

Long-term Effects of Dietary Marine ω -3 Fatty Acids upon Plasma and Cellular Lipids, Platelet Function, and Eicosanoid Formation in Humans

Clemens von Schacky, Sven Fischer, and Peter C. Weber

Medizinische Klinik Innenstadt der Universität München, D-8000 Munich 2, Federal Republic of Germany

Abstract

We studied the incorporation and metabolism of eicosapentaenoic (EPA) and docosahexaenoic acid in six human volunteers who supplemented their normal Western diet for 5 mo daily with 10–40 ml of cod liver oil, rich in ω -3 polyunsaturated fatty acids. EPA and docosahexaenoic acid were incorporated into the total phospholipids of plasma, platelets, and erythrocytes in a dose- and time-dependent manner. During ω -3 fatty acid ingestion serum triacylglycerols were lowered and platelet aggregation upon low doses of collagen was reduced. Concomitantly, formation and excretion of prostanoids showed a characteristic change. As measured in serum from whole clotted blood, thromboxane A_3 was formed in small amounts, whereas thromboxane A_2 formation was reduced to 50% of control values. Excretion of the main urinary thromboxane A metabolites was unaltered in subjects with low basal excretion rates, but decreased markedly in two subjects with high control values. As determined from the main urinary metabolite, prostaglandin I_3 was formed from EPA at rates up to 50% of unaltered prostaglandin I_2 formation. The biochemical and functional changes observed lasted for the entire supplementation period of 5 mo and were reversible within 12 wk after cessation of cod liver oil intake. Favorable changes induced by long-chain ω -3 fatty acids include a dose-related and sustained shift of the prostaglandin I/thromboxane A balance to a more antiaggregatory and vasodilatory state.

Introduction

The low incidence of atherosclerotic and chronic inflammatory diseases in Eskimos native to Greenland (1) has been attributed to their traditional diet consisting almost exclusively of marine food, rich in the two ω -3 fatty acids eicosapentaenoic acid (EPA)¹ and docosahexaenoic acid (2). Furthermore, during supplementation of an otherwise unchanged Western diet with ω -3 fatty acids, a favorable pattern of serum lipids (3–5), reduced platelet aggregability (6–8), a prolonged bleeding time (8), and a reduced blood pressure response to pressor hormones (8) have been ob-

served in studies with human volunteers. In addition, in animal models, fish oils have been found to reduce the size and sequelae of experimental myocardial (9) and cerebral infarction (10), prevent proteinuria and prolong survival in murine lupus (11), and retard rat mammary tumor growth (12).

Arachidonic acid (AA) and EPA are precursors of eicosanoids, which have a broad spectrum of biological activities and have been implicated in the pathogenesis of atherothrombotic and inflammatory processes (13–15). In contrast to studies in human endothelial cell cultures (16), and in rats (17), dietary EPA is transformed in vivo in humans to prostaglandin I_3 (18), which is as active (19) as the vasodilatory and antiaggregatory prostaglandin I_2 (20), derived from AA. Thromboxane A_3 , formed in small quantities after dietary EPA in human platelets (21), in addition to reduced thromboxane A_2 production is, however, far less proaggregatory than thromboxane A_2 (19, 22). Formation of the three series prostanoids would therefore be a means to alter favorably the prostaglandin I/thromboxane A balance, which has been claimed to play a role in the pathogenesis of vascular disorders (23). Reduction of serum cholesterol (3, 4) and triacylglycerol (3, 5) observed in humans after dietary ω -3 fatty acids may represent other beneficial effects.

Almost all of the human studies with ω -3 fatty acids were short-term studies with doses ranging from 50 mg of EPA per day (24) to 120 g of fish oil per day (up to 40% of the daily caloric intake) (3, 5). In one study, functional changes disappeared after 3 wk of ω -3 fatty acid supplementation, despite sustained biochemical changes (25). In another report, reductions in platelet counts were observed after very high doses (3). Although a clinical study on patients with coronary artery disease showed persistent effects of a fish oil supplement on serum lipids (26), it has not been clear whether functional and biochemical changes could be sustained for longer time periods. In addition, the time and dose-related incorporation of dietary ω -3 fatty acids into different blood cells in vivo has not been evaluated. Therefore, we measured the effects of cod liver oil supplementation in various dosages on the fatty acid composition in several compartments and on eicosanoid formation in a long-term study in human volunteers.

Methods

Study design. Six healthy male volunteers (ages 26–36 yr, weight 63–88 kg) were advised to withhold all drugs known to interfere with eicosanoid formation or lipid metabolism during the study period of 40 wk. Cod liver oil (Möller A/S, Oslo, Norway) was ingested in addition to an otherwise unchanged Western diet according to Figs. 1–6: i.e., 4 wk 10 ml, 4 wk 20 ml, 4 wk 40 ml, and 8 wk 20 ml per day without a pause. The cod liver oil contained (relative percentage): saturated fatty acids, 17.3; monoenes, 50.4; EPA, 9.4; and docosahexaenoic acid, 13.8. Dietary advice restricting the intake of any food before, during, or after the study period was not given. At each visit, 24-h urine samples were collected, a standardized questionnaire was filled out, body weight was measured, and blood was drawn after an overnight fast from an antecubital vein.

