blødningsdag til neste menstruasjons 1. blødningsdag (= tiden mellom to menstruasjoners begynnelse)? . 153 — ☐ dager

Pleier/pleide menstruasjonen å være regelmessig ..... 155 — Ja ☐ Nei ☐

Bruker De vanligvis smertestillende tabletter under menstruasjonen? ..... 156 — Ja ☐ Nei ☐

PLAGER FOR MENSTRUASJON

Har De før menstruasjon noen av disse plagene:
– Er De nedtrykt (deprimert) eller irritabel?
Sett kryss i ruten der «Ja» passer best. 157
- Ubetydelig ..... ☐ 1
- Merkbart ..... ☐ 2
- Plagsomt ..... ☐ 3

– Har De smertefulle bryst?
Sett kryss i ruten der «Ja» passer best. 158
- Ubetydelig ..... ☐ 1
- Merkbart ..... ☐ 2
- Plagsomt ..... ☐ 3

– Har De hovne hender/føtter, vektøkning, eller følelse av å «ese ut»?
Sett kryss i ruten der «Ja» passer best. 159
- Ubetydelig ..... ☐ 1
- Merkbart ..... ☐ 2
- Plagsomt ..... ☐ 3

Forsvinner plagene når menstruasjonen kommer? ..... 160 — Ja ☐ Nei ☐

Bruker De mot slike plager:
- vanndrivende tabletter? ..... 161 — Ja ☐ Nei ☐
- andre medisiner? ..... 162 — Ja ☐ Nei ☐

SVANGERSKAP

Hvor mange barn har De født? ..... 163 — Antall ☐

Hvor gammel var De første gang De var gravid? ..... 164 — ☐ år

PREVENSJON

Bruker eller har De brukt P-piller eller spiral? ..... 166 — Ja ☐ Nei ☐

Hvis ja, hvor mange år har De tilsammen brukt:
- P-piller? ..... 167 — ☐ år
- Spiral? ..... 169 — ☐ år

Hvor gammel var De da De begynte med:
- P-piller? ..... 171 — ☐ år
- Spiral? ..... 173 — ☐ år

Hvis De har sluttet med P-piller, uteble da menstruasjonen i mer enn 6 måneder uten at De var gravid? ..... 175 — Ja ☐ Nei ☐

Har de måttet slutte med P-piller fordi De fikk høyt blodtrykk? ..... 176 — Ja ☐ Nei ☐

KREFTPRØVE

Hvor mange ganger har De fått tatt kreftprøve (celleprøve) fra livmorhalsen siste 3 år? ... 177 — Antall prøver ☐

Hvor mange år siden er det siden siste prøve? ..... 178 — ☐ år

Deres kommentarer: 179

Takk for hjelpen! Husk å postlegge skjemaet idag!
Tromsøundersøkelsen 1986-7

LUNDBLAD TRYKKERI A S • TROMSØ

ADDITIONAL QUESTIONS THE TROMSØ HEALTH SURVEY, 1986 - 87.

English translation; Mrs. Anne Clancy and Mr. Kevin McCafferty

Cardiovascular heart and circulatory diseases, on which the surveys of 1974 and 1979-80 focused, are a very varied category of diseases whose causes are still partly unknown. In Tromsø we are therefore trying to obtain a more complete description of factors which may be important for the course of these diseases, such as diet, psychological pressure, "stress", social conditions and the occurrence of disease in relatives. Such a description is also important in the search for factors that contribute to cancer, a group of diseases which we will also be trying to combat in the coming years.

When you were called in, you received a questionnaire which you handed in at the survey. The present questionnaire asks for further information about your health and includes questions on various diseases and physical and psychological complaints. We have included questions on pregnancy, birth and menstruation.

In addition, we are interested in obtaining information on the public use of medical services in order to find out how to improve the health service.

We hope that you will take the trouble to fill in yet another questionnaire and return it to "Tromsø Board of Health" in the enclosed envelope. All information will be treated in strict confidence. If you have any comments to make on the survey, you may write them down in the space provided on the last page of the questionnaire.

Yours sincerely

Tromsø Board of Health

Department of Medicine, University of Tromsø

GENERAL STATE OF HEALTH

How is your health? *Tick the appropriate box.*	*Yes*
Very bad	☐
Bad	☐
Neither good nor bad, "middling"	☐
Good	☐
Excellent	☐

ILLNESS

Have you/ have you had: Tick "*yes*" or "*no*" for each question.	*Yes*	*No*
The skin disease psoriasis?	☐	☐
Asthma?	☐	☐
Allergic eczema?	☐	☐
Hay fever?	☐	☐
Chronic bronchitis?	☐	☐
Stomach ulcer?	☐	☐
Duodenal ulcer?	☐	☐
Your appendix removed?	☐	☐
An operation for a stomach ulcer?	☐	☐
Chronic rheumatoid arthritis?	☐	☐
Cancer?	☐	☐
Epilepsy?	☐	☐
Migraine?	☐	☐

INFECTIONS

How many times in the last 6 months have you had infections like a cold, influenza (flu) diarrhoea/vomiting, or similar illnesses ? *Number of times* :.....

Have you had one of these infection in the past 14 days?	*Yes*	*No*
	☐	☐

ILLNESS IN PARENTS OR SIBLINGS

Tick the appropriate box for relatives that have, or have had the following illnesses:

	Mother	Father	Brother	Sister
Cerebral stroke or brain haemorrhage:	☐	☐	☐	☐
Diabetes:	☐	☐	☐	☐
Rheumatoid arthritis:	☐	☐	☐	☐
Cancer:	☐	☐	☐	☐
Psoriasis:	☐	☐	☐	☐
Stomach or duodenal ulcer:	☐	☐	☐	☐
Asthma:	☐	☐	☐	☐

Tick the appropriate box if neither your parents nor siblings have or have had any of the above illnesses.	*Yes*	*No*
	☐	☐

MEDICINES

Have you during the last year used tablets/ sprays or had injections for asthma or allergies?	*Yes*	*No*
	☐	☐

Have you used any of the following medicines in the past 14 days?	*Yes*	*No*
Painkillers:	☐	☐
Antipyretics (to reduce fever):	☐	☐
Eczema ointment:	☐	☐
Blood pressure medication:	☐	☐
Heart medication:	☐	☐
Sleeping tablets:	☐	☐
Nerve tablets:	☐	☐
Migraine medication:	☐	☐
Epilepsy medication:	☐	☐
Other medicines:	☐	☐

CONTACT DUE TO OWN HEALTH OR ILLNESS

How many visits have you made during the past year due to your own health or illness ?

	Number of visits
To a GP(general practitioner):	.....
To a specialist, (non hospital):	.....
Emergency GP	.....
Medical officer at work :	.....
Physiotherapist:	.....
Chiropractor:	.....
Nature healer(homeopath etc.):	.....
Hospital outpatient department	.....
Number of hospital admissions in the past year:	.....

DIET

How many slices of bread do you usually eat daily ?

Tick the most appropriate box.	*Yes*
Less than 2 slices	☐
2 - 4 slices	☐
5 - 6 slices	☐
7 -12 slices	☐
13 or more slices	☐

What type of milk do you usually drink?

Tick the most appropriate box.	*Yes*
Do not drink milk	☐
Full cream milk (ordinary or curdled)	☐
Light milk	☐
Skimmed milk (ordinary or curdled)	☐

How many glasses/cups of milk do usually drink daily?

	Yes
Less than I glass/cup	☐
1 - 2 glasses/cups	☐
3 - 4 glasses/cups	☐
5 or more glasses/cups	☐

FISH

How often do you eat cod, coal fish, red snapper or other lean fish for dinner or in a sandwich?

Tick the most appropriate box	*Yes*
Less than once a week	☐
Once a week	☐
Twice a week	☐
3 or more times a week	☐

How often do you eat cod/pollock or other lean fish for dinner or in a sandwich?

Tick the most appropriate box.	*Yes*

Less than once a week	☐
Once a week	☐
Twice a week	☐
3 or more times a week	☐

How often do you eat fat fish, such as herring, halibut, mackerel, salmon or trout for dinner or in a sandwich?

Tick the most appropriate box	*Yes*
Less than once a week	☐
Once a week	☐
Twice a week	☐
3 or more times a week	☐

Do you take cod liver oil regularly?

Tick the most appropriate box	*Yes*
No	☐
'Dark-time'(mid-winter)	☐
All year	☐

BREAKFAST

Do you usually eat breakfast every day?	*Yes*	*No*
	☐	☐

DINNER

How often do you eat meat for dinner?

