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Dietary docosahexaenoic acid reduces the thromboxane/prostacyclin synthetic ratio in humans

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The effect of fish oils, rich in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), on eicosanoid production in vivo has been extensively studied, but data on the effects of dietary DHA alone on the synthesis of thromboxane (TXA_2) and prostacyclin (PGI_2) in humans are lacking. We quantified the effect of an isocaloric shift in DHA intake from trace (low-DHA diet) to about 6% of total fatty acids (high-DHA diet), ca. 2 en%, on the excretion of 11-dehydrothromboxane B_2 (11-$DTXB_2$) and 2, 3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ (PGI_2-M). In a longitudinal study, seven healthy men, living in a metabolic unit, were fed a 30% fat low-DHA diet for 30 days, then a high-DHA diet containing 6g/day of DHA for 90 days. A control group of four subjects remained on the low-DHA diet for the duration of the study (120 days). Three-day urine pools were collected at the end of each dietary period (around day 30 and day 120) and analyzed for eicosanoids by gas chromatography-electron capture negative ion-tandem mass spectrometry. Mean excretion of 11-$DTXB_2$ was 590 ± 256 ng/d (SD; n = 7) with the low-DHA diet, and 385 ± 148 ng/d (n = 7) with the high-DHA diet, a 35% reduction (P = 0.013, n = 11 including the control group, when log transformed data were used). Production of 11-$DTXB_2$ in the control group was unchanged. Mean excretion of PGI_2-M was 229 ± 73 ng/day (SD; n = 7) and 210 ± 102 ng/day with the low-DHA and the high DHA diet, respectively (a nonsignificant reduction). This study confirms that the synthesis of TXA_2 is more open to diet-induced modulation than the synthesis of PGI_2. The observed reduction of 11-$DTXB_2$ excretion may be associated with measurable effects on several physiologically significant cellular functions, such as platelet aggregation in vivo and inflammation in response to immune challenges. (J. Nutr. Biochem. 9:88–92, 1998) *Published by Elsevier Science Inc. 1998*

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Introduction

Based on results of almost two decades of research with humans and animal models, most scientists today will acknowledge the protective influence of dietary n-3 polyunsaturated fatty acids (PUFA) against cardiovascular disorders.[1] Populations whose diet has a consistently low n-6/n-3 fatty acid ratio display, together with depressed eicosanoid production, a marked reduction in the incidence of degenerative diseases affecting the vascular system.[2–5] Dietary n-6 PUFA have also been implicated in tumor promotion whereas fatty acids of the n-3 family have been associated with tumor inhibition.[6–9] n-3 PUFA are believed to exert their influence in the prevention of both cancer and cardiovascular disease by influencing eicosanoid-mediated processes.[10,11]

Thromboxane A_2 (TXA_2) is a vasoconstrictor and a powerful inducer of platelet aggregation, and prostacyclin (PGI_2) is a platelet antagonist and one of the most potent hypotensive agent. They are both directly derived, through the cyclooxygenase pathway, from arachidonic acid (20:4n-6), and their synthetic ratio is considered a biochemical marker of risk for thrombosis.[12] Given the overriding importance of TXA_2 and PGI_2 in vascular biology, the possibility of modulating their synthetic level through dietary means has been object of numerous investiga-

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tions.[13–22] Fish oil has been most often used and it was found to be an effective inhibitor of arachidonate metabolism. Fish oil is a rich source of both eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids. Thus, whereas its effect on eicosanoid modulation is reasonably well documented, data on the biochemical effects of DHA alone in humans are lacking.

In a typical American diet, the intake of DHA is estimated to be less than 0.1 g/day.[23] However, intakes of up to 10 g/d have been shown to have no adverse effects.[24,25] We measured the effect of replacing, for 90 days, 2% of calories in a reference (low-DHA) diet, where the DHA content was near zero, with an isocaloric amount from DHA on the excretion of the major metabolite of thromboxane A_2 (11-dehydrothromboxane B_2, 11-DTXB$_2$) and prostacyclin (2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$, PGI$_2$-M).