Platelet preparation and aggregation. Blood was obtained by veni-

Address reprint requests to Dr. Weber, Medizinische Klinik Innenstadt der Universität München, Ziemssenstrasse 1, D-8000 Munich 2, Federal Republic of Germany. Dr. von Schacky's present address is Divisions of Hematology-Oncology, 13 West, New York VA Medical Center, 408 First Avenue, New York 10010.

Received for publication 26 November 1984 and in revised form 21 May 1985.

1. Abbreviations used in this paper: AA, arachidonic acid; EPA, eicosapentaenoic (timnodonic) acid.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/85/10/1626/06 \$1.00

Volume 76, October 1985, 1626–1631

puncture with minimal venous stasis through 19-gauge butterfly sets. Platelets were prepared according to published procedures (27, 28). In short, fresh blood was anticoagulated with 0.15 volume of acid citrate dextrose (85 mM trisodium citrate, 111 mM dextrose, 71 mM citric acid); platelet-rich plasma was prepared by centrifugation (200 g) in the presence of prostaglandin I₂ (5 ng/ml) to prevent platelet activation and adjusted with autologous platelet poor plasma to a final concentration of 250,000 platelets/ μ l. In a two-channel aggregometer (Fresenius, Bad Homburg, Federal Republic of Germany) platelets were exposed for 8 min to 0.75 or 0.25 μ g/ml collagen of a single batch (Hormon Chemie, Munich) or saline (as control) 45–60 min after venipuncture. The change of the transmission of light after addition of the aggregating agents was recorded as a percentage, 100% corresponding to a change of 0.14 optical density.

Fatty acid analyses. Plasma free fatty acids and fatty acids in phospholipids of plasma, of washed erythrocyte ghosts, and of platelets were extracted, methylated, and quantitatively determined as previously described (6, 29). Briefly, EDTA-coated tubes were filled with fresh whole blood, briefly shaken, and centrifuged at 3,000 g for 10 min. Plasma was removed and frozen (-20°C) until analyzed. 2 ml of packed red cells was washed twice in saline, lysed by freeze-thawing twice and addition of 10 vol of H₂O, and centrifuged twice at 15,000 g. The pellet was resuspended in saline and frozen until analyzed. Platelet-rich plasma was prepared as described above and platelets were washed twice in Tyrode's-Hepes-EGTA-glucose buffer containing prostaglandin I₂ (5 ng/ml) (27, 28).

Plasma free fatty acids were extracted (30) and methylated in 75 μ l of methanol with diazomethane. Plasma, erythrocyte ghost (31), and platelet lipids (32) were extracted and phospholipids purified by column chromatography (29). The fatty acid methyl esters were prepared with methanol/H₂SO₄ (70°C, 2 h). Fatty acid methyl esters were analyzed by gas liquid chromatography on a Packard 419 gas chromatograph equipped with two glass columns (length 2 m, 3 mm i.d.) packed with 10% SP 2340 on 100–120-mesh Supelcoport (Supelchem, Griesbach). Carrier gas was N₂; temperature was programmed at 2°C/min from 150 to 200°C. Peaks were identified by comparison to a known standard mixture of fatty acid methyl esters (Sigma Chemical Co., Munich). The amounts of individual fatty acids were calculated as relative percentage with the evaluated fatty acids set as 100%.

Analysis of prostaglandin I metabolites. Urinary excretion of 2,3-dinor-6-keto prostaglandin F_{1 α} (prostaglandin I₂-M) and of Δ 17-2,3-dinor-6-keto prostaglandin F_{1 α} (prostaglandin I₃-M), the major urinary metabolites of endogenously formed prostaglandin I₂ and prostaglandin I₃, respectively, was analyzed by combined capillary gas chromatography-mass spectrometry as described previously (18). In short, 100 ml of urine was extracted with a highly specific method (33). The methylester-methoxime-trimethylsilylether derivatives were prepared and analyzed on a Finnigan MAT 44S gas chromatography/mass spectrometry system equipped with a fused silica wall-coated, open tubular capillary column (Carbowax CP 51, length 50 m, 0.23 mm i.d.; Chrompack, Lörrach). Operating conditions were: injection port, 280°C; interface, 270°C; ion source, 200°C; electron impact energy, 80 eV; current of emission, 0.9 mA; electron multiplier voltage, 1.8 kV. For quantification of prostaglandin I₃-M, the total excretion of prostaglandin I₂-M and prostaglandin I₃-M (prostaglandin I₂₊₃-M) was assessed after catalytic hydrogenation of the urinary extract containing deuterated prostaglandin I₂-M as internal standard. Prostaglandin I₃-M was calculated by subtracting the separately measured amount of prostaglandin I₂-M from the total amount of hydrogenated prostaglandin I₂₊₃-M (18).

Analysis of thromboxane A metabolites. In serum from clotted whole blood (10 ml, 1 h, 37°C) immunoreactive thromboxane B_{2/3} (thromboxane B_{2/3}) was assessed after acidification, extraction, and purification by high performance liquid chromatography as described previously (27, 34). We used a specific thromboxane B₂ antiserum (a gift from L. Levine, Brandeis University, Waltham, MA, USA), standard thromboxane B₂ (purchased from Upjohn, Kalamazoo, MI), and [³H]thromboxane B₂ (New England Nuclear, Dreieich, Federal Republic of Germany) with a specific activity of 150 Ci/mmol.