Tick the appropriate box	*Yes*
Less than once a week	☐
Once or twice a week	☐
3 - 4 times a week	☐
5 or more times a week	☐

How often do you use fat like butter, margarine, mayonnaise, etc. with your dinner?

Tick the most appropriate box	*Yes*
Less than once a week	☐
Once or twice a week	☐
3 - 4 times a week	☐
5 or more times a week	☐

Do you usually eat vegetables with your dinner?	*Yes*	*No*
	☐	☐

FRUIT

How often do you usually eat fruit ?

Tick the appropriate box.

Less than once a week	☐
About once a week	☐
2 - 3 times a week	☐
4 - 5 times a week	☐
More or less	☐

ALCOHOL

Are you a teetotaller?	*Yes*	*No*
	☐	☐

If "*not*", how often do you drink beer?

Tick the most appropriate box	*Yes*
Never or just a few times a year	☐
Once or twice a month	☐
About once a week	☐
2 - 3 times a week	☐
More or less daily	☐

How often do you drink wine ?

Tick in the most appropriate box	*Yes*
Never or just a few times a year	☐
Once or twice a month	☐
About once a week	☐
2 - 3 times a week	☐
More or less daily	☐

How often do you drink spirits ?

Tick the appropriate box	*Yes*
Never or just a few times a year	☐
Once or twice a month	☐
Approximately once a week	☐
2 or 3 times a week	☐
More or less daily	☐

Approximately how often in the past year have you drunk alcohol corresponding to at least 5 small bottles of beer, a bottle of wine, or a quarter bottle of spirits?

Tick the most appropriate box	*Yes*
Not at all the past year	☐
A few times	☐
Once or twice a month	☐
3 or more times a week	☐

PHYSICAL ACTIVITY

How often do you take part in physical activity lasting at least 20 minutes, which makes you perspire or become breathless?

Tick the appropriate box.	*Yes*
Rarely or never	☐
Weekly	☐
Several times a week	☐
Daily	☐

If you usually take part in this type of activity at least weekly, how much time do you spend exercising?

Tick the most appropriate box.	*Yes*
Less than 30 minutes a week	☐
Between 30 minutes and one hour weekly	☐
Between 1 and 2 hours a week	☐
More than 2 hours a week	☐

CHANGE IN DIETARY HABITS AND OTHER HABITS

Have you changed any of the following habits during the last 5 years?

Tick the appropriate box.	*Use now*		
	More	*As before*	*Less*
Dietary fat	☐	☐	☐
Soya margarine or oil	☐	☐	☐
Skimmed or low fat milk	☐	☐	☐
Coffee intake	☐	☐	☐
Alcohol intake	☐	☐	☐
Physical activity	☐	☐	☐

MARRIAGE / PARTNER

	Yes	*No*
Are you married or 'living together?	☐	☐

How old were you when you first married or moved in with a partner? *age:* .....

HOUSEHOLD

How many persons live in your household ?
Number of persons : .....

	Yes	*No*
Is anyone in your household 10 years or younger?	☐	☐
Does anyone in your household need special care/assistance? *(Other than the children)*	☐	☐

SCHOOLING

How many years schooling have you had? (include secondary and folk high schools)
Number of years : .....

EMPLOYMENT

Have you had paid work this past year?

Tick the appropriate box	*Yes*
Full-time work	☐
Part-time work	☐
Unpaid work	☐

How much house work do you normally do yourself ?

Tick the appropriate box	*Yes*
All or almost all	☐
At least half	☐
More than a quarter	☐
Less than a quarter	☐

BACK AND JOINTS CONDITIONS

	Yes	*No*
During this last year have you suffered from backache that has lasted longer than 4 weeks ?	☐	☐
If "*yes*", does the pain improve when you exercise?	☐	☐
Have you suffered from morning stiffness in your back lasting more than 30 minutes?	☐	☐

During the past 3 years have you suffered from pain in any of the following joints lasting more than 30 minutes ?	*Yes*	*No*
Knees	☐	☐
Elbows	☐	☐
Innermost finger joints	☐	☐
Other joints	☐	☐

If "*yes*", have you suffered from stiff joints in the mornings lasting more than 30 minutes?	*Yes*	*No*
	☐	☐

NECK HEAD AND SHOULDER COMPLAINTS

How often do you suffer headache?

Tick the appropriate box	*Yes*
Rarely or never	☐
Once or twice a month	☐
Once or twice a week	☐
Daily	☐

How often do you suffer pain in the neck or shoulder?

Tick the appropriate box	*Yes*
Rarely or never	☐
Once or twice a month	☐
Once or twice a week	☐
Daily	☐

Do these complaints inhibit your ability to work?

Tick the appropriate box.	*Yes*
Little or no effect	☐
To some degree	☐
To a large degree	☐
Cannot do ordinary work	☐

Have your back, shoulders, and /or neck ever been x-rayed? *Yes* ☐ *No* ☐

SLEEPLESSNESS / LOSS OF CONSCIOUSNESS

Have you ever suffered from sleeplessness? *Yes* ☐ *No* ☐

If *"yes"*, at what time of the year do you usually suffer from sleeplessness?

Tick the appropriate box	*Yes*
No particular time	☐
Especially during the 'dark time'	☐
Especially during the arctic summer (midnight sun)	☐
Especially in spring and autumn	☐

Have you at any time during the last 12 twelve months suffered from tiredness that has affected your work performance? *Yes* ☐ *No* ☐

Have you suffered from sudden loss of consciousness in the past year? *Yes* ☐ *No* ☐

Have you noticed sudden changes in your pulse rate or heartbeat in the past year? *Yes* ☐ *No* ☐

REACTION TO PROBLEMS

If you have major personal problems, do you expect to get help and support from your spouse or family? *Yes* ☐ *No* ☐

In the last year, have you long felt a need to seek help with personal problems, without doing so? *Yes* ☐ *No* ☐

During the past 2 weeks have you felt unable to cope with your problems?

Tick the appropriate box	*Yes*
Seldom or never	☐
Sometimes	☐
Often	☐
Nearly always	☐

During the past 2 weeks have you felt unhappy or depressed?

Tick the appropriate box	*Yes*
Seldom or never	☐
Sometimes	☐
Often	☐
Nearly always	☐

Do you ever feel lonely?

Tick the appropriate box	*Yes*
Very often	☐
Sometimes	☐
Rarely or never	☐

THE REMAINING SECTION OF THE QUESTIONNAIRE APPLIES TO WOMEN ONLY.

MENSTRUATION

How old were you when you started menstruating? *age:* .....

When did you start your last period? (*date, month, year*) ....

How many days usually pass from the first day of one period to the first day of your

next period (the time lapsed between the start of two periods)?

Number of days: .....

Do/did you menstruate regularly? *Yes* ☐ *No* ☐

Do you usually need pain-killers during menstruation? *Yes* ☐ *No* ☐

PRE-MENSTRUAL TENSION

Do you have any of the following complaints before your period?

Are you depressed or irritable?

Tick the appropriate box	*Yes*
Hardly at all	☐
Noticeably	☐
Very much so	☐

Are your breasts painful?

Tick the appropriate box	*Yes*
Hardly at all	☐
Noticeably	☐
Very much so	☐

Do you have swollen hands/feet, put on weight, or feel bloated?

Tick the appropriate box.	*Yes*
Hardly at all	☐
Noticeably	☐
Very much so	☐

Do the complaints disappear when you get your period? *Yes* ☐ *No* ☐

What type of medication do you use for these complaints?

Tick the appropriate box.	*Yes*
Diuretics	☐
Other medicines	☐

PREGNANCY

How many children have you had?

Number of children : .....

How old were you when you got pregnant for the first time? *Age*: .....

CONTRACEPTION

Do you now use or have you ever used the contraceptive pill or an intrauterine device? *Yes* ☐ *No* ☐

If *"yes"*, for how many years altogether have you used:

	Number of years
The pill:	.....
An intrauterine device:	.....

How old were you when you started using:

The pill:	*age:* .....
An intrauterine device:	*age:* .....

If you stopped taking the pill, did 6 months or more pass without menstruating (having a period), without your being pregnant? *Yes* ☐ *No* ☐

Did you have to stop taking the pill due to high blood pressure? *Yes* ☐ *No* ☐

CERVICAL SMEAR TEST

How many times have you had a cervical smear test in the last 3 years?

