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Originals

Effects of short-term high dose intake of evening primrose oil on plasma and cellular fatty acid compositions, α-tocopherol levels, and erythropoiesis in normal and Type 1 (insulin-dependent) diabetic men

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Summary. In addition to their usual diet, nine Type 1 (insulin-dependent) diabetic men and ten male control subjects took 20 g d,α-tocopheryl acetate enriched evening primrose oil (14.45 g 18:2c,ω6, 1.73 g 18:3c,ω6, 400 mg d,α-tocopheryl acetate) daily for one week. At start, diabetic patients had more 14:0, 15:0 and 18:2c,ω6, and less 16:0, 16:1c,ω7, 18:1c,ω7, 18:3c,ω6, 20:3c,ω9, 20:3c,ω6, 20:4c,ω6 and 22:6c,ω3 in plasma, erythrocytes and/or platelets. Furthermore, they had lower 16:1c,ω7/16:0, 18:1c,ω7/16:0, and 20:4c,ω6/20:3c,ω6 ratios and a higher 20:3c,ω6/18:3c,ω6 ratio. In diabetic patients, α-tocopherol levels in erythrocytes were lower, whereas those in plasma were normal. In both groups, oil intake changed fatty acid profiles. Most markedly, 20:3c,ω6 increased, whereas the ratios 20:3c,ω6/18:3c,ω6 and 20:4c,ω6/20:3c,ω6 decreased. 20:4c,ω6 increased in control subjects, but not in diabetic patients. Erythrocytes and platelets responded differently in their fatty acid profiles. α-tocopherol rose in plasma and, although less for diabetic patients, in erythrocytes. In diabetic patients as well as in control subjects, erythrocyte count, haemoglobin level, mean corpuscular haemoglobin content and concentration increased and glycosylated haemoglobin percentage decreased without an apparent decline in blood glucose levels. Plasma β-thromboglobulin and platelet factor 4 decreased, especially in diabetic patients. In conclusion, diabetic patients had abnormal fatty acid patterns, suggesting an impaired Δ9, Δ6 and Δ5 desaturation and an enhanced chain-elongation, and had lower erythrocyte α-tocopherol levels; and short-term high dose intake of evening primrose oil increased 20:3c,ω6 in both groups, but 20:4c,ω6 only in control subjects, gave fatty acid responses which were different for erythrocytes and platelets, enhanced erythropoiesis, and lowered indices of in vivo platelet activation.

Key words: Type 1 (insulin-dependent) diabetes mellitus, evening primrose oil, fatty acids, α-tocopherol, erythropoiesis, platelet function.

Certain dietary fatty acids have a favourable influence on the pathogenesis of the long-term complications of diabetes mellitus. For example, intake of sardine oil normalises lowered membrane fluidity of erythrocytes [1] and use of cod-liver oil [2] or linoleic acid (18:2c,ω6) enriched diet [3] corrects disturbed platelet aggregation. Both oils are rich in eicosapentaenoic acid (20:5c,ω3) and docosahexaenoic acid (22:6c,ω3). Furthermore, a 18:2c,ω6-rich diet was reported to inhibit the progression of diabetic retinopathy [4, 5]; and supplementation with evening primrose oil (EPO), which especially contains gamma-linolenic acid (18:3c,ω6) in addition to a large amount of 18:2c,ω6, has been suggested to improve diabetic neuropathy [6].

Abnormal fatty acid compositions are found in plasma lipids [7, 8], erythrocytes [7, 8], platelets [8, 9] and adipose tissue [10] of patients with Type 1 (insulin-dependent) diabetes mellitus. An increased level of 18:2c,ω6 [8, 9] along with decreased levels of its metabolites 18:3c,ω6 [7, 9] and dihomo-gamma-linolenic acid (20:3c,ω6) [8, 9] suggests an impaired activity of the enzyme Δ6 desaturase, as has been shown in in vitro and in vivo experiments with insulin deficient rats [11, 12].

The present study was designed to evaluate the effect of dietary 18:3c,ω6 supplementation on the fatty acid composition of plasma-, erythrocyte- and platelet lipids from Type 1 diabetic patients by administration of EPO. Additionally, plasma- and erythrocyte α-tocopherol levels were determined, as patients with Type 1 diabetes mellitus are reported to have abnormalities in vitamin E metabolism [13] and the EPO containing capsules were enriched with α-tocopheryl acetate. To minimise potential influences due to, amongst others, changes in diabetic control, dietary habits and life style, we performed a short-term supplementation study with a relatively high dose of the oil.

