Pilot Study on ω-3 Fatty Acids in Type I Diabetes Mellitus

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Patients with diabetes mellitus are prone to develop vascular complications. Because ω-3 polyunsaturated fatty acid (ω3FA) intake has a potential protective effect on cardiovascular disease, we studied the influence of ω3FA supplementation (5.4 g eicosapentaenoic acid and 2.3 g docosahexaenoic acid daily) for 4 wk in 13 well-controlled type I (insulin-dependent) diabetic subjects on a vascular risk profile. Each subject served as his/her own control in a pre- and post-ω3FA-intake phase. In plasma and platelets, phospholipids eicosapentaenoic acid and docosahexaenoic acid increased at the expense of arachidonic acid and linoleic acid. There was no significant change in blood pressure and glycosylated proteins. Only small changes were noted in blood glucose levels and insulin dose. Side effects were not noted. Triglycerides decreased significantly in the first 14 days, and total cholesterol increased slightly, probably because of an elevation of high-density lipoprotein cholesterol, although low-density lipoprotein cholesterol remained unchanged. Platelet aggregation induced by low doses of ADP and collagen, which was higher in diabetic than nondiabetic subjects, decreased during ω3FA intake to levels of healthy control subjects. Thromboxane production after ADP- and collagen-induced platelet aggregation decreased significantly. Thromboxane liberation during clotting of whole blood and urinary excretion of thromboxane were markedly lowered during ω3FA supplementation. The results show that even short-term intake of ω3FAs leads to beneficial changes of vascular risk factors without significant changes in glucose homeostasis. *Diabetes* 39:369–75, 1990

Patients with diabetes mellitus are prone to develop vascular complications because of macro- and microangiopathy, which leads to unacceptably high morbidity and mortality. Education, self control, and classic dietary and even highly sophisticated therapeutic measures, however, are insufficient to prevent development or progression of diabetic complications (1). Recently, a protective potential of ω-3 polyunsaturated fatty acids (ω3FAs) on cardiovascular diseases has been suggested by epidemiological studies in Eskimos (2), Japanese (3), and subjects in the Netherlands (4). Besides lowering blood lipids, blood pressure, and platelet aggregation, the ω3FA eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids reduce formation of proaggregatory and vasoconstrictory thromboxane A_2 (TXA_2) and give rise to a small amount of a biologically inactive TXA_3 in humans. Moreover, large amounts of prostaglandin I_3 (PGI_3) almost as vasodilatory and antiaggregatory as PGI_2 are formed dose dependently without depressing prostacyclin formation (2,5–7). Increased adhesiveness and aggregation concomitant with increased formation of TXA_2 are thought to be involved in the pathogenesis of atherosclerosis and its complications in diabetes. If feasible and effective, ω3FAs would be a highly attractive approach toward prophylaxis of atherosclerosis in diabetes. We therefore studied the effect of oral intake of EPA and DHA by type I (insulin-dependent) diabetic subjects on glucose and lipid metabolism, platelet aggregability, and thromboxane production.

RESEARCH DESIGN AND METHODS

Thirteen type I diabetic patients gave their informed written consent to participate in this study with the approval of the Ethics Committee of the University. All patients were highly motivated and well trained and had been under our regular control for at least 2 yr. Therefore, study effects in our investigation are very unlikely (for clinical details, see Table 1). Seven patients were treated with continuous subcutaneous insulin infusion (CSII) and 6 by subcutaneous insulin injections at least twice daily. All patients performed self-

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TABLE 1
Clinical details of diabetic patients

Patient	Sex	Age (yr)	Duration of diabetes (yr)	Ideal body weight (%)	C-peptide (ng/ml)		Insulin pump	Smoking	Retinopathy	Albuminuria (μg/min)	Hypertension	Neuropathy	
					0 min	6 min						Peripheral	Autonomic
B.U.	M	19	4	97	0.30	0.40	Y	−	−	2.0	−	−	−
B.B.	F	30	25	87	0.15	0.15	N	+	++	1338.0	+	+	++
B.M.	M	27	9	94	0.15	0.18	Y	+	−	3.7	−	−	−
B.I.	F	33	19	93	0.15	0.15	N	−	−	7.5	−	+	++
D.C.	M	27	10	95	0.15	0.15	Y	+	−	2.4	−	+	−
D.N.	M	52	14	105	1.30	2.50	N	−	+	6.6	+	++	++
H.A.	M	34	4	98	0.15	0.15	Y	−	−	0.2	−	−	−
H.E.	M	43	18	90	0.15	0.15	N	−	++	2.6	+	−	−
M.N.	M	22	10	92	0.15	0.15	Y	+	−	4.9	−	+	−
M.B.	M	23	13	95	0.15	0.15	Y	−	++	6.0	−	−	−
P.W.	M	23	4	118	0.15	0.15	N	−	FH	1.5	+	−	+
W.E.	F	27	16	96	0.15	0.15	N	+	−	11.1	−	−	−
Z.U.	M	23	6	98	0.26	0.28	Y	−	−	1.4	−	−	+