Number of times: .....

How many years is it since you had your last cervical smear test ?

Number of years: .....

Comments

.....

.....

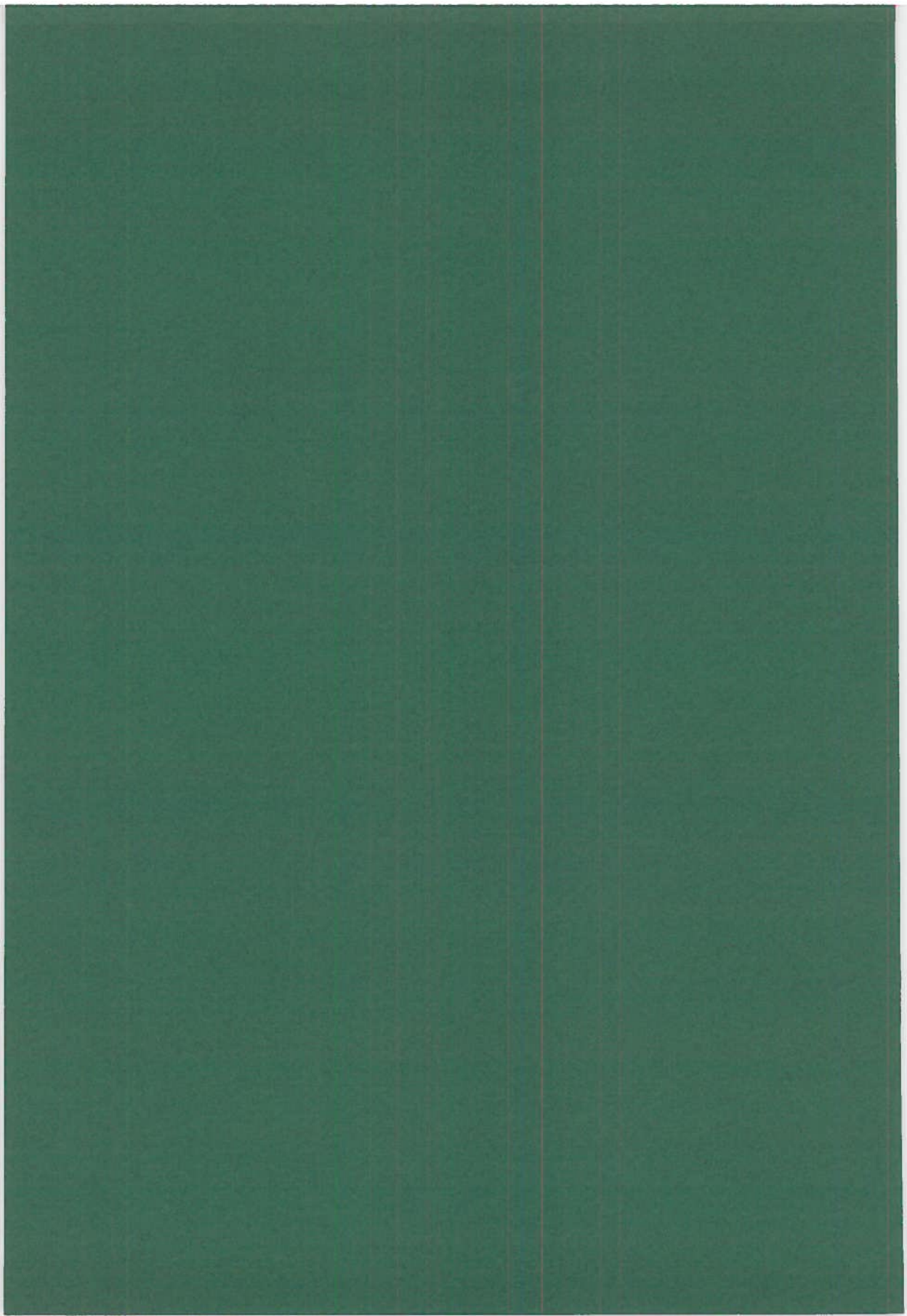
Thank you for your help! Remember to post the questionnaire today!

The Tromsø survey 1986-1987.

APPENDIX III

Questionnaire 1 The Nordland Health Study 1987-1988

Original version



Helseundersøkelsen i Nordland

1988-89.

Helseundersøkelsen i Nordland pågår fremdeles, og du er en av dem som har benyttet muligheten til en ekstra helsesjekk.

Ved frammøte til undersøkelsen fikk du utlevert et spørreskjema som skulle sendes til Fylkeslegen i Nordland. Vi kan ikke se å ha mottatt ditt skjema ennå. Derfor sender vi deg et nytt skjema og håper at du har anledning til å fylle det ut. Svarkonvolutt følger vedlagt. Porto er allerede betalt.

I tillegg til at undersøkelsen gir mulighet til å forebygge hjerte-karsykdom, bidrar den til øke våre kunnskaper om bl.a. kroniske sykdommer, arbeidsmiljøet og hvordan helsetjenesten fungerer. Det er derfor svært viktig at flest mulig svarer.

Alle opplysninger som du gir på skjemaet, vil bli behandlet strengt fortrolig.

På forhånd takk for hjelpen!

Med vennlig hilsen

Fylkeslegen i Nordland Universitetet i Tromsø

Statens helseundersøkelser

LILLEBLAD GRAFISK A/S TROMSØ

A FAMILIE

Har en eller flere av foreldre eller søsken hatt hjerteinfarkt (sår på hjertet) eller angina pectoris (hjertekrampe)? 12

B EGEN SYKDOM

Har De, eller har De hatt:

Hjerteinfarkt? 13
Angina pectoris (hjertekrampe)? 14
Hjerneslag? 15
Sukkersyke? 16

Er De under behandling for:

Høyt blodtrykk? 17

Bruker De:

Nitroglycerin? 18

C SYMPTOMER

Får De smerter eller ubehag i brystet når De:

Går i bakker, trapper eller fort på flat mark? 19
Går i vanlig takt på flat mark? 20

Dersom De får smerter eller vondt i brystet ved gange, pleier De da:

Stoppe? 21
Saktne farten? .....
Fortsette i samme takt? .....

Dersom De stopper eller saktner farten, går da smertene bort:

Etter mindre enn 10 minutter? 22
Etter mer enn 10 minutter? .....

Har De vanligvis:

Hoste om morgenen? 23
Oppspytt fra brystet om morgenen? 24

D MOSJON

Bevegelse og kroppslig aktivitet i Deres fritid. Dersom aktiviteten varierer mye, f.eks. mellom sommer og vinter, så ta ett gjennomsnitt. Spørsmålet gjelder bare det siste året.

Sett kryss i den ruten som passer best.

Leser, ser på fjernsyn eller annen stillesittende beskjeftigelse? 25

Spaserer, sykler eller beveger Dem på annen måte minst 4 timer i uken? .....
(Her skal De også regne med gang eller sykling til arbeidsstedet, søndagsturer m.m.)

Driver mosjonsidrett, tyngre hagearbeid e.l.? ..
(Merk at aktiviteten skal vare i minst 4 timer i uken.)

Trener hardt eller driver konkurranseidrett regelmessig og flere ganger i uken? .....

E SALT/FETT

Hvor ofte bruker De salt kjøtt eller salt fisk til middag?

Sett kryss i den ruten som passer best.

Aldri eller sjeldnere enn en gang i måneden 26
Inntil en gang i uken .....
Inntil to ganger i uken .....
Mer enn to ganger i uken .....

Hvor ofte pleier De å strø ekstra salt på middagsmaten?

Sett kryss i den ruten som passer best.

Sjelden eller aldri 27
Av og til eller ofte .....
Alltid eller nesten alltid .....

Hva slags margarin eller smør bruker De vanligvis på brødet?

Sett kryss i den ruten som passer best.

Bruker ikke smør eller margarin på brød 28
Smør .....
Hard margarin .....
Myk (Soft) margarin .....
Smør/margarin blanding .....

Hva slags fett blir vanligvis brukt til matlaging i husholdningen Deres?

Sett kryss i den ruten som passer best

Smør eller hard margarin 29
Myk (Soft) margarin eller olje .....
Smør/margarin blanding .....

F RØYKING

Røyker De daglig for tida? 30

Dersom svaret er «JA», svar da på dette:

Røyker De sigaretter daglig? 31
(håndrullede eller fabrikkfremstilte)

Dersom De ikke røyker sigaretter nå, svar da på dette:

Har De røykt sigaretter daglig tidligere? 32

Dersom De svarte «JA», hvor lenge er det da siden De sluttet?