To measure thromboxane B₂ and thromboxane B₃ separately, lipids of 5 ml of serum from clotted whole blood were extracted (27), methylated with diazomethane, and purified by thin-layer chromatography with the organic phase of ethylacetate/isooctane/acetic acid/H₂O (110:50:20:100). Thromboxane B₂ and thromboxane B₃ were eluted with methanol and analyzed as methylester-methoxime-trimethylsilylether derivatives by combined capillary gas chromatography (capable of separating thromboxane B₂ and thromboxane B₃) mass spectrometry (21).

The urinary excretion of immunoreactive 2,3-dinor-thromboxane B_{2/3} in 24 h was assessed as described previously (34). 30 ml of urine was acidified and extracted, and eicosanoids were fractionated by reverse-phase high performance liquid chromatography. We used [³H]-thromboxane B₂ as a tracer and thromboxane B₂ antiserum, which had 50–60% cross-reactivity with authentic 2,3-dinor thromboxane B₂. Cross-reactivity with 2,3-dinor thromboxane B₃ could not be assessed due to the lack of authentic standard.

Other analyses. A standardized questionnaire concerning protocol adherence, side effects, and state of health was filled out for each volunteer at each visit.

Serum cholesterol, high density lipoprotein cholesterol (both Boehringer, Mannheim), and triacylglycerol (Merck, Darmstadt) and all other determinations (blood counts and the remaining serum data, and urine data) were performed with routine methods in the central laboratory at our hospital.

Statistics. Data were analyzed by the two-tailed paired *t*-test on control at week 0 versus the respective time point.

Results

Fatty acid changes in plasma, platelets, and red cells. Plasma free fatty acids (Fig. 1 *a*) displayed dose-related changes of EPA and docosahexaenoic acid, with insignificant changes in both linoleic acid and AA. In plasma phospholipids (Fig. 1 *b*), EPA and docosahexaenoic acid increased rapidly in a dose-related manner and linoleic acid and AA decreased, but AA only at the higher doses of cod liver oil. AA, EPA, and linoleic acid returned to control values within 8 wk after cessation of the cod liver oil supplementation. In platelet phospholipids (Fig. 1 *c*) EPA and docosahexaenoic acid increased slower than in plasma phospholipid fatty acids, with the decrease of AA accounting almost completely for the exchange. Linoleic acid remained unchanged. Changes in plasma and platelet fatty acid composition reached their extremes with maximum dosing of cod liver oil, at 12 wk. In erythrocyte membranes (Fig. 1 *d*) EPA and docosahexaenoic acid were increasing up to week 20, 8 wk after the reduction of the dose of cod liver oil. Docosahexaenoic acid had even not returned to control values 20 wk after cessation of cod liver oil. In plasma, platelet, and erythrocyte phospholipids, the sum of palmitic, stearic, and oleic acid remained constant at between 58 and 68% of the fatty acids throughout the study. In plasma free fatty acids, the sum of these three fatty acids varied insignificantly between 70 and 77%.

Triacylglycerol levels in serum decreased closely related to the dose of cod liver oil, whereas cholesterol and high density lipoprotein cholesterol did not change significantly during the study (Fig. 2).

Platelet aggregation. Platelet aggregation (Fig. 3) in platelet-rich plasma upon low doses of collagen (0.25 and 0.75 μ g/ml) was persistently decreased during the period of cod liver oil with a nadir at the maximum dosing of cod liver oil, and returned to control values at the end of the study. Platelet counts decreased from a control value of 181,000 \pm 36,000 to 151,200 \pm 26,700 at week 20 (*P* < 0.05) (see Fig. 6).