Retinopathy was characterized as follows: −, no background changes; +, background changes; ++, preproliferative retinopathy with laser treatment; FH, fundus hypertonicus. Albuminuria was measured with an immunoturbidimetric method (8). For hypertension: +, systolic pressure >145 mmHg and/or diastolic pressure >95 mmHg on at least 3 occasions. For peripheral neuropathy: −, no pathological finding; +, mild changes; ++, severe changes. For autonomic neuropathy: + or ++, decrease or loss, respectively, of R-R variability during different maneuvers.

monitoring of blood glucose (SMBG) and were trained in self-adjustment of the insulin dose to achieve near normoglycemia. Diabetic retinopathy was assessed by funduscopy and fluorescein angiography. All patients had normal serum creatinine levels. Three women and 10 men, each carefully matched for age and sex, served as control subjects (age 29 ± 8 yr, % ideal body wt 97 ± 3, 6 smokers and 7 nonsmokers).

Study design. Except for insulin, no other therapy was taken for at least 2 wk before and during the study. The patients kept an exact record of carbohydrate intake, insulin requirement, and blood glucose values. They continued their normal daily activities except for extreme exercise. To maintain good blood glucose control, the patients were allowed to change their carbohydrate intake and/or insulin dose.

The study consisted of three phases: a prephase of 7 days, a capsule phase of 28 days, and a postphase of 14 days. During the prephase, four daily blood glucose profiles with at least four determinations were obtained. The patients collected urine for 24 h on 2 days, measured the volume, and stored the sample at −20°C. At the end of the prephase, an extensive clinical examination took place, including an electrocardiogram to establish cardiac autonomic neuropathy, a Schellong test, and tests for ophthalmologic and neurological status. Blood was collected from an antecubital vein with minimal stasis in the fasted state before insulin application in the patients on conventional treatment or before bolus injection in the patients on CSII.

During the capsule phase, patients ingested 18 capsules/day containing a total of 5.4 g EPA and 2.3 g DHA as a supplement to their otherwise unchanged diet (50% carbohydrate, 15% protein, and 35% fat). Eight blood glucose profiles (at least 4 determinations/day) were performed during the 28 days of dietary intake of EPA and DHA. Furthermore, 24-h urine was collected on days 1, 2, 7, and 28, and blood was drawn on days 14 and 28.

In the postphase, no capsule intake occurred. SMBG was continued, however. Urine was collected during the last 24 h of this phase, and blood was drawn on the last day.

Capsules (1.2 g each) containing 300 mg EPA and 130 mg DHA and no cholesterol were obtained from PGE Technology (Marblehead, MA). The oil was from sardines, and EPA and DHA were in the form of triglycerides. The fatty acid profile was C14:0, 4.0%; C16:0, 5.3%; C16:1, 8.1%; C18:0, 0.4%; C18:1, 10.1%; C18:2, 1.7%; C18:3, 1.0%; C18:4, 5.3%; C20:1, 3.0%; C20:4, 1.0%; C20:5, 28.0%; C22:1, 1.8%; C22:5, 3.8%; C22:6, 12.0%; and others, 13.0%.

Parameters of glucose homeostasis. Glucose was measured by a glucose oxidase method in the clinic and by SMBG with a reflectometer. Stable glycosylated hemoglobin (HbA_1) was determined by agar gel electrophoresis (Glytrac, Corning, Palo Alto, CA), and glycosylated albumin and total glycosylated serum proteins were assayed by Isolab's Gluc-Affin system (Akron, OH).