Mindre enn 3 måneder? 33
3 måneder – 1 år? .....
1–5 år? .....
Mer enn 5 år? .....

Skal besvares av de som røyker nå eller som har røykt tidligere:

Hvor mange år til sammen har De røykt daglig? 34

Hvor mange sigaretter røyker eller røykte De daglig?
Gi opp antallet sigaretter daglig 36
(håndrullede + fabrikkframstilte)

Røyker De noe annet enn sigaretter daglig?

Sigarer eller serutter/sigarillos? 40
Pipe? 41

Dersom De røyker pipe, hvor mange pakker tobakk (50 gram) bruker De i pipen på en uke?

Gi opp gjennomsnittlig tall på pakker i uken 42

G KAFFE

Hvor mange kopper kaffe drikker De vanligvis hver dag?

Sett kryss i den ruten som passer best.

Drikker ikke kaffe, eller mindre enn en kopp 45
1 – 4 kopper .....
5 – 8 kopper .....
9 eller flere kopper .....

Hva slags kaffe drikker De vanligvis hver dag?

Kokekaffe 46
Filterkaffe 47
Pulverkaffe 48
Koffeinfri kaffe 49
Drikker ikke kaffe 50

H ARBEID

Har De i de siste 12 månedene fått arbeidsledighetstrygd? 51

Er De for tiden sykemeldt, eller får De attføringspenger? 52

Har De full eller delvis uførepensjon? 53

Har De vanligvis skiftarbeid eller nattarbeid 54

Har De i det siste året hatt:

Sett kryss i den ruten som passer best.

For det meste stillesittende arbeid? 55
(f.eks. skrivebordsarb., urmakerarb., montering)
Arbeide som krever at De går mye? .....
(f.eks. ekspeditørarb., lett industriarb., undervisn.)
Arbeide der De går og løfter mye? .....
(f.eks. postbud, tyngre industriarb., bygningsarb.)
Tungt kroppsarbeid? .....
(f.eks. skogsarb., tungt jordbruksarb., tungt bygningarb.)

Er husmorarbeid hovedyrket Deres? 56

I ETTERUNDERSØKELSE

Har noen i husstanden Deres (utenom Dem selv) vært innkalt til nærmere undersøkelse hos lege etter den siste hjertekarundersøkelsen? 57

Dersom denne helseundersøkelsen viser at De bør undersøkes nærmere: Hvilken almenpraktiserende lege ønsker De da å bli henvist til?

Skriv navnet på legen her →

..... 58

Ingen spesiell lege .....

61

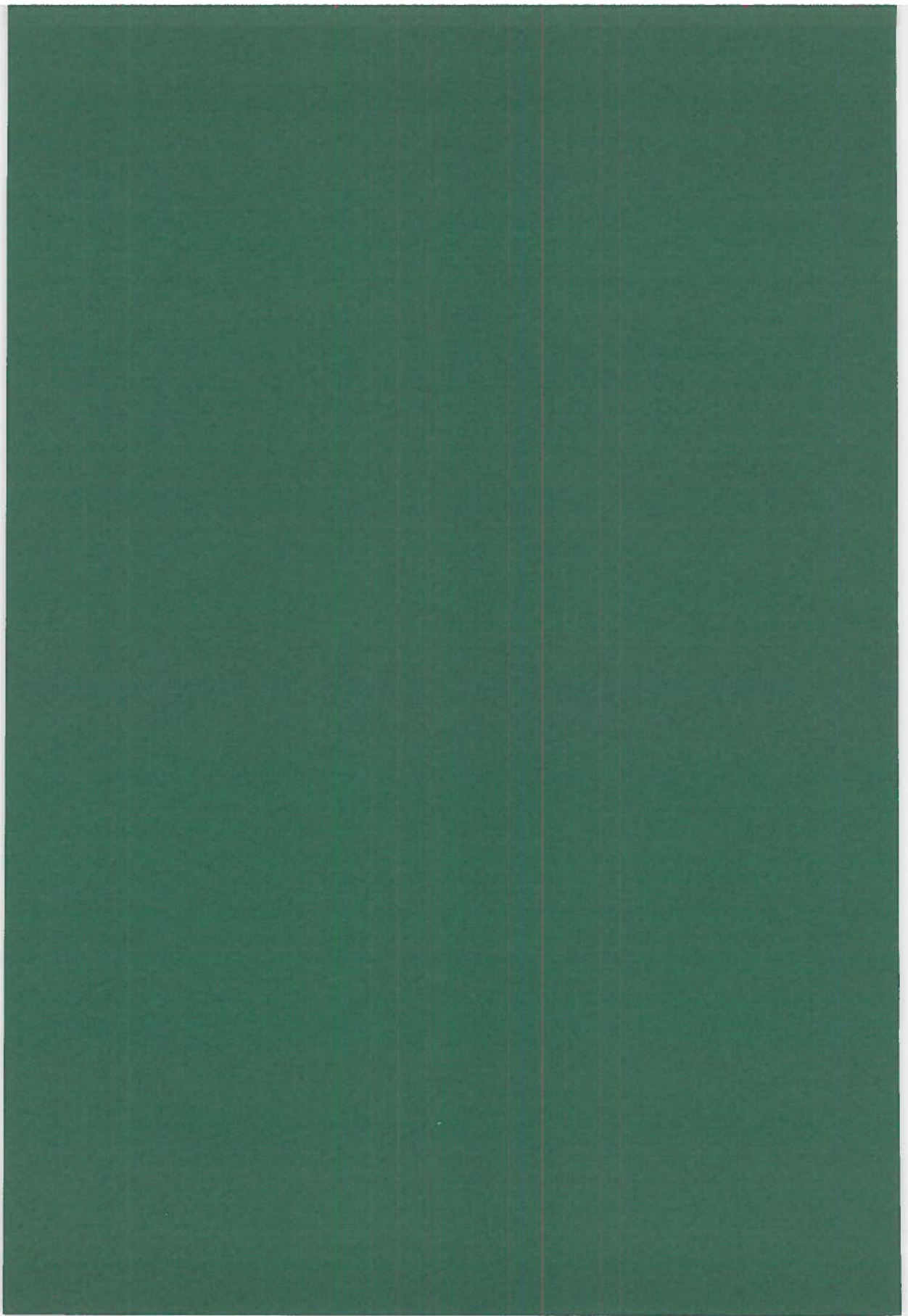
Ikke skriv her

Ikke skriv her

APPENDIX IV

Questionnaire 1 The Nordland Health Study 1987-1988

Original version



Tilleggsspørsmål til Helseundersøkelsen i Nordland 1988–1989.

Hovedformålet med den undersøkelsen du idag har gjennomgått er å undersøke risiko for hjerte- karsykdommer. Dette er imidlertid en mangeartet sykdomsgruppe med tildels dårlig kjente årsaksforhold.
For å finne mer ut av årsakene til hjertekarsykdommer og andre hyppige kroniske sykdommer er det nødvendig å få mer kunnskap om bl.a. vaner, helseforholdene generelt og arbeidsmiljøet.

I dette spørreskjemaet ønsker vi derfor å stille deg en rekke spørsmål om forhold som vi tror kan ha betydning for risikoen for å få bl.a. hjertekarsykdom og kreft. Hvis du er i tvil om hva du skal svare, sett kryss i den ruten som passer best.

Ved å delta på denne undersøkelsen bidrar du til å finne mer ut om de forhold som er av betydning for helse og sykdom. Opplysningene du gir vil bare bli brukt til forskning og blir behandlet strengt fortrolig.

Det utfylte skjema sendes i vedlagte svarkonvolutt.
Portoen er betalt.

På forhånd takk for bidraget!

Med vennlig hilsen

Fylkeslegen i Nordland — Universitetet i Tromsø

Statens helseundersøkelser

PERSONALIA

	Ja	Nei
Er du gift eller samboende?	☐	☐

I hvilket fylke er du født?

.....

Hvor mange års skolegang har du (ta også med folkeskole og ungdomskole)? ..... år

Hvor mange personer bor det i din husstand?

Antall: .....

	Ja	Nei	Vet ikke
Er to eller flere av dine besteforeldre av finsk ætt	☐	☐	☐
Er to eller flere av dine besteforeldre av samisk ætt	☐	☐	☐

HELSE OG SYKDOM

Hvordan er din helsetilstand?