Methods and materials

Subjects

Initially twelve healthy male volunteers, 20 to 40 years old, were recruited from the San Francisco Bay area. Those who met preliminary selection criteria were given a physical examination and were subjected to hematological and chemical screening. Those with test results within normal ranges were entered into the study. All subjects were within 20% of ideal body weight based on 1983 Metropolitan Life tables.[26] They were housed at the Human Nutrition Suite, Western Human Nutrition Research Center, ARS, USDA, Presidio of San Francisco, CA, for the duration of the study (120 days). They were required not to ingest any drug formulation containing aspirin or other anti-inflammatory agents, and to report any medication prescribed by a physician during the study for evaluation for possible effects on the variables under investigation. All procedures were approved by the University of California, Davis, Human Subjects Committee and by the USDA/ARS Human Studies Review Committee.

Controlled diets

The diets consisted of natural foods purchased from local food suppliers. All nutrients were provided by the diets in amounts to meet or exceed the Recommended Daily Allowances (RDA)[27] as estimated from data in the USDA Handbook 8.[28] Vitamin E was also supplied as supplement at twice the RDA. Energy contribution (en%) from proteins, carbohydrates and lipids was virtually identical in the two diets: 15%, 55% and 30%, respectively. The low-DHA diet had a saturated: monounsaturated: polyunsaturated (S:M:P) ratio 1.0/1.4/1.2. In the high-DHA diet 2% of calories was provided by DHA through the use of DHASCO, a natural triglyceride (produced from algae by the Martek Biosciences Corporation of Columbia, MD USA) which is 40% DHA. Thus, about 15 g of DHASCO per day was added isocalorically to the diet. The resulting S:M:P ratio in the high-DHA diet was 1.0/1.2/1.0. No food or food supplement other than what was provided by the study was allowed.

U.S. Department of Agriculture Handbood 8[28] and in-house nutrient databases were utilized to determine the nutrient composition of the diets. *Table 1* shows the mean fatty acid composition of the two diets from the analysis of representative composites of both diets based on a 5-day menu cycle. The energy values of the meals were adjusted, daily if necessary, to maintain the participants' body weights. We made adjustments so that the fatty acid composition in the diet remained constant. Further details concerning the diets can be obtained from the authors.

Table 1 Fatty acids (%) in the two diets[1]

Fatty acid	Diet	
	Low-DHA	High-DHA
12:0 (lauric)	0.73 ± 0.30	1.29 ± 0.30
14:0 (myristic)	2.17 ± 0.84	4.32 ± 0.74
16:0 (palmitic)	16.32 ± 1.56	16.61 ± 1.57
16:1n-9	0.72 ± 0.39	0.92 ± 0.41
18:0 (stearic)	7.53 ± 0.93	7.08 ± 0.97
18:1*trans*, all isomers[2]	6.96 ± 0.99	6.17 ± 0.95
18:1n-9*cis* (oleic)	26.00 ± 1.69	26.64 ± 1.76
18:1n-7	1.97 ± 0.24	1.67 ± 0.26
18:1n-5	2.11 ± 0.38	1.52 ± 0.42
18:2*trans,trans* + 19:0	0.58 ± 0.13	0.54 ± 0.14
18:2n-6 (linoleic)	28.34 ± 2.24	21.57 ± 2.68
18:3n-3 (α-linolenic)	3.21 ± 0.35	2.60 ± 0.41
22:0 (behenic)	0.23 ± 0.01	0.24 ± 0.01
20:5n-3 (EPA)	0.33 ± 0.04	0.35 ± 0.04
22:6n-3 (DHA)	*	6.51 ± 0.49
Total	97.20 ± 0.27	98.03 ± 0.38
Unknowns and Trace FA	2.80 ± 0.27	1.97 ± 0.38

[1]Average values (±SD) from the analysis of 5-day representative composites.
[2]Fatty acid analyses were performed by a modification of the procedures described by Slover and Lanza (1979) in *J. Amer. Oil Chem. Soc.* **56,** 933–943
*Trace.

Experimental design

All volunteers were first fed the low-DHA diet for 30 days. On day 31 they were divided into two groups. Group A, eight subjects, were placed on the high-DHA diet obtained as described above. The other four subjects (Group B) remained on the low-DHA diet for the duration of the study (120 days) and functioned as a control group.