A glucagon test was performed to assess a possible rest function of the endocrine pancreas. Glucagon hydrochloride (1 mg, Novo, Copenhagen) was injected intravenously in the fasted diabetic subject. Blood was drawn before and 6 min after the glucagon injection. C-peptide concentration was determined by radioimmunoassay (RIA; IRE, Fleurus, Belgium) with limit of detection <0.15 ng/ml. Albumin in 24-h urine was determined by an immunoturbidimetric method with a Hitachi analyzer type 704 (normal values 0–20 μg/min; 8).

Ex vivo aggregation of platelets and measurement of thromboxane. Platelet-rich plasma (PRP) was prepared by differential centrifugation and adjusted to a platelet number of 2×10^5. Aggregation was induced with 166 and 333 μg arachidonic acid (AA)/ml PRP, 0.3 and 0.6 μg collagen/ml PRP, and 0.5 and 1.0 μg ADP/ml PRP (9). Aggregation was stopped 5 min after addition of the aggregating substance by acidification with 1 N HCl (final pH 3.5).

ADP (Serva, Heidelberg, FRG) was dissolved in 0.9% NaCl and stored frozen (−20°C) at concentrations of 1 mg/ml. Collagen reagent (Hormon-Chemie, Munich, FRG), containing 1 mg/ml suspended collagen fibrils, was stored at 4°C. Only one batch was used for the whole study. AA (Serva) was suspended in ethanol-*n*-hexane (1:9 vol/vol) at a concentration of 10 mg/ml and stored at −80°C under N_2 atmosphere. Immediately before use, the ethanol-*n*-hexane mixture was evaporated under N_2, and the AA was resuspended in 0.1 M Tris buffer (pH 7.4) with 10% ethanol. Ethanol in the final concentration used had no influence on platelet aggregation. All reagents used were of analytical grade.

Thromboxane extraction was started by organic acid solvents as described (10). Thromboxane was determined by RIA with standard TXB_2 and a specific antibody from Paesel (Frankfurt, FRG) and [^{3}H]TXB_2 from Du Pont-NEN (Boston, MA; 9,11).

The method introduced by Patrono et al. (12) was used to liberate thromboxane from clotted whole blood. Blood (10 ml) was drawn from an antecubital vein by plastic syringe and immediately transferred into two glass tubes. The blood was allowed to clot at 37°C for 30 min, then it was centrifuged at 2000 × *g* for 10 min, and the serum was kept at −80°C until assayed for thromboxane by RIA (9,11).

Urine was collected for 24 h and stored at −20°C. A urine probe of 2 ml was acidified with 1 N HCl to pH 3 and passed through a reverse-phase octadodecylsilylsilica cartridge (Sep-Pak C-18, Waters, Milford, CT) and then prewashed successively with 5 ml 96% ethanol, water, and 0.1 N acetic acid. The cartridge was then washed with 5 ml 15% ethanol and 5 ml petroleum ether. The prostanoids were eluted with 2 ml ethylacetate and evaporated to dryness. The residue was dissolved in 2 ml 0.1 M phosphate-buffered saline (pH 7.4) containing 0.1% gelatine, and thromboxane was measured by RIA (9,11). Values in urine from healthy people by this method are comparable with values obtained after fractionation by reverse-phase high-performance liquid chromatography (13).

Fatty acid analysis. Plasma and washed platelets were extracted with 2,6-di-tert-butyl-*p*-cresol at a final concentration of 0.2% (14). Phospholipids were purified by open-column chromatography (15). Fatty acid methyl esters were prepared by acid hydrolysis (3 N HCl in methanol; Supelco, Sulzbach/Taunus, FRG) or 1 h at 75°C under N_2. Analysis was performed on an HP 5890 A gas chromatograph (Hewlett-Packard, Taufkirchen, FRG) equipped with a Durabond 225 30-m × 0.25-mm fused silica capillary column (ICT, Carlo Erba, Frankfurt, FRG). Peaks were identified by comparison to standard fatty acid methyl esters (Sigma, Munich). Values are given as relative percentage of the fatty acids set as 100%.

Routine laboratory parameters were determined by standard laboratory methods with the Hitachi analyzer 705.

For statistical evaluation, analysis of variance for repeated measurements and a subsequent Wilcoxon rank test for paired samples were used in the diabetic patients. To compare the results in the diabetic group with those in the control group, the Wilcoxon-Mann-Whitney test for unpaired samples was used.

RESULTS

The intake of the capsules was surprisingly well tolerated. None of the patients dropped out of the study, and none had intermittent illnesses. No adverse effects of capsule intake were observed, and no changes occurred in blood pressure. The routine laboratory parameters for hepatic, kidney, and bone marrow function remained unchanged.