- Dårlig ☐1
- Hverken god eller dårlig ☐2
- Bra ☐3
- Utmerket ☐4

Har du eller har du hatt:
Kryss av for hver sykdom.

	Ja	Nei
Hudsykdommen psoriasis	☐	☐
Astma	☐	☐
Allergisk eksem	☐	☐
Høysnue	☐	☐
Kronisk bronkitt	☐	☐
Leddgikt (revmatisk artritt)	☐	☐
Bechterews sykdom	☐	☐
Brystkreft	☐	☐
Kreft på livmorhalsen	☐	☐
Annen kreftsykdom	☐	☐
Epilepsi (fallesyke)	☐	☐
Migrene	☐	☐

Hvor mange av dine egne tenner har du igjen? Antall .....

Hvor mange ganger har du hatt vondt i halsen eller influensa med høy feber det siste året? .....

MAGEPLAGER

	Ja	Nei
Har du vært plaget med sure oppstøt, halsbrann eller brystsvie?	☐	☐
Har du vært plaget med sterke smerter eller verk øverst i magen?	☐	☐
Hvis «Ja»: Forandrer smertene seg når du spiser?	☐	☐

Hvis smertene forandrer seg ved spising, blir de:

- mindre plagsomme ☐1
- verre ☐2

	Ja	Nei
Har du søkt lege på grunn av slike plager?	☐	☐

Hvor gammel var du første gang du fikk slike plager? ..... år

	Ja	Nei
Har du eller har du hatt sår på magesekken eller tolvfingertarmen?	☐	☐

Hvis «Ja»: Hva slags sår?

- Sår på magesekk ☐1
- Sår på tolvfingertarmen ☐2
- Vet ikke ☐3

Hvilket år ble diagnosen stillet første gang? 19 .....

	Ja	Nei
Er såret påvist ved røntgenundersøkelse?	☐	☐
Er såret påvist ved gastroskopi (direkte undersøkelse av magen gjennom bøyelig rør)?	☐	☐
Ble du innlagt i sykehus for såret?	☐	☐
Er du operert for såret?	☐	☐

Hvis «Ja»: I hvilket år ble du operert? 19 .....

HOFTEPLAGER

	Ja	Nei
Har du medfødt hoftelidelse?	☐	☐
Hvis «Ja»: Sitter lidelsen i		
Høyre hofte	☐1	
Venstre hofte	☐2	
Begge hofter	☐3	
Hvor gammel var du da hoftelidelsene ble oppdaget?	..... år	
	Ja	Nei
Er du operert i hoften?	☐	☐

	Ja	Nei	Vet ikke
Ble du som spebarn behandlet for hoftefeil med gips eller med pute mellom beina	☐	☐	☐

Kryss av for familiemedlemmer som har eller har hatt hoftefeil:

Farfar ☐1 Farmor ☐2 Søster ☐3 Far ☐4
Morfar ☐5 Mormor ☐6 Bror ☐7 Mor ☐8

PLAGER I HODE, NAKKE OG SKULDRE

Hvor ofte er du plaget av hodepine?
- Sjelden eller aldri ☐1
- En eller flere ganger i måneden ☐2
- En eller flere ganger i uken ☐3
- Daglig ☐4

Hvor ofte er du plaget av smerter i nakke eller skuldre?
- Sjelden eller aldri ☐1
- En eller flere ganger i måneden ☐2
- En eller flere ganger i uken ☐3
- Daglig ☐4

Reduserer plagene i hodet, nakken eller skuldre din arbeidsevne?
- Aldri, eller i ubetydelig grad ☐1
- I noen grad ☐2
- I betydelig grad ☐3
- Klarer ikke vanlig arbeid ☐4

BEVISTLØSHET / HJERTEPLAGER

	Ja	Nei
Har du siste år hatt anfall med plutselig og fullstendig tap av bevissthet?	☐	☐
Hvis «Ja»: Falt du om?	☐	☐
	Antall	
Hvor mange anfall har du hatt siste år?	.....	
	Ja	Nei
Har du hatt anfall med plutselig endring i pulsen eller hjerterytmen siste år?	☐	☐
Hvis «Ja»: Hvordan var hjerteslagene?		
Raskere enn normalt	☐1	
Langsommere enn normalt	☐2	
Uregelmessige	☐3	
	Ja	Nei
Ble du uvel, kvalm e.l. under anfallet?	☐	☐
	Antall	
Hvor mange slike anfall har du hatt siste år?	.....	

SYKDOM HOS FAMILIE

Kryss av for de slektninger som har eller har hatt noen av sykdommene:

	Far	Mor	Bror	Søster
Hjerneslag eller hjerneblødning	☐	☐	☐	☐
Sukkersyke	☐	☐	☐	☐
Hudsykdommen psoriasis	☐	☐	☐	☐
Magesår eller tolvfingertarmsår	☐	☐	☐	☐
Astma	☐	☐	☐	☐
Leddgikt (revmatoid artritt)	☐	☐	☐	☐
Bechterews sykdom	☐	☐	☐	☐
Brystkreft		☐		☐
Annen kreftsykdom	☐	☐	☐	☐
Epilepsi (fallesyke)	☐	☐	☐	☐
Migrene	☐	☐	☐	☐
Ingen av sykdommene ovenfor	☐	☐	☐	☐

KONTAKT MED HELSETJENESTEN

Hvor mange besøk har du hatt siste år på grunn av egen helse eller sykdom? Svar på hvert enkelt spørsmål.	Antall besøk
Hos vanlig lege	.....
Hos spesialist utenfor sykehus	.....
På legevakta	.....
Hos bedriftslege	.....
Hos sykepleier på sykestue	.....
Hos fysioterapeut	.....
Hos kiropraktor	.....
Hos naturmedisiner (homeopat, soneterapeut o.l.)	.....
Hos tannlege	.....
På sykehusets poliklinikk	.....
Antall innleggelser på sykehus siste år	.....
Antall hjemmebesøk av lege til familien siste år	.....

Har du søkt hjelp på grunn av plager fra hode, nakke og skuldre det siste året?	Ja	Nei
Hos vanlig lege	☐	☐
Hos spesialist utenfor sykehus	☐	☐
På legevakta	☐	☐
Hos bedriftslege	☐	☐
Hos kiropraktor	☐	☐
Hos naturmedisiner (homeopat, soneterapeut o.l.)	☐	☐
På sykehusets poliklinikk	☐	☐
	Antall	
Antall innleggelser på sykehus siste år p.g.a. plager fra hode, nakke og skuldre	.....	

Hva gjorde du siste gang du hadde vondt i halsen eller influensa med høy feber?
- Oppsøkte lege for å få behandling ☐1
- Oppsøkte lege for å få sykemelding ☐2
- Ventet til det hele gikk over av seg sjøl ☐3
- Brukte mine egne måter å bli frisk på ☐4

BRUK AV RØNTGEN-UNDERSØKELSE

	Ja	Nei
Har du vært til røntgenundersøkelse de siste 5 år?	☐	☐

Hvis «Ja»:

Hvor mange ganger har du vært til røntgenundersøkelse av:	Antall ganger
nakken	.....
korsryggen (veikryggen)	.....
magesekk/tolvfingertarm	.....
tykktarm	.....
tenner	.....

TILFREDSHET MED HELSETJENESTEN

Hvor lang tid tar det vanligvis å få time hos vanlig lege i den kommunen der du bor?
- 0-4 dager ☐1
- 5-8 dager ☐2
- 9-14 dager ☐3
- Mer enn 14 dager ☐4
- Vet ikke ☐5

Er det vanskelig eller lett å få lege til sykebesøk i hjemmet når det er behov for det?
- Lett ☐1
- Vanskelig ☐2
- Svært vanskelig ☐3
- Vet ikke ☐4

Er det lettere eller vanskeligere å få lege til sykebesøk enn for 5 år siden?
- Lettere nå ☐1
- Uforandret ☐2
- Vanskeligere nå ☐3
- Vet ikke ☐4

Synes du at almenpraktiserende leger i din kommune tar seg nok tid til å snakke med pasientene?
- Nok tid ☐1
- Dårlig tid ☐2
- Svært dårlig tid ☐3
- Vet ikke ☐4

Er du alt i alt fornøyd eller misfornøyd med almenlege-tjenesten i din bostedskommune?
- Godt fornøyd ☐1
- Fornøyd ☐2
- Misfornøyd ☐3
- Vet ikke ☐4

Tror du almenlegetjenesten alt i alt har blitt bedre eller dårligere i løpet av de siste 5 år i din kommune?
- Bedre nå enn før ☐1
- Bedre for 4-5 år siden ☐2
- Uforandret ☐3
- Vet ikke ☐4

	Ja	Nei
Bor du i gangavstand til et vanlig legekontor?	☐	☐

	Ja	Nei	Ikke aktuelt
Har fylket (Nordland fylkeskommune) nektet deg innleggelse på sykehus utenfor Nordland når din vanlige lege mente det var nødvendig?	☐	☐	☐

Har du siste 5 årsperiode latt være å søke hjelp hos tannlege, lege, sykehuspoliklinikk eller fysioterapeut p.g.a. egenandeler? Kryss av for hvert spørsmål.	Ja	Nei
Tannlege	☐	☐
Vanlig lege	☐	☐
Poliklinikk	☐	☐
Fysioterapeut	☐	☐

KONTAKT I NÆRMILJØET

	Antall timer
Hvor mange timer bruker du på lokal foreningsvirksomhet (som idrettlag, politiske lag, religiøse eller andre foreninger) i en vanlig arbeidsuke?	.....