Eicosanoids. Prostaglandin I₃-M, the major urinary metab-

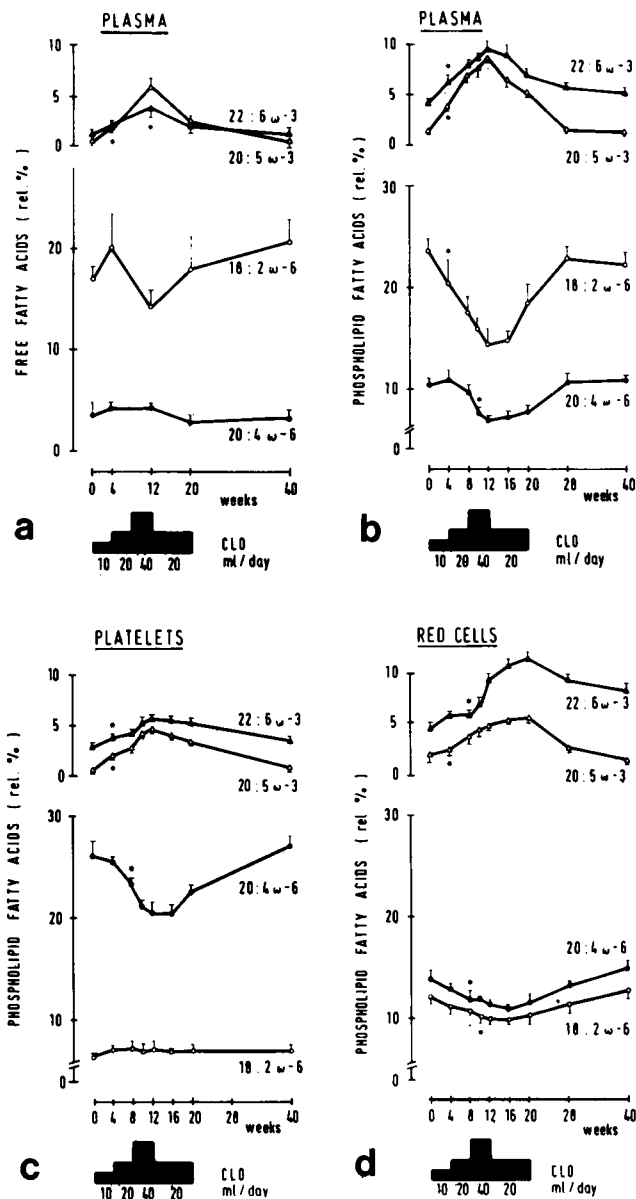


Figure 1. Fatty acids (relative %, mean±SEM, $n = 6$) in (a) plasma, (b) plasma phospholipids, (c) platelet phospholipids, and (d) erythrocyte membrane phospholipids before, during, and after ingestion of dietary cod liver oil (CLO) in various dosages. Palmitic, stearic, and oleic acid remained constant throughout the study. *, $P < 0.05$, paired t test as compared to time 0.

olite of endogenous prostaglandin I_3 , was detectable in urine only during the intake of cod liver oil. Related to the intake of cod liver oil, prostaglandin I_3 -M amounted up to 50% of prostaglandin I_2 -M, the major metabolite of prostaglandin I_2 . Production of prostaglandin I_2 was unaltered throughout the study, as measured by the urinary excretion of prostaglandin I_2 -M (Fig. 4). Excretion of immunoreactive 2,3-dinor thromboxane $B_{2/3}$ remained unaltered during the study in four volunteers with low control values of 2,3-dinor thromboxane B_2 , whereas in the two volunteers with high control values (2,310 and 3,266 ng/g creatinine, respectively), it decreased to ~25% of control values and returned thereafter to prestudy values (Fig. 5). Immunoreactive thromboxane $B_{2/3}$ in serum from clotted whole blood

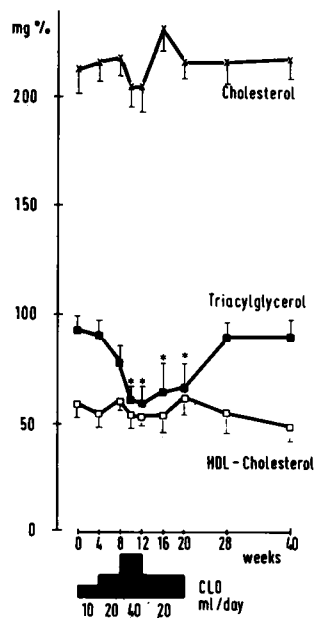


Figure 2. Serum lipids (mg%, mean±SEM, $n = 6$) before, during, and after dietary intake of cod liver oil (CLO) at the indicated HDL, high density lipoprotein. *, $P < 0.05$, paired t test as compared to time 0.

decreased quickly and was dose related during cod liver oil supplementation, and had not yet returned to control values 8 wk after cessation of cod liver oil. Thromboxane B_2 concentrations, measured in the same serum samples by gas chromatography/mass spectrometry, were lower in absolute terms, but paralleled the radioimmunologic determinations (Fig. 6). Thromboxane B_3 was detectable in small quantities in all samples during cod liver oil, exact quantification however was not possible due to the lack of deuterated standard thromboxane B_3 .

Safety and routine laboratory data included in a standardized questionnaire, heart rate, body weight, complete blood counts, sodium, potassium, calcium, uric acid, kidney and liver function parameters in serum, urinary excretion of sodium, potassium, and calcium, and plasma coagulant activity were unchanged throughout the study. No adverse effects of the intake of cod liver oil were observed; no volunteer became ill during the study.

Discussion

In this long-term study, we evaluated the effects of cod liver oil in various dosages in healthy volunteers. Administration of cod

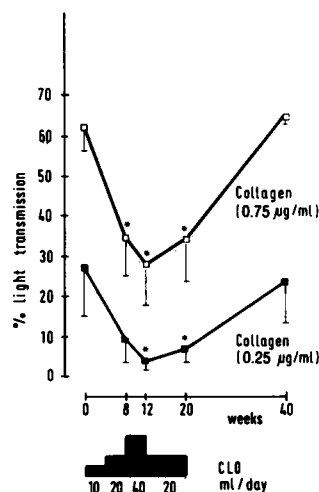


Figure 3. Platelet aggregation on 0.25/0.75 $\mu\text{g/ml}$ collagen (% light transmission, mean±SEM, $n = 6$) before, during, and after intake of dietary cod liver oil (CLO). *, $P < 0.05$, paired t test as compared to time 0.