The blood glucose values were controlled by the patients and were very similar throughout the study (Table 2). There was a small increase in insulin requirement and carbohydrate intake, but these changes were probably of little clinical significance. The levels for HbA_1, glycosylated albumin, and glycosylated total serum proteins did not change significantly during the study (Table 2). Microalbuminuria was in the normal range in 12 diabetic patients and did not change during capsule intake. In one diabetic patient, the albumin excretion rate was high (985 ± 216 μg/min) and increased (to 1525 ± 148 μg/min) during the study.

During the entire study, the lipid levels were within the normal range (Fig. 1). Triglycerides decreased from days 1 to 14 (86 ± 11 vs. 75 ± 11 mg/L, $P < 0.05$) and thereafter increased again with no significant differences between days 1 and 28, 1 and 42, and 28 and 42. Total cholesterol increased slightly (day 1 vs. 42, 204 ± 7 vs. 219 ± 11 mg/L, $P < 0.05$), probably because of an elevation of high-density lipoprotein cholesterol (day 1 vs. 28 and 42, 54 ± 3 vs. 61 ± 4 mg/L, $P < 0.01$). The slight increase in low-density lipoprotein cholesterol was not statistically significant.

The composition of the plasma phospholipids changed

TABLE 2
Parameters of metabolic control of diabetic patients

	HbA_1 (%)	Glycosylated albumin (%)	Glycosylated total protein (%)	Carbohydrate intake (g/wk)	Mean blood glucose (mg/dl)	Insulin used (U/wk)	Increase in insulin requirement (%)
Day 1 (before)	9.2 ± 0.5	14.1 ± 1.3	17.4 ± 1.2	1300 ± 97	129 ± 10	325 ± 28	
Day 14	9.0 ± 0.5	15.3 ± 1.8	16.9 ± 1.1	1323 ± 96	142 ± 10	346 ± 10	5 ± 3
Day 28	8.9 ± 0.5	14.6 ± 1.3	17.5 ± 1.2	1327 ± 100	145 ± 9	340 ± 29*‡	5 ± 2
Day 42 (after)	8.8 ± 0.5	14.8 ± 1.9	17.3 ± 1.6	1352 ± 98	149 ± 9†	332 ± 29	2 ± 2

Values are means ± SE. Carbohydrate intake was calculated by addition of all carbohydrates eaten during that week. Mean blood glucose during 1 wk was calculated by addition of all blood glucose values measured during that week divided by number of measurements. Insulin used was obtained by adding all units administered during that week.
*$P \leq 0.05$ vs. values before capsule intake.
†$P \leq 0.01$.
‡$P \leq 0.05$ vs. day 42.

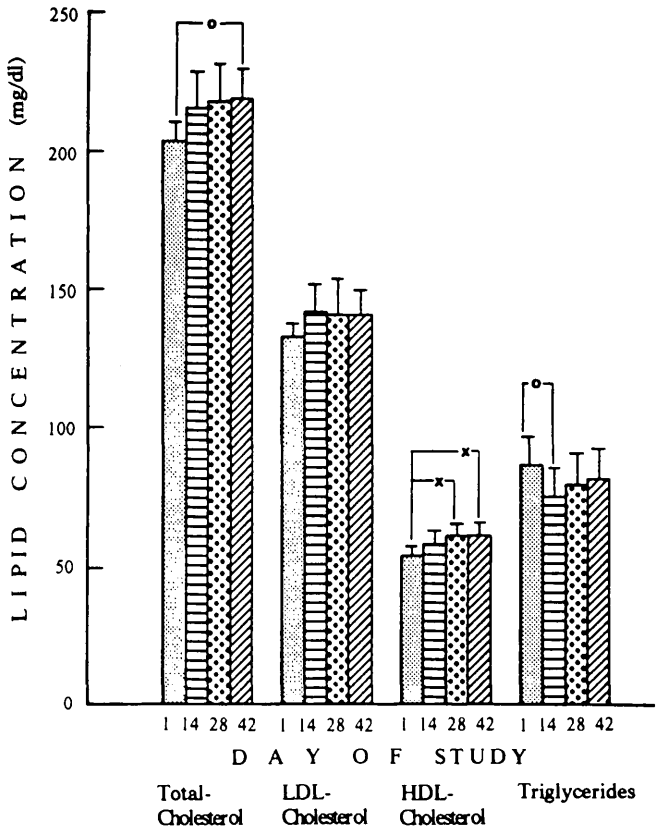

FIG. 1. Total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol and triglycerides before (day 1), during (days 14 and 28), and after (day 42) ω-3 fatty acid intake in diabetic subjects (n = 13). Values are means ± SE. °$P < 0.05$. ˣ$P < 0.01$.