Urine collection

For eicosanoid analysis, 24-hr urine samples were collected in polyethylene bottles and kept refrigerated during the collection period. Urine was collected for 3 consecutive days around day 30 and day 120. After the 24-hr collections were completed, urine volumes were measured, and 2% portions of each 24-hr (3 consecutive day) collection were pooled and stored at −70°C until analyses could be performed. The participants, who had no access to normal toilet facilities, were under constant supervision and received detailed instructions about urine collection.

Measurement of 11-DTXB$_2$ and PGI$_2$-M

Analyses of 11-dehydrothromboxane B_2 (11-DTXB$_2$) and 2, 3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ (PGI$_2$-M) were performed on 10-mL aliquots of the 72-hr urine pools prepared as described above. This enabled us to assess the mean daily total synthesis of TXA_2 and PGI_2 during the 72-hr periods. Quantification was achieved by capillary gas chromatography-electron capture negative ion-tandem mass spectrometry (GC-ECNI-MS-MS). Detailed procedures, instrumental conditions and method validations have been described.[29,30] As reported, the interassay relative standard deviation (precision) for the 11-DTXB$_2$ assay was 1–2%[29] and about 5% for PGI$_2$-M.[30] To avoid any adverse consequences of day-to-day changes in instrument performance, we conducted all the analyses in a paired fashion, i.e., urine samples collected around day 30 and day 120 from the same subject were processed

Table 2 Mean urinary excretion rates (ng/24 hr) of 11-dehydrothromboxane B_2 during the last 3 days of each dietary treatment (range in parenthesis, $n = 2$)[1]

	Diet			
	Low-DHA		High-DHA	%
Subject	Day 30	Day 120	Day 120	Decrease[3]
13†	847 (824–869)	981 (968–993)		
14	899 (883–915)		426 (380–473)	53
18	530 (527–532)		613 (605–621)	−16
19	348 (342–354)		210 (199–221)	40
20	495 (490–500)		340 (332–348)	31
21	1004 (994–1015)		540 (536–543)	46
23	406 (393–418)		284 (280–288)	30
24	446 (439–452)		282 (278–285)	37
25[2]	289 (288–290)	356 (355–358)		
26[2]	470 (470–471)	551 (537–565)		
27[2]	960 (928–992)	783 (775–791)		

[1]One subject (group A) withdrew from the study.
[2]Control subjects (group B)
[3]Percent decrease calculated as [(low-DHA minus high-DHA)/low-DHA] × 100.

Table 3 Mean urinary excretion rates (ng/24 hr) of 2,3-Dinor-6-oxo-prostaglandin $F_{1\alpha}$ during the last 3 days of each dietary treatment (range in parenthesis, $n = 2$)[1]

	Diet			
	Low-DHA		High-DHA	%
Subject	Day 30	Day 120	Day 120	Decrease[3]
13†	225 (210–239)	185 (179–190)		
14	245 (244–246)		135 (135–135)	45
18	246 (233–259)		334 (333–336)	−36
19	126 (121–131)		100 (98–103)	21
20	193 (180–207)		160 (156–163)	17
21	286 (284–289)		352 (333–371)	−23
23	166 (161–172)		140 (124–156)	16
24	342 (310–373)		247 (241–253)	28
25[2]	298 (297–298)	520 (476–565)		
26[2]	312 (302–322)	317 (308–326)		
27[2]	262 (259–265)	259 (241–278)		

[1]One subject (group A) withdrew from the study.
[2]Control subjects (group B).
[3]Percent decrease calculated as [(low-DHA minus high-DHA)/low-DHA] × 100.

simultaneously and analyzed by GC-tandem mass spectrometry the same day.

Statistical analysis

We evaluated the 11-DTXB$_2$ and PGI$_2$-M 24-hr excretion rates and the docosahexaenoic acid intakes using the SAS Institute's (Cary, NC USA) PROC MIXED package. P values less than 0.05 were considered statistically significant.