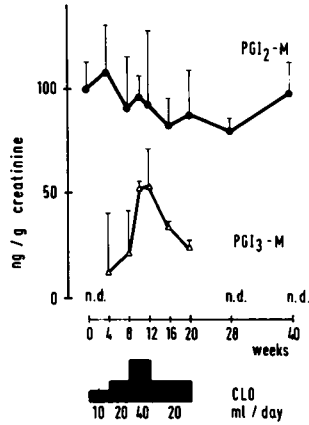


Figure 4. Endogenous prostaglandin I₂ and prostaglandin I₃ production as measured by the urinary metabolites prostaglandin I₂-M (PGI₂-M) and PGI₃-M (ng/g of creatinine, mean±SEM, n = 6) before, during, and after intake of dietary cod liver oil (CLO). The metabolites were measured by combined gas chromatography/mass spectrometry.

liver oil for 5 mo induced changes in the fatty acid composition in the observed compartments and significant alterations in the spectrum of the biologically highly active prostanoids derived from either endogenous AA or EPA, which are sustained during the intake of cod liver oil and reversible 12 wk after cessation.

Lipid changes

Fatty acid changes. EPA and docosahexaenoic acid increased approximately in parallel in all phospholipids analyzed. Synthesis of docosahexaenoic acid from ingested EPA in considerable amounts thus seems unlikely during simultaneous ingestion of EPA and docosahexaenoic acid. In human volunteers, after 3.6 g of 75% pure EPA-ethyl ester per day for 4 wk, an increase of EPA, but unchanged levels of docosahexaenoic acid in platelets or erythrocytes, have been found (7). Results in primates suggest a very low capacity to convert α -linolenic acid to EPA and docosahexaenoic acid (35). In human volunteers, however, EPA increased to a small extent in serum phospholipids after dietary α -linolenic acid (36). In the present study, docosahexaenoic acid decreased more slowly than EPA subsequent to dose reduction (plasma, platelets) or cessation (erythrocytes) of cod liver oil, indicating some type of docosahexaenoic acid retention. Docosahexaenoic acid measurements during fetal development and in newborns also suggest that primates possess a mechanism to selectively retain docosahexaenoic acid (35, 37).

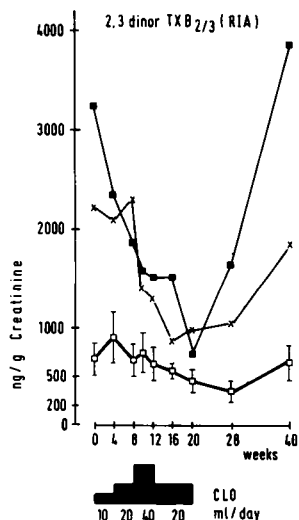


Figure 5. Endogenous thromboxane A_{2/3} production as measured by the urinary metabolites 2,3-dinor thromboxane (TX) B_{2/3} (ng/g of creatinine, mean±SEM, n = 4) before, during, and after dietary intake of cod liver oil (CLO). The metabolites were measured radioimmunologically (RIA).

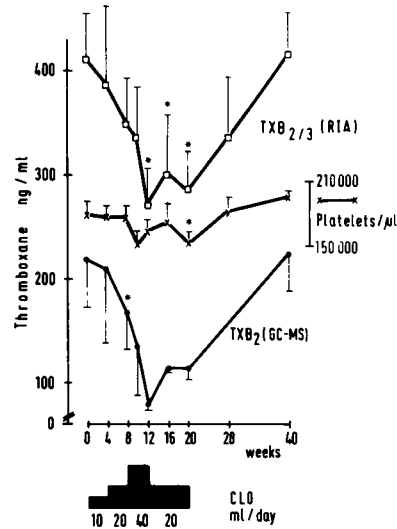


Figure 6. Platelet count (platelets/ μ l) in blood and thromboxane formation in serum of clotted whole blood (ng/ml, mean±SEM) before, during, and after intake of dietary cod liver oil (CLO) in various dosages. Thromboxane (TX) B_{2/3} (n = 6) was measured radioimmunologically (RIA), thromboxane B₂ (n = 3) was measured by combined gas chromatography/mass spectrometry (GC-MS). *, P < 0.05, paired t test as compared to time 0.

In vitro an avid incorporation of radioactive AA and EPA and, to a lesser extent, of labeled docosahexaenoic acid into platelet and erythrocyte phospholipids can be observed (38–41). In vivo, however, in spite of considerable amounts of EPA and docosahexaenoic acid in plasma free fatty acids, the fatty acid changes in platelet and, even more so, in erythrocyte phospholipids occurred with delay. In erythrocytes EPA and docosahexaenoic acid continued to increase despite reduction of the dose of cod liver oil. Thus, in vivo in humans only a limited exchange between cellular and plasma AA, EPA, and docosahexaenoic acid may contribute to the actual fatty acid pattern in blood cell phospholipids. In that this exchange is probably the only influence on cellular phospholipid fatty acid composition after cell formation, it may well be that, in this respect, cells are defined to a considerable extent during cell formation.

In phospholipids, polyunsaturated fatty acids are located almost exclusively in the sn-2 position of the glycerol moiety (40), and ω -6 and ω -3 polyunsaturated fatty acids compete for the same binding sites. During ingestion of cod liver oil, EPA and docosahexaenoic acid increased in all phospholipids studied; the ω -6 fatty acids, however, were not exchanged proportionately. For example, linoleic acid was reduced in plasma and erythrocytes, but was unaffected in platelets. This finding excludes simple competition between the ω -6 and ω -3 fatty acids for the sn-2 position of the glycerol moiety as the only regulatory mechanism.