significantly during capsule intake. EPA and DHA increased markedly, mainly at the expense of linoleic acid, whereas AA decreased less dramatically (Table 3). Fourteen days after cessation of EPA/DHA supplementation, plasma levels of DHA and EPA were still higher, whereas AA was lower than at the beginning of the study ($P < 0.005$). Linoleic acid, however, reached its starting value. Saturated fatty acids remained unchanged during the whole study (not shown). Platelet membrane phospholipids were altered in a way very similar to plasma phospholipids during and after ω3FA intake (Table 3). However, changes of EPA/DHA and AA were more sluggish.

Platelet counts did not change during ω3FA intake. Ex vivo platelet aggregation induced by ADP (1.0 μg/ml), collagen (0.6 μg/ml), and AA (166 and 333 μg/ml) was not significantly different between platelets from diabetic patients and matched healthy control subjects. No significant changes occurred during the study (Table 4). However, platelet aggregation induced by 0.5 μg/ml ADP or 0.3 μg/ml collagen was significantly higher in the diabetic patients and decreased during ω3FA intake, so that the difference from healthy control subjects was no longer significant (day 28; Table 4). Fourteen days after omittance of ω3FA supplementation, aggregation with 0.5 μg/ml ADP had again increased, although aggregation with collagen remained at the decreased level.

Thromboxane production. Basal thromboxane production during ex vivo ADP- and collagen-induced aggregation was higher in platelets from diabetic patients than from healthy control subjects (Table 5). However, during the ω3FA intake, these values decreased significantly without reaching levels of healthy control subjects and increased after stopping ω3FA intake.

When AA was used as the aggregating substance, the amount of thromboxane produced by platelets from diabetic subjects was significantly lower than from control subjects (Table 5). Although a small increase was seen during the diet, this increase was not significant, and the values remained significantly lower than platelets of the control subjects (Table 5).

The amount of thromboxane liberated during clotting of whole blood according to the method of Patrono et al. (12) was significantly higher in blood from diabetic patients than control subjects (Fig. 2). A decrease in thromboxane production was observed in the diabetic patients on EPA/DHA, which was reversible 14 days after cessation of ω3FA supplementation. In addition, thromboxane excretion in urine over 24 h was higher in diabetic patients than control subjects (Fig. 3). During the EPA/DHA intake, a significant decrease could be noted on day 28, followed by a significant increase on omission of ω3FAs.

TABLE 3
Plasma and platelet phospholipid fatty acids in 13 insulin-dependent diabetic patients before (day 1), during (days 14 and 28), and after (day 42) supplementation of eicosapentaenoic acid and docosahexaenoic acid to an otherwise unchanged diet

	Day 1	Day 14*	Day 28*	Day 42
Plasma				
Linoleic acid (C18:2ω6)	22.15 ± 2.1	16.00 ± 2.0	16.56 ± 2.3	21.59 ± 1.7
Arachidonic acid (C20:4ω6)	9.30 ± 1.1	7.93 ± 0.8	7.67 ± 0.9	8.20 ± 0.9†
Eicosapentaenoic acid (C20:5ω3)	0.73 ± 0.2	7.36 ± 1.5	7.32 ± 2.0	1.70 ± 0.4†
Docosahexaenoic acid (C22:6ω3)	2.23 ± 0.4	4.38 ± 0.8	4.69 ± 1.1	4.40 ± 0.8†
Platelets				
Linoleic acid (C18:2ω6)	6.63 ± 0.7	6.06 ± 0.9	5.89 ± 0.9	6.55 ± 1.0
Arachidonic acid (C20:4ω6)	28.55 ± 2.1	23.44 ± 2.5	21.93 ± 3.1	25.37 ± 3.3†
Eicosapentaenoic acid (C20:5ω3)	0.60 ± 0.3	4.60 ± 1.2	5.16 ± 1.3	1.66 ± 0.6†
Docosahexaenoic acid (C22:6ω3)	1.52 ± 0.3	2.15 ± 0.4	2.33 ± 0.6	2.16 ± 0.4†

Values are means ± SE in percentages.
*$P < 0.001$ vs. day 1, except platelet linoleic acid.
†$P < 0.01$ vs. day 1.