Table 1. Individual characteristics of the Type 1 (insulin-dependent) diabetic men

Patient no.	Age (years)	Diabetes duration (years)	BMI (kg/m^2)	Initial GsHb (%)[a]	Insulin dose (U)				
					morning		evening		total daily
					S	I	S	I	dose
1	41	5	23.1	6.6	8	18	2	12	40
2	29	3	21.7	7.8	10	12	6	-	28
3	28	4	21.4	7.5					46[b]
4	22	3	21.9	9.5	14	16	14	-	44
5	21	8	24.2	8.3	18	42	5	11	76
6	22	0.3	20.9	8.5	6	20	-	-	26
7	21	3	25.1	9.0					34[c]
8	30	10	20.0	7.3	14	46	14	4	78
9	50	15	24.3	9.3	13	29	-	-	42

BMI: body mass index (weight/height^2); GsHb: glycosylated haemoglobin; S: short-acting insulin; I: intermediate-acting insulin
[a] range in control subjects 5.8–6.6%; [b] treated by continuous subcutaneous insulin infusion during 13 months; [c] treated by continuous subcutaneous insulin infusion during 20 months

Subjects and methods

Diabetic patients and control subjects

Nine Type 1 diabetic men of whom the individual characteristics are shown in Table 1, and ten apparently healthy male members of the hospital staff (aged 24–54 years, median 30 years) were studied. All diabetic patients were ketosis-prone. None of them had nephropathy, proliferative retinopathy or other relevant disease. The groups did not differ for body weight (control subjects: 65.5–85.7 kg, median 76.2 kg) and body mass index (control subjects: 20.0–26.3 kg/m^2, median 22.8 kg/m^2). All participants in the study denied smoking, excessive alcohol intake and the use of any medication.

Protocol

During one week, each participant took twice daily 10 g EPO, that was enriched with 20 mg/g d,α-tocopheryl acetate (Efamol, Efamol Ltd., London, UK). The fatty acid composition of the oil by our own analysis was: 14:0, 0.06; 16:0, 6.99; 18:0, 1.64; 20:0, 0.23; 22:0, 0.06; 16:1c,ω7, 0.07; 18:1c,ω9, 8.94; 18:1c,ω7, 0.72; 20:1c,ω9, 0.14; 18:2c,ω6, 72.40; 18:3c,ω6, 8.74 (mol/100 mol). The participants were asked not to change their dietary habits and usual alcohol intake during the period of EPO intake and the week thereafter. At the end of the EPO use period, a small increase in body weight was found in the group of control subjects (median increase 0.4 kg, $p < 0.05$).

The protocol was approved by the medical ethical committee of the hospital. Written informed consent was obtained from every participant.

Methods

At the start and at the end of the EPO intake period, and at one week thereafter, venous blood samples with and without EDTA and heparine as anticoagulant were collected without occlusion 1–3 h after breakfast. On the day before blood sampling, the diabetic patients collected fingerprick samples for blood glucose measurements at 03.00 hours, 07.00 hours (fasting), 1.5 h after breakfast, 11.00 hours, 1.5 h after lunch, 17.00 hours, 1.5 h after dinner, and at 23.00 hours.

EDTA-anticoagulated blood served for the isolation of platelet-poor plasma, platelets and erythrocytes and for the preparation of blood cell profiles that were made by a Coulter Counter Model S-plus IV (Coulter Electronics Ltd., Luton, Bedfordshire, UK) at least 1 h after venipuncture [14]. Ten ml samples were centrifuged at 250 *g* for 10 min. The platelet-rich plasma was collected and recentrifuged for 10 min at 1500 *g*. The supernatant (platelet-poor plasma) was removed and the platelet-pellet was washed three times with 2 ml of 0.9% sodium chloride solution. The erythrocytes were washed three times with 5 ml 0.9% sodium chloride solution. After each centrifugation at 800 *g* for 10 min, the buffy coat was removed as completely as possible. The erythrocytes were finally resuspended to a haematocrit of about 50%, and counted in a Coulter Counter Model S-plus IV. Fatty acid profiles of plasma, the erythrocyte suspension (approximately 50%) and the whole platelet-pellet were performed by a previously described capillary gas chromatographic method [15], using an apolar stationary phase. For technical reasons 23:0, 24:0, and 24:1c,ω9 could not reliably be analysed. α-tocopherol in plasma and vitamin E in erythrocyte suspension were determined by high performance liquid chromatography with fluorescence detection [16, 17]. As the qualitatively and quantitatively major part of vitamin E in erythrocytes is α-tocopherol [18], vitamin E is referred to in the text as α-tocopherol. β-thromboglobulin and platelet factor 4 were measured in platelet-poor plasma by radioimmunoassay (Amersham, Buckinghamshire, UK, and Abbott Laboratories, North Chicago, Ill, USA, respectively). Fructosamine concentrations were determined in plasma [19] with a Multistat III (Instrumentation Laboratory, Spokane, Wash, USA). The reagent was carbonate buffer (0.1 mol/l, pH 10.35) containing 0.25 mmol/l nitroblue tetrazolium chloride. Protein-based standards and control sera were obtained from Instruchemie BV, Hilversum, The Netherlands.