	Antall
Hvor mange familier/husstander i nabolaget kjenner du så godt at dere besøker hverandre av og til?	.....

Har du i løpet av de siste 14 dagene snakket med:	Ja	Nei
noen i familien om gleder og sorger	☐	☐
noen i familien om helsespørsmål	☐	☐
andre utenom familien om gleder og sorger	☐	☐
andre utenom familien om helsespørsmål	☐	☐

	Ja	Nei
Har noen spurt deg om råd når det gjelder sykdom og helse de siste 14 dager?	☐	☐

Hender det ofte at du føler deg ensom?
- Nei ☐1
- Av og til ☐2
- Ofte ☐3

Hvor ofte er du vanligvis sammen med venner i fritiden?
- Daglig/nesten daglig ☐1
- 2-3 ganger pr. uke ☐2
- Ca. 1 gang pr. uke ☐3
- 1-2 ganger pr. måned ☐4
- Sjeldnere enn en gang pr. måned ☐5

KOSTHOLD

Hvor mange brødskiver spiser du vanligvis daglig?
- Mindre enn 2 skiver ☐1
- 2-4 skiver ☐2
- 5-6 skiver ☐3
- 7-12 skiver ☐4
- 13 eller flere skiver ☐5

Hva slags melk drikker du vanligvis?
- Drikker ikke melk ☐1
- Helmelk, søt eller sur ☐2
- Lettmelk, søt eller sur ☐3
- Skummet melk, søt eller sur ☐4

Hvor mange glass/kopper melk drikker du vanligvis daglig?
- Mindre enn ett glass/kopp ☐1
- 1-2 glass/kopper ☐2
- 3-4 glass/kopper ☐3
- 5 eller flere glass/kopper ☐4

Hvor ofte spiser du vanligvis frukt?
- Sjeldnere enn en gang i uken ☐1
- Omtrent en gang i uken ☐2
- 2-3 ganger i uken ☐3
- 4-5 ganger i uken ☐4
- Omtrent daglig ☐5

Hvor ofte spiser du vanligvis grønnsaker til middag eller som egen rett?
- Sjeldnere enn en gang i uken ☐1
- Omtrent en gang i uken ☐2
- 2-3 ganger i uken ☐3
- 4-5 ganger i uken ☐4
- Omtrent daglig ☐5

Hvor ofte spiser du gulrøtter?
- Sjeldnere enn en gang i uken ☐1
- Omtrent en gang i uken ☐2
- 2-3 ganger i uka ☐3
- Mer enn 3 ganger i uka ☐4

Hvor ofte spiser du poteter til middag i løpet av en uke?
- Sjeldnere enn 4 ganger i uken ☐1
- 4-5 ganger i uken ☐2
- 6-7 ganger i uken ☐3

Hvor mange poteter spiser du vanligvis til hvert middagsmåltid?
- Mindre enn en ☐1
- 1-2 ☐2
- 3-4 ☐3
- 5 eller flere ☐4

Hvor ofte bruker du fett (smør, margarin, remulade, majones og lignende) til eller på middagsmaten?
- Sjeldnere enn en gang i uken ☐1
- 1-2 ganger i uken ☐2
- 3-4 ganger i uken ☐3
- 5 eller flere ganger i uken ☐4

Hvor ofte spiser du torsk/sei eller annen mager fisk til middag?
- Sjeldnere enn en gang i uken ☐1
- 1 gang i uken ☐2
- 2 ganger i uken ☐3
- 3 ganger i uken ☐4
- 4 eller flere ganger i uken ☐5

Hvor ofte spiser du feitere fisk slik som uer, kvejte, sild, makrell, laks eller ørret til middag?
- Sjeldnere enn en gang i uken ☐1
- 1 gang i uken ☐2
- 2 ganger i uken ☐3
- 3 ganger i uken ☐4
- 4 eller flere ganger i uken ☐5

Hvor mange skiver spiser du der pålegget består av feit fisk (sild, sardiner, makrell, laks o.l.)?
- Mindre enn en skive i uka ☐1
- 1-2 skiver i uka ☐2
- 3-6 skiver i uka ☐3
- 1-2 skiver om dagen ☐4
- 3-4 skiver om dagen ☐5
- 5 eller flere skiver daglig ☐6

	Ja	Nei
Tar du tran, tranpiller eller fiskeoljekapsler for tida?	☐	☐

Hvor mye cola-drikker drikker du i uka?
- Drikker ikke cola-drikker ☐1
- Mindre enn 0.5 liter pr. uke ☐2
- 0.5 - 1.5 liter pr. uke ☐3
- Mer enn 1.5 liter pr. uke ☐4

ALKOHOL

	Ja	Nei
Er du total avholdsmann/kvinne?	☐	☐

Hvis «Nei»:

Hvor ofte pleier du å drikke øl?
- Aldri, eller bare noen få ganger i året ☐1
- 1-2 ganger i måneden ☐2
- Omtrent 1 gang i uken ☐3
- 2-3 ganger i uken ☐4
- Omtrent daglig ☐5

Hvor ofte pleier du å drikke vin?
- Aldri, eller bare noen få ganger i året ☐1
- 1-2 ganger i måneden ☐2
- Omtrent 1 gang i uken ☐3
- 2-3 ganger i uken ☐4
- Omtrent daglig ☐5

Hvor ofte pleier du å drikke brennevin?
- Aldri, eller bare noen få ganger i året ☐1
- 1-2 ganger i måneden ☐2
- Omtrent 1 gang i uken ☐3
- 2-3 ganger i uken ☐4
- Omtrent daglig ☐5

Omtrent hvor ofte har du i løpet av siste år drukket en mengde alkohol tilsvarende minst 5 halvflasker øl, en hel flaske vin eller ¼ flaske brennevin?
- Ikke siste år ☐1
- Noen få ganger ☐2
- 1-2 ganger i måneden ☐3
- 1-2 ganger i uken ☐4
- 3 eller flere ganger i uken ☐5

MOSJON

Hvor ofte utfører du fysisk aktivitet av minst 20 minutters varighet som fører til at du blir svett eller andpusten?
- Sjelden eller aldri ☐1
- Ukentlig ☐2
- Flere ganger i uka ☐3
- Daglig ☐4

Dersom du vanligvis utfører slik aktivitet minst en gang i uka, hvor mye tid bruker du ukentlig til slik aktivitet?
- Mindre enn 30 minutter i uka ☐1
- Mellom 30 minutter og 1 time i uka ☐2
- Mellom 1 og 2 timer i uka ☐3
- Mer enn 2 timer i uka ☐4

Har du endret din fysiske aktivitet i løpet av de siste 5 år?
- Jeg drev mer fysisk aktivitet før ☐1
- Det har ikke vært noen endring ☐2
- Jeg drev mindre fysisk aktivitet før ☐3

REAKSJONER PÅ PROBLEMER

	Ja	Nei
Hvis du får store personlige problemer, regner du da med å få hjelp og støtte fra ektefelle, samboer eller familie?	☐	☐
Har du i lengere tid følt behov for å oppsøke noen på grunn av personlige problem siste år, uten at du har tatt slik kontakt?	☐	☐

Blir du utålmodig eller irritert når du må vente?
- Svært irritert ☐1
- Noe irritert ☐2
- Ikke irritert ☐3

Er du stort sett fornøyd med tilværelsen?
- Meget fornøyd ☐1
- Ganske fornøyd ☐2
- Litt misfornøyd ☐3
- Meget misfornøyd ☐4

Har du i de siste 14 dager følt deg ulykkelig og nedtrykt (deprimert)?
- Aldri eller sjelden ☐1
- Av og til ☐2
- Ofte ☐3
- Nesten hele tida ☐4

Under kommer noen påstander vi ber deg ta stilling til.
For hver påstand skal du sette ett kryss i en rute, alt etter hvor enig du er i påstanden.