TABLE 4
Ex vivo aggregation of platelets from diabetic and nondiabetic control subjects

Aggregates (μg/ml)	Day 1	Day 14	Day 28	Day 42	Control
ADP					
0.5	40 ± 5*	37 ± 5*	32 ± 5†	44 ± 8*†	24 ± 3
1.0	51 ± 5	52 ± 5	52 ± 5	56 ± 6	54 ± 4
Collagen					
0.3	38 ± 8*	35 ± 7*	34 ± 8	33 ± 9	20 ± 6
0.6	50 ± 5	56 ± 5	53 ± 6	56 ± 5	41 ± 6
Arachidonic acid					
166	27 ± 11	36 ± 11	34 ± 10	38 ± 12	38 ± 9
333	59 ± 8	61 ± 8	71 ± 3	68 ± 6	67 ± 6

Values are means ± SE. Aggregation is expressed as percent increase in light transmission, whereby the light transmission of platelet-rich plasma is set at 0%, and light transmission of platelet-poor plasma is set at 100%.
*$P \leq 0.05$ vs. control subjects.
†$P < 0.05$.

DISCUSSION

The daily intake of a rather large amount of EPA/DHA over 4 wk was, as in healthy subjects (13), tolerated without any side effects. The changes in plasma and platelet phospholipid composition confirmed compliance in taking the prescribed ω3FA capsules.

Glucose homeostasis, expressed as degree of HbA_1, glycosylated albumin, and total glycosylated plasma proteins, was not altered throughout the study, demonstrating that patients successfully kept blood glucose under control by self-monitoring and self-adjustment of their daily insulin dose. Mean blood glucose and HbA_1 levels during the study were comparable with those before the study. The small changes in insulin dose (2–5%) and a slight increase in carbohydrate intake were probably of no clinical significance.

Body weights remained constant. Glauber et al. (16) reported elevated basal hepatic glucose output and unchanged peripheral glucose disposal with an increase of fasting and postprandial blood glucose. In addition, a marked decrease of meal-stimulated insulin release during ω3FAs in Glauber's non-insulin-dependent (type II) diabetic patients was noted that, however, could not be confirmed by others (17). They gave a warning when recommending dietary ω3FA in type II diabetes. Their number of patients was small, however, and proper metabolic control before the study was not achieved. Insulin resistance as a cause of deteriorated glucose metabolism during ω3FA diet in type II diabetes has not been demonstrated in animal experiments (18).

The plasma lipid concentrations were normal in our type I diabetic patients, which contrasts with many overweight type II diabetic patients. High-density cholesterol levels were slightly increased in this study by intake of ω3FAs. In nondiabetic subjects, plasma high-density lipoprotein cholesterol has been reported to be unchanged (19), decreased (20), or increased (21). ω3FAs were especially effective in almost normalizing plasma cholesterol in patients with hyperlipidemia, and a transient reduction of serum triglycerides was observed (22). Previously, reduction of triglycerides were not observed in type I diabetic patients (23), whereas they were sustained in type II diabetic patients after fish oil supplementation (17,24). Although the changes in plasma lipids might be small, they can be highly effective in reducing vascular complications in high-risk individuals (25,26).

During ω3FA supplementation, EPA and DHA were incorporated into plasma phospholipids at the expense of linoleic acid and AA, into platelet phospholipids (however, in a more

TABLE 5
Thromboxane production after ex vivo platelet aggregation

Aggregates (μg/ml)	Day 1	Day 14	Day 28	Day 42	Control
ADP		$P < 0.01$		$P < 0.01$	
0.5	5.1 ± 2.0†	2.9 ± 1.0	2.1 ± 0.5†	5.5 ± 1.5†	1.7 ± 0.8
			$P < 0.05$		
		$P < 0.01$		$P < 0.05$	
1.0	8.8 ± 1.8	5.4 ± 1.3	5.0 ± 1.1	7.3 ± 1.5	7.0 ± 1.3
		$P < 0.05$	$P < 0.05$		
Collagen		$P < 0.05$	$P < 0.01$		
0.3	8.6 ± 1.8*	7.2 ± 1.0	6.0 ± 1.3	6.9 ± 1.6	5.2 ± 1.9
0.6	15.7 ± 2.2*	11.9 ± 1.6*	9.4 ± 1.3	11.2 ± 1.7	8.7 ± 2.3
		$P < 0.05$			
Arachidonic acid					
166	172 ± 52*	192 ± 37*	210 ± 61†	211 ± 10	293 ± 38
333	427 ± 50*	430 ± 49†	463 ± 28*	425 ± 41*	585 ± 36