Heparine-anticoagulated blood was used for the measurement of glycosylated haemoglobin by a colorimetric method [20].

Serum was prepared from venous blood by centrifugation at 1500 *g* for 10 min. Serum parameters of renal and liver function, and the concentrations of cholesterol and triglycerides were measured by a Sequential Multiple Analyzer Plus Computer (SMA-C, Technicon, Tarrytown, NY, USA). Serum levels of free T_4 and thyroid stimulating hormone were determined by radioimmunoassay tests (Amersham International plc, Amersham, UK).

Statistical analysis

Statistical analyses were performed by Wilcoxon two sided rank sum test for paired and unpaired data; p values ≤ 0.05 were considered significant. Linear regression line parameters were calculated according to Deming [21].

Results

Fatty acid compositions

The fatty acid profiles before, at the end of one week EPO intake and at one week thereafter are given in

Table 2. Relative amounts (median values) of plasma fatty acids from Type 1 diabetic men and healthy male control subjects at the start (A), at the end of a one week period of evening primrose oil intake (B), and at one week thereafter (C)

Fatty acids	Diabetic patients ($n=9$)			Control subjects ($n=10$)		
	A	B	C	A	B	C
(mol/100 mol)						
ΣSFA	32.88	32.44	34.17^{a}	32.14	32.31	32.85^{c}
14:0	1.03	0.95	1.64^{r}	1.03	1.09	1.28^{d,p}
15:0	0.39	0.37	0.44^{q}	0.34	0.33	0.36
16:0	22.53	22.37	23.64^{a,r}	22.70	22.23	22.92
18:0	7.66	7.67	7.48	7.15	7.58	7.32
20:0	0.27	0.25	0.25	0.28	0.30	0.27
22:0	0.87	0.84	0.76	0.83	0.80	0.85
ΣMUFA	20.78	17.06^{b}	20.79^{r}	23.92	19.75^{d}	22.74^{r}
16:1c,ω7	1.29^{E}	1.17	1.44	1.86	1.37^{c}	2.00^{r}
18:1c,ω9	17.23	14.16^{b}	17.34^{r}	18.81	15.77^{d}	17.86^{r}
18:1c,ω7	1.90	1.74^{c}	1.75^{q}	2.56	2.32	2.56
20:1c,ω9	0.18	0.16	0.18	0.23	0.15^{a}	0.15
ΣPUFA	46.45	48.56	44.60^{r}	43.63	47.57^{d}	43.49^{r}
18:2c,ω6	37.50^{E}	39.29	36.44^{r}	32.75	35.88^{d}	32.45^{r}
18:3c,ω6	0.26^{H}	0.80^{c}	0.33^{r}	0.48	1.07^{d}	0.48^{s}
20:2c,ω6	0.23	0.21^{b}	0.21^{b}	0.29	0.22^{b}	0.21^{b}
20:3c,ω9	0.09^{H}	0.07^{b}	0.08	0.19	0.11^{c}	0.13^{c}
20:3c,ω6	0.94^{E}	1.45^{c}	1.26^{a,r}	1.34	1.67^{d}	1.44^{s}
20:4c,ω6	4.96^{H}	4.48	4.38	5.88	6.88^{d}	6.02^{q}
20:5c,ω3	0.31	0.28	0.29	0.50	0.37	0.48
22:4c,ω6	0.15	0.17	0.15	0.19	0.19	0.20
22:5c,ω6	0.08	0.08	0.08	0.10	0.09	0.10
22:5c,ω3	0.48	0.42	0.44	0.52	0.51	0.53
22:6c,ω3	1.19	1.08	1.02	1.24	1.17	1.24
ΣFAc,ω6	44.15	46.50^{a}	42.70^{r}	40.73	45.66^{d}	41.13^{r}
ΣFAc,ω3	2.04	1.97	1.86	2.26	1.98	2.27

ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; ΣFAc,ω6, total ω6 fatty acids; ΣFAc,ω3, total ω3 fatty acids.
p-value diabetic patients versus control subjects: $^{E}p<0.02$; $^{H}p<0.002$
p-value B or C versus A: $^{a}p<0.05$; $^{b}p<0.02$; $^{c}p<0.01$; $^{d}p<0.002$
p-value C versus B: $^{p}p<0.05$; $^{q}p<0.02$; $^{r}p<0.01$; $^{s}p<0.002$

Table 3. Relative amounts (median values) of erythrocyte fatty acids from Type 1 diabetic men and healthy male control subjects at the start (A), at the end of a one week period of evening primrose oil intake (B), and at one week thereafter (C)

Fatty acids	Diabetic patients ($n=9$)			Control subjects ($n=10$)		
	A	B	C	A	B	C
(mol/100 mol)						
ΣSFA	46.38	45.73	46.50	45.30	45.62	46.73^{c,p}
14:0	0.54^{I}	0.32	0.58	0.31	0.32	0.64^{d,s}
15:0	0.38^{I}	0.26	0.35	0.27	0.27	0.33^{b,r}
16:0	24.57	22.24	24.84	23.64	23.46	25.35^{c,r}
18:0	18.19	19.17^{c}	18.16	18.46	18.26	17.63^{d,s}
20:0	0.49	0.50	0.48	0.51	0.50	0.49
22:0	2.28	2.44^{a}	2.32^{p}	2.46	2.38^{a}	2.23^{d,r}
ΣMUFA	14.06	14.74	14.61	15.37	14.76^{b}	15.01^{a}
18:1c,ω9	11.98	11.92^{a}	11.81^{a}	12.43	11.82^{c}	12.39
18:1c,ω7	1.97^{D}	2.08	1.69	2.27	2.19	2.00
20:1c,ω9	0.28	0.30	0.30	0.28	0.27	0.28
ΣPUFA	38.84	40.04	38.48	39.70	40.06	39.18^{q}
18:2c,ω6	13.85^{G}	13.22	13.13	11.65	11.88	11.96
18:3c,ω6	tr	tr	tr	tr	tr	tr
20:2c,ω6	0.29	0.33	0.29	0.31	0.30	0.28
20:3c,ω9	0.31	0.34	0.25	0.36	0.29	0.26^{a}
20:3c,ω6	1.63	1.84^{c}	1.84^{c}	1.62	1.72^{d}	1.68^{r}
20:4c,ω6	13.54^{H}	14.51	13.89	14.66	15.01	14.53
20:5c,ω3	0.38	0.37	0.34	0.47	0.48	0.49
22:4c,ω6	2.93	3.05^{b}	2.96	3.16	3.17	3.01^{c,p}
22:5c,ω6	0.41	0.41	0.42	0.45	0.44^{c}	0.42^{b}
22:5c,ω3	2.45	2.43	2.38	2.49	2.42	2.32^{a,p}
22:6c,ω3	3.01^{D}	3.04	3.07	3.90	3.80	3.69^{p}
ΣFAc,ω6	32.83	33.55	32.87	32.63	32.92	31.77^{p}
ΣFAc,ω3	5.68	5.94	5.98	6.96	6.57	6.47^{c}

ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; ΣFAc,ω6, total ω6 fatty acids; ΣFAc,ω3, total ω3 fatty acids; tr, trace amount.
p-value diabetic patients versus control subjects: $^{D}p<0.05$; $^{G}p<0.005$; $^{H}p<0.002$; $^{I}p<0.001$
p-value B or C versus A: $^{a}p<0.05$; $^{b}p<0.02$; $^{c}p<0.01$; $^{d}p<0.002$
p-value C versus B: $^{p}p<0.05$; $^{q}p<0.02$; $^{r}p<0.01$; $^{s}p<0.002$

median values for plasma in Table 2, for erythrocytes in Table 3 and for platelets in Table 4. In the erythrocyte fatty acid compositions, expressed in absolute amounts, some significant ($p<0.05$) changes were noted, which were not accompanied by corresponding changes in relative amounts. From the start to the end of the EPO intake period, and subsequently until one week thereafter, these alterations were in median values (nmol/10^8 cells) for the control group: in total fatty acids: 58.97 to 56.33 to 56.86 ($p<0.01$ versus at the start), in total saturated fatty acids: 26.95 to 25.72 ($p<0.05$) to 26.47 ($p<0.02$ versus at the start), in 18:0: 10.89 to 10.88 to 10.17 ($p<0.02$ versus at the start, and versus at one week EPO use), and in total monounsaturated fatty acids: 8.44 to 8.43 to 8.24 ($p<0.01$ versus at the start). For the diabetic group, the following changes were found in 20:4c,ω6: 8.29 to 8.46 to 8.18 ($p<0.05$ versus at one week EPO use). In the diabetic group, inverse linear correlations were observed between glycosylated haemoglobin levels and erythrocyte 18:2c,ω6 contents (for absolute amounts $r=-0.72$, $p<0.05$; for relative amounts $r=-0.59$, $0.05<p<0.10$), as well as between glycosylated haemoglobin levels and plasma 18:2c,ω6 levels ($r=-0.66$, $0.05<p<0.10$). The median values of desaturase and chain-elongation product/precursor ratios and the essential fatty acid status ratio 20:3c,ω9/20:4c,ω6 [22] in plasma, erythrocytes and platelets are shown in Table 5.