Results

The average weights of the subjects did not vary significantly during the 120 days of the study. One subject in Group A withdrew from the study. The mean excretion of 11-DTXB$_2$ was 590 ± 256 ng/day (SD; $n = 7$) with the low-DHA diet, and 385 ± 148 ng/day ($n = 7$) with the high-DHA diet, a 35% reduction ($P = 0.013$ by ANOVA including the control group, total $n = 11$, on log transformed data). Production of 11-DTXB$_2$ in the control group was unchanged. Excretion of 11-dehydrothromboxane B$_3$ at day 120 by the men on the high-DHA diet was not observed, even when we operated under conditions of maximum sensitivity, i.d., with straight electron capture negative ion mass spectrometry (no MS-MS). The mean excretion of PGI$_2$-M was 229 ± 73 ng/day (SD; $n = 7$) and 210 ± 102 ng/day with the low-DHA and the high-DHA diet, respectively (a non-significant reduction). Excretion values for individual subjects are shown in *Tables 2* and *3*. Values for each of the 11 volunteers who completed the study are also shown graphically in *Figures 1* and *2*.

Discussion

This study is, to our knowledge, the first attempt to define the influence of DHA alone on eicosanoid production in vivo in normal humans eating normal diets. Specifically, the study quantifies the effect of an isocaloric shift in DHA intake from near zero (low-DHA diet) to about 6% of total fatty acids (high-DHA diet), ca. 2 en%, on the production of vasoactive eicosanoids in seven male volunteers. Available evidence from this study and the literature does not allow us to conclude whether DHA is a weaker or a stronger depressor of TXA$_2$ production than EPA. Clearly, however, and unlike fish oil,[18,21,22] DHA did not reduce PGI$_2$, as determined by measuring the excretion of its urinary me-

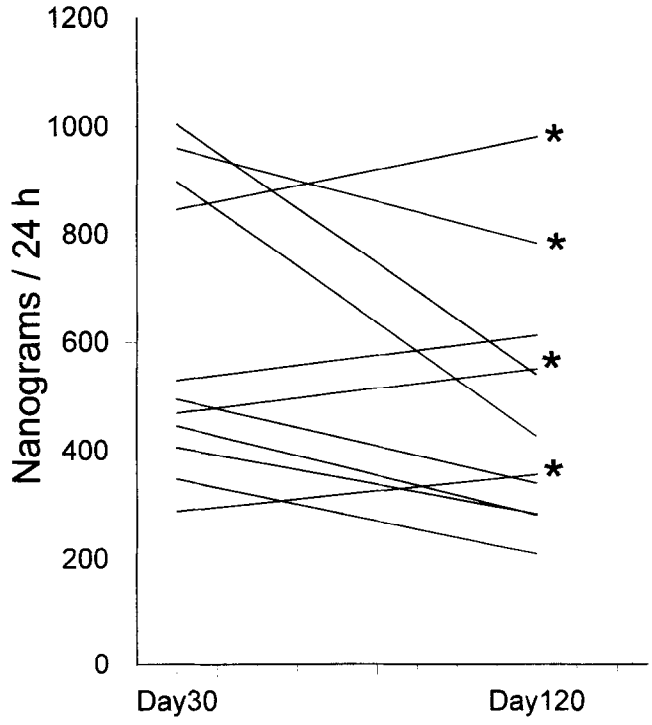

Figure 1 Mean 11-dehydrothromboxane B$_2$ daily excretion rates of the 11 subjects who completed the study. The asterisked lines correspond to the subjects in the control group (see Methods and materials section).

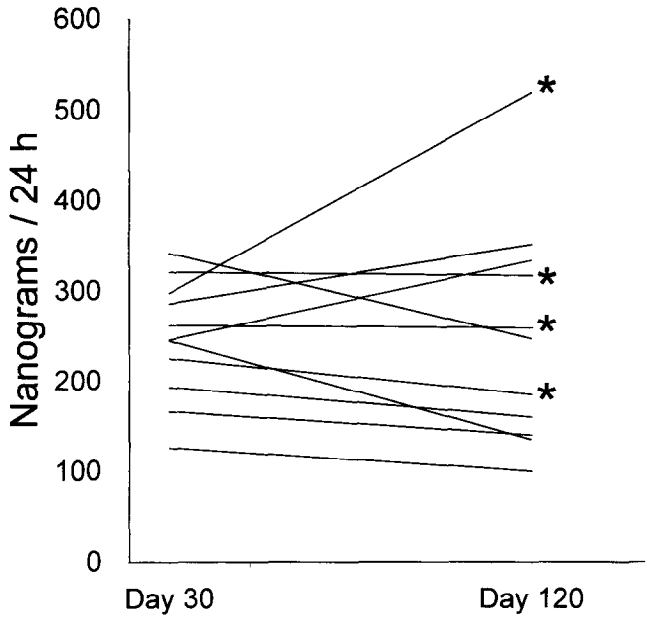

Figure 2 Mean 2,3-dinor-6-oxo-prostaglandin $F_{1\alpha}$ daily excretion rates of the 11 subjects who completed the study. The asterisked lines correspond to the subjects in the control group (see Methods and materials section).