Values are means ± SE in nanograms per milliliter.
*$P = 0.05$, †$P = 0.01$, vs. control values.

delayed fashion), and at the sole expense of AA. Saturated fatty acids remained unaltered. These findings are in keeping with results in volunteers and type I diabetic women (13,27) and point to a specific regulation of fatty acid composition of plasma and platelet phospholipids also in diabetic patients.

Compared with healthy control subjects, ex vivo platelet aggregation and concomitant TXB_2 production on stimulation with ADP (0.5 μg/ml) and collagen (0.3 μg/ml) were increased in diabetic patients, which markedly contrasts with recent findings where in vivo and ex vivo platelet functions were examined (28). Low concentrations of these aggregating substances are more suitable to discern differences in platelet aggregation than high concentrations that have been used previously (29). Whenever tested, platelet aggregation was induced with AA. This rules out inadvertent ingestion of cyclooxygenase inhibitors as a cause for observed differences in platelet aggregability. AA-induced aggregation was comparable in healthy control subjects and diabetic patients.

Addition of ω3FAs to the diet of the diabetic patients reduced platelet aggregation in response to 0.5 μg/ml ADP, reversible 2 wk after cessation. Aggregation in response to 1 μg/ml ADP or to 0.3 or 0.6 μg/ml collagen, however, was not affected. In a preliminary report, higher threshold doses for platelet aggregation by collagen and AA were observed in diabetic patients during ω3FA supplementation (30). Thromboxane formation in PRP stimulated with low doses of agonist can be considered a model of submaximal platelet stimulation. Response to all stimuli used was decreased

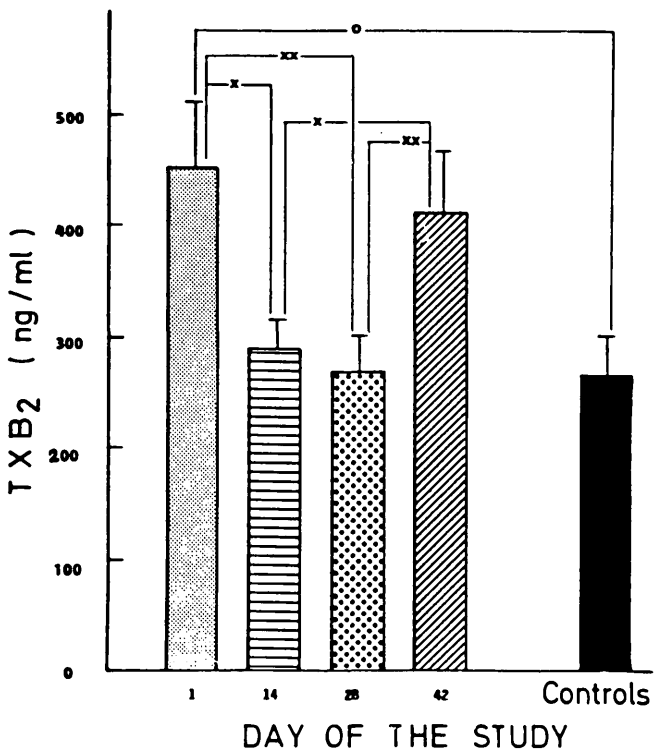

FIG. 2. Thromboxane (TXB_2) production during clotting of whole blood for 30 min at 37°C. Measurement was performed with radioimmunoassay in serum after centrifugation. Values are means ± SE. Controls are age- and sex-matched healthy subjects. °$P < 0.05$. ˣ$P < 0.01$. ˣˣ$P < 0.001$.