Cholesterol and triglycerides. Cod liver oil reduced in a dose-dependent manner triacylglycerol levels in serum to 66% of control values, indicating, that a 4–8.3 g/day ω -3 fatty acid supplement may be sufficient to reduce the synthesis of apoprotein B, as observed in human volunteers with 24 g/day (5). With a maximum dose of 8.3 g of ω -3 fatty acids in normocholesterolemic volunteers, a lowering of serum cholesterol, comparable to that in patients with elevated cholesterol levels after far higher doses of ω -3 fatty acids (3–5), could not be expected. Nevertheless

ω -3 fatty acids are as potent as ω -6 fatty acids in lowering serum cholesterol levels (3).

Platelet aggregation

Ex vivo platelet aggregation upon low-dose collagen was reduced during cod liver oil. The platelet count decreased, but never went below the physiologic range. Aggregation studies were performed in adjusted platelet-rich plasma (27) using a single batch of collagen. In short-term studies (up to 4 wk) comparable effects were seen (6, 7, 18, 42, 43), but in a 11-wk trial platelet aggregation upon 0.5–4 μ g/ml collagen returned to normal at 6 wk (25). This may be due to different sources of ω -3 fatty acids used (fish or cod liver oil) or to methodologic differences (27, 43). The decrease in platelet aggregation paralleled the increase of ω -3 fatty acids and the decrease of AA by \sim 20% in platelet phospholipids. In serum from whole clotted blood thromboxane B_{2/3}, as measured by radioimmunoassay, and thromboxane B₂, as measured by gas chromatography/mass spectrometry, were reduced by 50%. In vitro, a ratio AA to EPA of 1:1 is necessary for a 50% inhibition of cyclooxygenation of AA (19). Therefore, the reduced concentration of AA in phospholipids cannot be the only limiting step of thromboxane formation. Other mechanisms such as changes in the physicochemical properties of membranes (40), impaired coupling of collagen to its receptors or differences in the release of AA and EPA from membrane phospholipids (6, 29) may contribute to the reduced aggregation and thromboxane formation.

Eicosanoid synthesis

We estimated eicosanoid biosynthesis via two approaches: (a) measurement of a specific urinary metabolite, thus assessing the endogenous biosynthesis of the respective eicosanoid and (b) measurement of eicosanoids in maximally stimulated samples ex vivo, thus assessing cellular capacity of eicosanoid production.

Prostaglandin I₃-M, the major urinary metabolite of prostaglandin I₃, formed in vivo from ingested EPA, was detected persistently during the intake period for cod liver oil, and increased in a dose-dependent manner with increasing amounts of cod liver oil. Interestingly, at the same time, endogenous production of prostaglandin I₂, as measured by its major urinary metabolite, prostaglandin I₂-M, was not reduced. These in vivo results are in contrast to results of studies with human endothelial cell cultures, where a reduced prostaglandin I₂ production upon stimulation with thrombin was observed after preincubation of the cells with EPA (17). Animal experiments are not applicable in this respect, because rats do not form prostaglandin I₃ after dietary EPA (16). In vivo in humans, however, the endogenous production of potent antiaggregatory and vasodilatory substances, i.e., the sum of prostaglandin I₂ and prostaglandin I₃, was increased during EPA in this and in a previous study (18).

Endogenous thromboxane A_{2/3} formation, as determined by radioimmunological measurement of urinary 2,3-dinor thromboxane B_{2/3} excretion, was largely unaltered during the ingestion period of cod liver oil in the four subjects with normal control levels (normal range in our laboratory 485 \pm 153 ng/g creatinine, n = 46). The excretion of 2,3-dinor thromboxane B₂ was severalfold higher before and after cod liver oil in two subjects, and this high excretion rate decreased during cod liver oil. This may be attributed to reduction of an increased in vivo platelet activation by ingestion of cod liver oil. In serum from whole clotted blood, the capacity of platelets to form thromboxane A₂ was reduced during period of intake of cod liver oil, with a concom-

itant formation of thromboxane A₃ in small amounts. Formation of thromboxane A₃, which is hardly (22), if at all (2, 19), proaggregatory, has previously been detected after dietary EPA in platelet rich plasma (21) or washed platelets (29). Thromboxane A₂ formation was reduced closely related to the dose of cod liver oil, as measured by combined gas chromatography/mass spectrometry. As measured by radioimmunoassay in the same samples, thromboxane B_{2/3} was higher in absolute terms, probably due to unspecific cross-reacting material, but followed the same characteristic pattern. Thus the capacity of platelets to form thromboxane was reduced during the ingestion of cod liver oil.

In experiments with human neutrophils, EPA enrichment by dietary means, and stimulation with Ca⁺⁺ ionophore resulted in formation of leukotriene B₅ in appreciable amounts (44, 45). Concomitant leukotriene B₄ formation was unchanged after 3–4 wk (44, 45), and decreased after 6 wk (45). In the present study and in other studies, thromboxane formation of EPA-enriched platelets was reduced upon stimulation ex vivo after less than 6 wk (3, 6, 8). These data point to differences in handling of EPA between different cells, and to time-dependent changes in cellular response to dietary alterations. The endogenous biosynthesis of thromboxane A_{2/3} was reduced if the controls were elevated, but formation of prostaglandin I₂ unchanged with an additional synthesis of prostaglandin I₃. Since ex vivo studies on EPA-enriched human endothelium have not been performed, it can only be speculated, that such differences pertain also to endothelial cells.