tabolite. The reduced capacity to synthesize TXA_2 associated with the high-DHA diet in this study may have resulted from increased levels of DHA, at the expense of arachidonic acid, in platelet lipid stores. This attractive hypothesis, however, has yet to be conclusively proved in humans. The most likely explanation is that DHA is sufficiently similar in structure to arachidonic acid to act as a competitive inhibitor of cyclo-oxygenase. This mechanism has been proposed first by Corey et al.[31] to explain the inhibitory effect of DHA on prostaglandin production in seminal vesicles. One should also keep in mind that our interpretation of the results is based on the assumption that the 11-$DTXB_2$ and PGI_2-M excretion rates substantially correlate with the synthetic rates of their respective metabolic precursors, TXA_2 and PGI_2, in platelets and endothelial cells. Virtually all TXA_2 originates from platelet lipid stores. Whereas endothelial cells are the major source of PGI_2, there is evidence that in rats the gastrointestinal tract is another site of PGI_2 synthesis from dietary precursor fatty acids.[32] It has not been demonstrated, however, that in humans PGI_2 is absorbed through the gut and thereby subjected to the catabolic pathway leading to PGI_2-M.

As shown in *Table 1*, the increase of DHA in the high-DHA diet was essentially at the expense of linoleic acid (18:2n-6). Thus, we cannot rule out that the effect on TXA_2 production was due, in part, to the small reduction of linoleic acid intake. However, we are inclined to attribute the reduced TXA_2 output with the high-DHA diet to DHA, whose intake increased from "trace" to about 2% of calories. This conclusion is supported by results of previous studies where only the n-3 PUFA intake was altered, whereas the n-6 PUFA intake was kept constant.[17,18] The amount of linoleic acid (>20%) present in both diets (*Table 1*) is deemed adequate for metabolic conversion. The large amount of linoleic acid may help explain why the metabolite of the trienoic thromboxane (TXA_3) was not detected in the urine of men on the high-DHA diet. The quantity of arachidonic acid (20:4n-6) was negligible in both diets.

This study confirms previous observations that the synthesis of thromboxane is more open to diet-induced modulation than the synthesis of prostacyclin.[13,25,33,34] More recently, in a fish oil supplementation (15 g/day) study conducted at the USDA Center in Beltsville, MD USA, the mean 11-$DTXB_2$ excretion was reduced by 38% while the mean excretion of PGI_2-M declined by only 22% after ten weeks, compared with a basal diet.[17,18] Sanigorski et al. made a similar observation in a rat study where the n-3 fatty acid was α-linolenic acid from linseed oil.[35] In that study 18:3n-3 selectively decreased TXA_2 production in vitro while having a negligible effect on PGI_2. The uniformly observed greater TXA_2 response may be related to the magnitude of diet-induced changes in arachidonate and eicosapentaenoate in platelet and endothelial lipid pools.

In 1985, von Schacky and Weber demonstrated, for the first time, the retroconversion of dietary DHA to EPA in humans, and recognized DHA as an important factor in the antithrombotic effect of fish oil.[36] Two years later Fischer et al.[37] determined that the EPA available from retroconversion is quickly transformed, like dietary EPA itself, to PGI_3. The pathways for PUFA biosynthesis have been reviewed by Sprecher et al.,[38] and the retroconversion of DHA to EPA in both humans and rats has been confirmed recently.[39,40] Our failure, in this study, to detect 11-$DTXB_3$ in subjects on the high-DHA diet would indicate that the retroconversion did not take place to a significant extent, possibly attributable to the moderate level of DHA intake. The effects of the high-DHA diet on plasma, platelet and tissue fatty acid composition, platelet function and blood clotting, plasma lipoprotein distribution, and apoproteins from this study are reported elsewhere.[41,42]

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