	Helt enig	Noe enig	Noe uenig	Helt uenig
Når jeg ikke føler meg bra, bør jeg snakke med lege eller annet helsepersonell	☐	☐	☐	☐
Jeg har stor kontroll over min egen helse	☐	☐	☐	☐
Hvis jeg blir syk, er det for det meste min egen adferd som avgjør hvor raskt jeg blir frisk igjen	☐	☐	☐	☐
Folk flest har problemer nok om en ikke også skal mase om alt som er skadelig for helsa	☐	☐	☐	☐
Jeg kan i stor grad unngå kreft og hjerteinfarkt, hvis jeg tar de riktige forholdsreglene	☐	☐	☐	☐
Jeg kan endre hvilken som helst vane, bare jeg bestemmer meg for det	☐	☐	☐	☐
Folk må ha lov til å skade sin egen helse, så lenge de ikke skader andre	☐	☐	☐	☐

ARBEIDSMILJØ

Hva er ditt nåværende hovedyrke?
- Hjemmeværende husmor ☐ 1
- Skoleelev/student ☐ 2
- Industri/verksted/anleggs/bygnings/sprengings/gruvearbeide ☐ 3
- Jordbruks/skogbruksarbeide ☐ 4
- Fisker/sjømann ☐ 5
- Kontor/handels/hotell/servicearbeide ☐ 6
- Helsearbeide ☐ 7
- Lærer/annet undervisningsarbeide ☐ 8
- Landtransport (sjåfør m.v) ☐ 9
- Arbeidsledig ☐10
- Under attføring ☐11
- Uføretrygdet/alderstrygdet/pensjonert ☐12
- Annet ☐13

- Angi evt. yrkesbetegnelse her:

.....

Resten av spørsmålene om ***arbeidsmiljø*** besvares bare av dem som er i lønnet arbeid.

Hvor mange år du har vært på din siste arbeidsplass? .....år

	Ja	Nei
Arbeider du i full stilling? (37 timer eller mer pr. uke)	☐	☐
Har du skiftarbeide?	☐	☐

Hvor ofte arbeider du overtid?
- Hver uke ☐1
- Hver måned ☐2
- Sjelden eller aldri ☐3

	Ja	Nei
Er reisetiden til og fra arbeidet samlet over 1 time	☐	☐
Må du i forbindelse med arbeidet overnatte utenfor hjemmet?	☐	☐

Hvis «Ja»: Hvordan overnatter du?
- I ordinær bopel/leilighet ☐1
- På hybel. ☐2
- På hotell/pensjonat ☐3
- I anleggsbrakke ☐4
- På annen måte ☐5

	Ja	Nei
Har du vært sykemeldt tilsammen mer enn 4 uker det siste året?	☐	☐

Hvordan trives du med det arbeidet du har nå?
- Meget godt ☐1
- Godt ☐2
- Dårlig ☐3
- Trives ikke ☐4

Er arbeidsoppgavene tilstrekkelig varierte?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Får du vite om du gjør en god jobb?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Er kontakten og samarbeidet med overordnede bra?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Er samarbeide og fellesskap bra på arbeidsplassen?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Får du hjelp og støtte når du har problemer i arbeidet?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Kan du påvirke arbeidsforholdene slik at du får et passende arbeidstempo?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Har du for mye å gjøre i ditt arbeide?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Stiller arbeidet for store krav til deg?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Er du redd for at ditt arbeid skal endres ved omorganisering, nye arbeidsmåter o.l.?
- Som oftest ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Er du blitt mobbet/trakassert på arbeidsplassen?
- Ofte ☐1
- Iblant ☐2
- Sjelden ☐3
- Aldri ☐4

Er du i ditt nåværende arbeid, eller har du i tidligere arbeid vært, utsatt for:

	Nåværende arbeid				Tidligere arbeid	
	Utsatt for		Medfører ubehag		Utsatt for	
	Ja	Nei	Ja	Nei	Ja	Nei
Støy	☐	☐	☐	☐	☐	☐
Vibrasjoner (utstyr, kjøretøy e.l.)	☐	☐	☐	☐	☐	☐
Dårlig klima (kulde, varme, trekk o.l.)	☐	☐	☐	☐	☐	☐
Stråling (røntgen, glødende metall, o.l.)	☐	☐	☐	☐	☐	☐
Dårlig belysning	☐	☐	☐	☐	☐	☐
Synskrevende arbeide	☐	☐	☐	☐	☐	☐
Tunge løft	☐	☐	☐	☐	☐	☐
Ensformig arbeide	☐	☐	☐	☐	☐	☐
Tobakksrøyk	☐	☐	☐	☐	☐	☐
Røyk (fra sveising, lodding o.l.)	☐	☐	☐	☐	☐	☐
Eksos	☐	☐	☐	☐	☐	☐
Gasser og løsemidler	☐	☐	☐	☐	☐	☐
Andre kjemikalier	☐	☐	☐	☐	☐	☐

RESTEN AV SKJEMAET BESVARES BARE AV KVINNER

Hvor gammel var du da du fikk menstruasjon første gang? .... år

Antall

Hvor mange barn har du født? ....

Dersom du har født barn, hvor gammel var du første gang du fødte? .... år

Hvor gammel var du da du fikk ditt siste barn? .... år

	Ja	Nei
Har du vært plaget av bekkenløsning under ett eller flere av dine svangerskap?	☐	☐

BRYSTUNDERSØKELSE

Hvor ofte undersøker du brystene dine selv?
Sett kryss i den ruten som passer best.
- Aldri ☐1
- 2-3 ganger pr. år ☐2
- 1 gang pr. måned ☐3
- Oftere enn en gang i måneden ☐4

	Ja	Nei
Har du søkt lege for kul i brystet?	☐	☐
Hvis «Ja»:		
Ble det tatt prøve av kulen?	☐	☐
Har du røntgenundersøkt (mammografert) brystene?	☐	☐

PREVENSJON

	Ja	Nei
Bruker du P-Piller nå?	☐	☐
Har du brukt P-Piller tidligere?	☐	☐

Hvis «Ja» på ett av de to spørsmålene over:
- Hvor gammel var du da du begynte med P-Piller? .... år
- Hvor mange år har du tilsammen brukt P-Piller? .... år
- Dersom du har født, hvor lenge brukte du P-Piller før første fødsel? .... år

Hvis du har sluttet å bruke P-Piller:
- Hvor gammel var du da du sluttet ? .... år

	Ja	Nei
Ble du anbefalt å slutte av medisinske årsaker?	☐	☐

KREFTPRØVE

Antall

Hvor mange ganger har du fått tatt kreftprøve (celleprøve) fra livmorhalsen siste 3 år? ....

Hvor mange år er det siden siste prøve ble tatt? .... år

ISM SKRIFTSERIE - FØR UTGITT:

1. Bidrag til belysning av medisinske og sosiale forhold i Finnmark fylke, med særlig vekt på forholdene blant finskættede i Sør-Varanger kommune.
   Av Anders Forsdahl, 1976. (nytt opplag 1990)

2. Sunnhetstilstanden, hygieniske og sosiale forhold i Sør-Varanger kommune 1869-1975 belyst ved medisinal-beretningene.
   Av Anders Forsdahl, 1977.

3. Hjerte-karundersøkelsen i Finnmark - et eksempel på en populasjonsundersøkelse rettet mot cardiovasculære sykdommer. Beskrivelse og analyse av etterundersøkelsesgruppen.
   Av Jan-Ivar Kvamme og Trond Haider, 1979.

4. The Tromsø Heart Study: Population studies of coronary risk factors with special emphasis on high density lipoprotein and the family occurrence of myocardial infarction.
   Av Olav Helge Førde og Dag Steinar Thelle, 1979.