In our study fatty acid changes and prostanoid formation have been found to be different from the results of experiments in vitro or in animals. It seems doubtful, therefore, that in vitro or animal studies with ω -3 fatty acids can be directly compared with those carried out after administration of ω -3 fatty acids to humans. We conclude, that in humans ω -3 fatty acids are incorporated and metabolized with a high degree of specificity. Besides lowering serum lipids, reducing platelet aggregability ex vivo and probably in vivo, and lowering blood pressure and blood vessel reactivity (10), dietary ω -3 fatty acids alter the spectrum of eicosanoids in a favorable manner.

Acknowledgments

We thank the volunteers for participation. The expert technical assistance of B. Böhlig, E. Schmidt, U. Katzner, and I. Kurzmann is gratefully acknowledged.

This study was supported in part by the Deutsche Forschungsgemeinschaft.

References

1. Kromann, N., and A. Green. 1980. Epidemiological studies in the Upernavik district, Greenland. *Acta. Med. Scand.* 208:401–406.
2. Bang, H. O., J. Dyerberg, and A. Nielsen. 1971. Plasma lipid and lipoprotein pattern in Greenlandic west coast Eskimos. *Lancet.* 1:1143–1145.
3. Goodnight, S. H., W. S. Harris, W. E. Connor, and D. R. Illingworth. 1982. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arteriosclerosis.* 2:87–113.
4. Ahrens, E. H., W. Insull, J. Hirsch, W. Stoffel, M. L. Peterson, J. W. Farquhar, T. Miller, and H. J. Thomasson. 1959. The effect on human serum-lipids of a dietary fat, highly unsaturated, but poor in essential fatty acids. *Lancet.* 1:115–119.
5. Illingworth, D. R., W. S. Harris, and W. E. Connor. 1984. Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. *Arteriosclerosis.* 4:270–275.