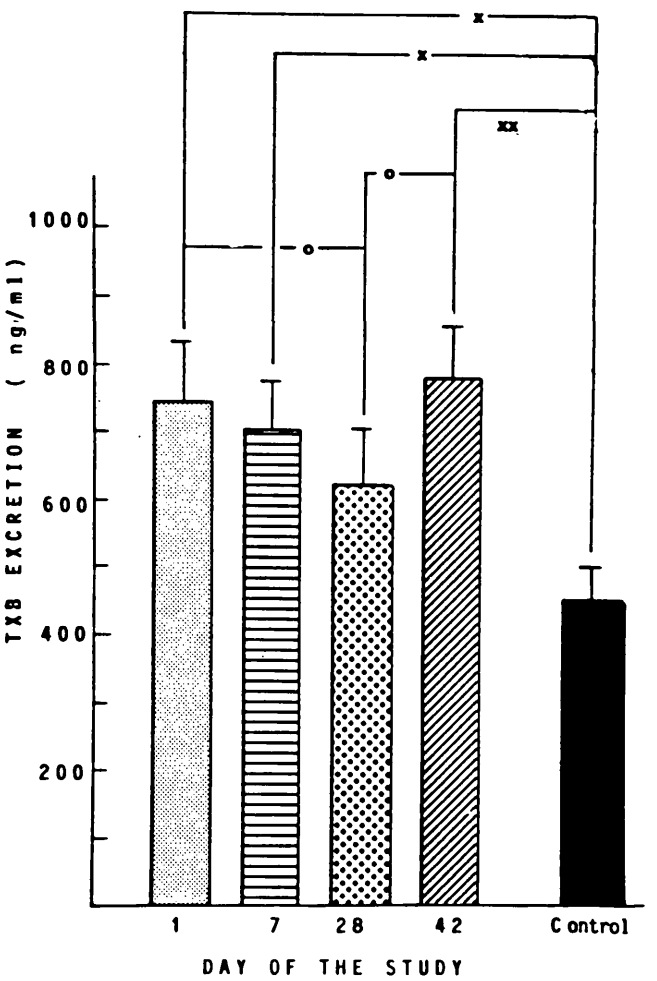

FIG. 3. Thromboxane (TXB) excretion in urine collected for 24 h. Values are means ± SE. °$P < 0.05$. ˣ$P < 0.01$. ˣˣ$P < 0.001$.

throughout the supplementation period. Fourteen days after cessation, this phenomenon was reversible for ADP but not for collagen. This may reflect elevated ω3FA levels in platelet phospholipids 14 days after cessation (Fig. 2) and/or different metabolic pathways in platelet response to the respective stimuli.

In clotted whole blood (30 min, 37°C), platelet activation is presumed to be maximal. Thus, platelets of diabetic patients have a higher capacity to form thromboxane than platelets of healthy control subjects (Fig. 2). This capacity was reduced to normal levels by ω3FA intake, consistent with previous findings in type I diabetic patients (23,31).

Thromboxane excretion was higher in diabetic patients than in healthy control subjects (Fig. 3). It was significantly reduced during the ω3FA supplementation period and reached its preintervention levels 2 wk after cessation. The RIA we used does not discern sufficiently between elevated levels of 2,3-dinorthromboxane and thromboxane. Moreover, cross-reactivity with EPA-derived thromboxane metabolites cannot be determined because of the lack of an authentic standard. TXA_3, however, is hardly if at all bioactive and is only formed in small quantities (7,32). Kidney thromboxane synthesis, measured as urinary thromboxane excretion, is thought to reduce kidney blood flow (33) and seems to be

augmented in diabetic nephropathy (34). 2,3-Dinorthromboxane formation (35) is elevated in patients likely to develop vascular complications (36). It can be markedly reduced by ω3FAs in volunteers (13) and patients with atherosclerosis (37). Both systemic and kidney thromboxane formation when elevated reflect detrimental pathophysiological processes in the diabetic patient. Our data indicate ω3FAs have a beneficial effect on at least one of these processes. Thus, addition of ω3FAs to the diet of diabetic patients reduces thromboxane formation on submaximal and maximal stimulation, which is reflected in vivo as reduced urinary excretion of thromboxane.

In conclusion, enhanced thromboxane formation and increased platelet aggregability also seen in metabolically well-adjusted type I diabetic patients without severe late complications can be markedly reduced by addition of an acceptable amount of ω3FAs to their otherwise unchanged diet. No side effects were observed. High-density lipoprotein cholesterol was increased, whereas glucose control and glycosylated proteins remained unchanged. The benefit of ω3FAs in reducing vascular risk remains to be tested in prospective studies with clinical criteria as end points. Such studies in diabetic patients are now warranted.

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