5. Reformer i distriktshelsetjenesten III: Hypertensjon i distriktshelsetjenesten.
   Av Jan-Ivar Kvamme, 1980.

6. Til professor Knut Westlund på hans 60-års dag, 1983.

7.* Blodtrykksovervåkning og blodtrykksmåling.
   Av Jan-Ivar Kvamme, Bernt Nesje og Anders Forsdahl, 1983.

8.* Merkesteiner i norsk medisin reist av allmennpraktikere - og enkelte utdrag av medisinalberetninger av kulturhistorisk verdi.
   Av Anders Forsdahl, 1984.

9. "Balsfjordsystemet." EDB-basert journal, arkiv og statistikksystem for primærhelsetjenesten.
   Av Toralf Hasvold, 1984.

10. Tvunget psykisk helsevern i Norge. Rettsikkerheten ved slikt helsevern med særlig vurdering av kontrollkommisjonsordningen.
    Av Georg Høyer, 1986.

11. The use of self-administered questionnaires about food habits. Relationships with risk factors for coronary heart disease and associations between coffee drinking and mortality and cancer incidence.
    Av Bjarne Koster Jacobsen, 1988.

12.* Helse og ulikhet. Vi trenger et handlingsprogram for Finnmark.
    Av Anders Forsdahl, Atle Svendal, Aslak Syse og Dag Thelle, 1989.

13. Health education and self-care in dentistry - surveys and interventions.
Av Anne Johanne Søgaard, 1989.

14. Helsekontroller i praksis. Erfaringer fra prosjektet helsekontroller i Troms 1983-1985.
Av Harald Siem og Arild Johansen, 1989.

15. Til Anders Forsdahls 60-års dag, 1990.

16. Diagnosis of cancer in general practice. A study of delay problems and warning signals of cancer, with implications for public cancer information and for cancer diagnostic strategies in general practice.
Av Knut Holtedahl, 1991.

17. The Tromsø Survey. The family intervention study. Feasibility of using a family approach to intervention on coronary heart disease. The effect of lifestyle intervention of coronary risk factors.
Av Synnøve Fønnebø Knutsen, 1991.

18. Helhetsforståelse og kommunikasjon. Filosofi for klinikere.
Av Åge Wifstad, 1991.

19. Factors affecting self-evaluated general health status - and the use of professional health care services.
Av Knut Fylkesnes, 1991.

20. Serum gamma-glutamyltransferase: Population determinants and diagnostic characteristics in relation to intervention on risk drinkers.
Av Odd Nilssen, 1992.

21. The Healthy Faith. Pregnancy outcome, risk of disease, cancer morbidity and mortality in Norwegian Seventh-Day-Adventists.
Av Vinjar Fønnebø, 1992.

22. Aspects of breast and cervical cancer screening.
Av Inger Torhild Gram, 1992.

23. Population studies on dyspepsia and peptic ulcer disease: Occurrence, aetiology, and diagnosis. From The Tromsø Heart Study and The Sørreisa Gastrointestinal Disorder Studie.
Av Roar Johnsen, 1992.

24. Diagnosis of pneumonia in adults in general practice.
Av Hasse Melbye, 1992.

25. Relationship between hemodynamics and blood lipids in population surveys, and effects of n-3 fatty acids.
Av Kaare Bønaa, 1992.

26. Risk factors for, and 13-year mortality from cardiovascular disease by socioeconomic status. A study of 44690 men and 17540 women, ages 40-49. **Av Hanne Thürmer, 1993.**

27. Utdrag av medisinalberetninger fra Sulitjelma 1891-1990. **Av Anders Forsdahl, 1993.**

28. Helse, livsstil og levekår i Finnmark. Resultater fra Hjerte-karundersøkelsen i 1987-88. Finnmark III. **Av Knut Westlund og Anne Johanne Søgaard, 1993.**

29. Patterns and predictors of drug use. A pharmacoepidemiologic study, linking the analgesic drug prescriptions to a population health survey in Tromsø, Norway. **Av Anne Elise Eggen, 1994.**

30. ECG in health and disease. ECG findings in relation to CHD risk factors, constitutional variables and 16-year mortality in 2990 asymptomatic Oslo men aged 40-49 years in 1972. **Av Per G. Lund-Larsen, 1994.**

31. Arrhythmia, electrocardiographic signs, and physical activity in relation to coronary heart risk factors and disease. The Tromsø Study. **Av Maja-Lisa Løchen, 1995.**

32. The Military service: mental distress and changes in health behaviours among Norwegian army conscript. **Av Edvin Schei, 1995.**

33. The Harstad injury prevention study: Hospital-based injury recording and community-based intervention. **Av Børge Ytterstad, 1995.**

34.* Vilkår for begrepsdannelse og praksis i psykiatri. En filosofisk undersøkelse. **Av Åge Wifstad, 1996.** (utgitt Tano Aschehoug forlag 1997)

35. Dialog og refleksjon. Festskrift til professor Tom Andersen på hans 60-års dag, 1996.

36. Factors affecting doctors´ decision making. **Av Ivar Sønbø Kristiansen, 1996.**

37. The Sørreisa gastrointestinal disorder study. Dyspepsia, peptic ulcer and endoscopic findings in a population. **Av Bjørn Bernersen, 1996.**

38. Headache and neck or shoulder pain. An analysis of musculoskeletal problems in three comprehensive population studies in Northern Norway. **Av Toralf Hasvold, 1996.**

39. Senfølger av kjernefysiske prøvespreninger på øygruppen Novaya Semlya i perioden 1955 til 1962. Rapport etter programmet "Liv". Arkangelsk 1994.
Av A.V. Tkatchev, L.K. Dobrodeeva, A.I. Isaev, T.S. Podjakova, 1996.

40. Helse og livskvalitet på 78 grader nord. Rapport fra en befolkningsstudie på Svalbard høsten 1988.
Av Helge Schirmer, Georg Høyer, Odd Nilssen, Tormod Brenn og Siri Steine, 1997.

41. Physical activity and risk of cancer. A population based cohort study including prostate, testicular, colorectal, lung and breast cancer.
Av Inger Thune, 1997.

42. The Norwegian - Russian Health Study 1994/95. A cross-sectional study of pollution and health in the border area.
Av Tone Smith-Sivertsen, Valeri Tchachtchine, Eiliv Lund, Tor Norseth, Vladimir Bykov, 1997.

43. Use of alternative medicine by Norwegian cancer patients
Av Terje Risberg, 1998.

44. Incidence of and risk factors for myocardial infarction, stroke, and diabetes mellitus in a general population. The Finnmark Study 1974-1989.
Av Inger Njølstad, 1998.

45. General practitioner hospitals: Use and usefulness. A study from Finnmark County in North Norway.
Av Ivar Aaraas, 1998.

45B Sykestuer i Finnmark. En studie av bruk og nytteverdi.
Av Ivar Aaraas, 1998.

46. No går det på helsa laus. Helse, sykdom og risiko for sykdom i to nord-norske kystsamfunn.
Av Jorid Andersen, 1998.

47. The Tromsø Study: Risk factors for non-vertebral fractures in a middle-aged population.
Av Ragnar Martin Joakimsen, 1999.

48. The potential for reducing inappropriate hospital admissions: A study of health benefits and costs in a department of internal medicine.
Av Bjørn Odvar Eriksen, 1999.

49. Echocardiographic screening in a general population. Normal distribution of echocardiographic measurements and their relation to cardiovascular risk factors and disease. The Tromsø Study.
Av **Henrik Schirmer, 2000.**

50. Environmental and occupational exposure, life-style factors and pregnancy outcome in artic and subartic populations of Norway and Russia.
Av Jon Øyvind Odland, 2000.

50B Окружающая и профессиональная экспозиция, факторы стиля жизни и исход беременности у населения арктической и субарктической частей Норвегии и России
Юн Ойвин Удлан 2000

51. A population based study on coronary heart disease in families. The Finnmark Study 1974-1989.
Av Tormod Brenn, 2000.

52. Ultrasound assessed carotid atherosclerosis in a general population. The Tromsø Study.
Av Oddmund Joakimsen, 2000.

53. Risk factors for carotid intima-media thickness in a general population. The Tromsø Study 1979-1994.
Av Eva Stensland-Bugge, 2000.

54. The South Asian cataract management study.
Av Torkel Snellingen, 2000.

55. Air pollution and health in the Norwegian-Russian border area.
Av Tone Smith-Sivertsen, 2000.

56. Interpretation of forearm bone mineral density. The Tromsø Study.
Av Gro K. Rosvold Berntsen, 2000.

De som er merket med * har vi dessverre ikke flere eksemplar av.