6. Siess, W., P. Roth, B. Scherer, I. Kurzmann, B. Böhlig, and P. C. Weber. 1980. Platelet membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. *Lancet*. 1:441-444.
7. Terano, T., A. Hirai, T. Hamazaki, S. Kobayashi, T. Fujita, Y. Tamura, and A. Kumagai. 1983. Effect of oral administration of highly purified eicosapentaenoic acid of platelet function, blood viscosity and red cell deformability in healthy human subjects. *Atherosclerosis*. 46:321-331.
8. Lorenz, R., U. Spengler, S. Fischer, J. Duhm, and P. C. Weber. 1983. Platelet function, thromboxane formation and blood pressure control during supplementation of the western diet with cod liver oil. *Circulation*. 67:504-511.
9. Culp, B. R., W. E. M. Lands, B. R. Lucchesi, B. Pitt, and J. Romson. 1980. The effects of dietary supplementation of fish oil on experimental myocardial infarction. *Prostaglandins*. 20:1021-1030.
10. Black, K. L., B. R. Culp, D. Madison, O. S. Randall, and W. E. M. Lands. 1979. The protective effects of dietary fish oil on focal cerebral infarction. *Prostaglandins Leukotrienes Med.* 5:257-268.
11. Prickett, J. D., D. R. Robinson, and A. D. Steinberg. 1981. Dietary enrichment with the polyunsaturated fatty acid eicosapentaenoic acid prevents proteinuria and prolongs survival in NZBxNZW F₁ mice. *J. Clin. Invest.* 68:556-559.
12. Karmali, R. A., J. Marsh, and C. Fuchs. 1984. Effects of omega-3 fatty acids on growth of a rat mammary tumor. *J. Natl. Cancer Inst.* 73:457-461.
13. Dyerberg, J., H. O. Bang, E. Stofferson, S. Moncada, and J. R. Vane. 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. *Lancet*. 1:117-119.
14. Majerus, P. W. 1983. Arachidonate metabolism in vascular disorders. *J. Clin. Invest.* 72:1521-1525.
15. Lewis, R. A., and K. F. Austen. 1984. The biologically active leukotrienes. *J. Clin. Invest.* 889-897.
16. Spector, A. A., T. L. Kaduce, P. H. Figard, K. C. Norton, J. C. Hoak, and R. L. Czervionke. 1983. Eicosapentaenoic acid and prostacyclin production by cultured human endothelial cells. *J. Lipid Res.* 24:1595-1604.
17. Hornstra, G., E. Christ-Hazelhof, E. Haddeman, F. ten Hoor, and D. H. Nugteren. 1981. Fish oil feeding lowers thromboxane and prostacyclin production by rat platelets and aorta and does not result in the formation of prostaglandin I₃. *Prostaglandins*. 21:727-738.
18. Fischer, S., and P. C. Weber. 1984. Prostaglandin I₃ is formed in vivo in man after dietary eicosapentaenoic acid. *Nature (Lond.)*. 307:165-168.
19. Needleman, P., A. Raz, M. S. Minkes, J. A. Ferrendelli, and H. Sprecher. 1979. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc. Natl. Acad. Sci. USA*. 76:944-948.
20. Moncada, S., R. Gryglewski, S. Bunting, and J. R. Vane. 1976. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature (Lond.)*. 263:663-665.
21. Fischer, S., and P. C. Weber. 1983. Thromboxane A₃ (TXA₃) is formed in human platelets after dietary eicosapentaenoic acid (C20:5 ω -3). *Biochem. Biophys. Res. Commun.* 116:1091-1099.
22. Gryglewski, R. J., J. A. Salmon, F. B. Ubatuba, B. C. Weatherly, S. Moncada, and J. R. Vane. 1979. Effects of all-*cis* 5,8,11,14,17-eicosapentaenoic acid and PGH₃ on platelet aggregation. *Prostaglandins*. 18:453-478.
23. Vane, J. R. 1982. Prostacyclin: a hormone with a therapeutic potential. The Sir Henry Dale Lecture for 1981. *J. Endocrinol.* 95:3P-45P.
24. Velardo, B., M. Lagarde, M. Guichardant, M. Dechavanne, M. Beylot, G. Sautot, and F. Berthezene. 1982. Decrease of platelet activity after intake of small amounts of eicosapentaenoic acid in diabetics. *Thromb. Haemostasis*. 48:344. (Lett.)
25. Thorngren, M., and A. Gustafson. 1981. Effects of 11-week increase in dietary eicosapentaenoic acid on bleeding time, lipids and platelet aggregation. *Lancet*. 2:1190-1193.
26. Saynor, R., D. Verel, and T. Gillot. 1984. The long term effect of dietary supplementation with fish lipid concentrate on serum lipids, bleeding time, platelets and angina. *Atherosclerosis*. 50:3-10.
27. Siess, W., P. Roth, and P. C. Weber. 1981. Stimulated platelet aggregation, thromboxane B₂ formation and platelet sensitivity to prostacyclin—a critical evaluation. *Thromb. Haemostasis* 45:204-207.
28. Siess, W., P. Cuatrecasas, and E. G. Lapetina. 1983. A role for cyclooxygenase products in the formation of phosphatidic acid in stimulated human platelets. *J. Biol. Chem.* 258:4683-4686.
29. Schacky, C. von, W. Siess, S. Fischer, and P. C. Weber. 1985. A comparative study of eicosapentaenoic acid metabolism by human platelets in vivo and in vitro. *J. Lipid Res.* 26:457-464.
30. Gerber, J. G., J. S. Barnes, and A. S. Nies. 1979. Measurement of arachidonic acid in the plasma by gas-liquid chromatography—flame ionization using dihomogamma-limolenic acid as an internal standard. *J. Lipid Res.* 20:912-914.
31. Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497-509.
32. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911-917.
33. Falardeau, P., J. A. Oates, and A. R. Brash. 1981. Quantitative analysis of two dinor metabolites of prostaglandin I₂. *Anal. Biochem.* 115:359-367.
34. Fischer, S., M. Struppler, B. Böhlig, C. Bernutz, W. Wober, and P. C. Weber. 1983. The influence of selective thromboxane synthetase inhibition with a novel imidazole derivative, UK-38,485, on prostanoid formation in man. *Circulation*. 68:821-826.
35. Neuringer, M., W. E. Connor, C. Van Petten, and L. Barstadt. 1984. Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. *J. Clin. Invest.* 73:272-279.
36. Mest, H. J., J. Beitz, H. U. Heinroth, and W. Förster. 1983. The influence of linseed oil on fatty acid pattern in phospholipids and thromboxane formation in platelets in man. *Klin. Wochenschr.* 61:187-191.
37. Crawford, M. A., A. G. Hassam, and G. Williams. 1976. Essential fatty acids and fetal brain growth. *Lancet*. 1:452-453.
38. Bills, T. K., J. B. Smith, and M. J. Silver. 1977. Selective release of arachidonic acid from the phospholipids of human platelets in response to thrombin. *J. Clin. Invest.* 60:1-6.
39. Neufeld, E. J., D. B. Wilson, H. Sprecher, and P. W. Majerus. 1983. High affinity esterification of eicosanoid precursor fatty acids by platelets. *J. Clin. Invest.* 72:214-220.
40. Stubbs, C. D., and A. D. Smith. 1984. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta.* 779:89-137.
41. Fischer, S., C. von Schacky, W. Siess, T. Strasser, and P. C. Weber. 1984. Uptake, release and metabolism of docosahexaenoic acid (DHA, C22:6 ω -3) in human platelets and neutrophils. *Biochem. Biophys. Res. Commun.* 120:907-918.
42. Brox, J. H., J. E. Killie, S. Gunnes, and A. Nordoy. 1981. The effect of cod liver oil and corn oil on platelets and vessel wall in man. *Thromb. Haemostasis*. 46:604-611.
43. Sanders, T. A. B., M. Vickers, and A. P. Haines. 1981. Effect on blood lipids and haemostasis of a supplement of cod-liver oil, rich in eicosapentaenoic acid and docosahexaenoic acids, in healthy young men. *Clin. Sci.* 61:317-324.
44. Strasser, T., S. Fischer, and P. C. Weber. 1985. Leukotriene B₃ is formed in human neutrophils after dietary eicosapentaenoic acid. *Proc. Natl. Acad. Sci. USA*. 82:1540-1543.
45. Lee, T. H., R. L. Hoover, J. D. Williams, R. I. Sperling, J. Ravalese, B. W. Spur, D. R. Robinson, E. J. Corey, R. A. Lewis, and K. F. Austen. 1985. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N. Engl. J. Med.* 312:1217-1224.