α-tocopherol

The individual α-tocopherol levels in erythrocytes before, at the end of one week EPO intake and at one week thereafter are depicted in Figure 1 (median values for diabetic patients: 4.17 to 4.72 to 4.63 nmol/10^{10} cells, and for control subjects: 5.47 to 6.86 to 5.95 nmol/10^{10} cells). The absolute and relative increases at the end of one week EPO use were less in the diabetic patients than in the control subjects ($p<0.01$ and $p<0.05$, respectively). The α-tocopherol con-

centration in plasma was increased at the end of one week EPO intake and returned to base-line level at one week thereafter (median values for diabetic patients: 23 to 47 ($p<0.01$) to 23 μmol/l ($p<0.01$), and for control subjects: 24 to 48 ($p<0.01$) to 29 μmol/l ($p<0.01$)).

Table 4. Relative amounts (median values) of platelet fatty acids from Type 1 diabetic men and healthy male control subjects at the start (A), at the end of a one week period of evening primrose oil intake (B), and at one week thereafter (C)

Fatty acids	Diabetic patients ($n=9$)			Control subjects ($n=10$)		
	A	B	C	A	B	C
(mol/100 mol)						
ΣSFA	42.99	40.99	43.36	43.91	40.34^{d}	$43.39^{b,s}$
14:0	0.56	0.37	1.23	0.84	0.25^{d}	1.22^{s}
16:0	17.48^{D}	16.92	18.22	19.32	14.65^{d}	18.52^{s}
18:0	19.55	19.61	19.09	19.09	19.13	19.06
20:0	1.72	1.63	1.66	1.53	1.69^{b}	1.38^{s}
22:0	3.49	3.39	3.25	3.38	3.62	3.00
ΣMUFA	17.28	17.41	17.56	17.68	17.39	17.46
18:1c,ω9	14.83	14.97	14.88	15.10	14.57	14.71
18:1c,ω7	1.53	1.61	1.60	1.81	1.88	1.86
20:1c,ω9	0.66	0.62	0.70	0.58	0.64	0.54
ΣPUFA	39.42	39.79	39.45	38.44	43.17^{d}	$40.25^{a,s}$
18:2c,ω6	11.03^{F}	13.64	9.90^{q}	9.16	11.37^{b}	9.51^{q}
18:3c,ω6	0.10	0.32^{c}	0.21	tr	0.34^{c}	0.20
18:3c,ω3	0.44	0.53	0.47	0.51	0.45	$0.86^{a,q}$
20:2c,ω6	0.41^{D}	0.44	0.46	0.31	0.35	0.33
20:3c,ω9	0.18	0.15	0.18	0.19	0.15^{b}	0.14
20:3c,ω6	1.27	1.68^{a}	1.68	1.23	1.53^{d}	$1.33^{a,s}$
20:4c,ω6	20.25	19.07	20.68	21.74	22.23	21.69
22:4c,ω6	1.91	1.98	2.01	1.98	2.30^{a}	2.05
22:5c,ω6	0.18	0.19	0.19	0.19	0.20	0.18
22:5c,ω3	1.52	1.49	1.46	1.54	1.74	1.56^{q}
22:6c,ω3	1.38	1.35	1.31	1.50	1.41	1.42
ΣFAc,ω6	36.39	35.87	35.59	35.02	39.45^{c}	36.31^{s}
ΣFAc,ω3	3.39	3.61	3.61	3.70	3.83	3.86^{a}

ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; ΣFAc,ω6, total ω6 fatty acids; ΣFAc,ω3, total ω3 fatty acids; tr, trace amount.
p-value diabetic patients versus controls: $^{D}p<0.05$; $^{F}p<0.01$
p-value B or C versus A: $^{a}p<0.05$; $^{b}p<0.02$; $^{c}p<0.01$; $^{d}p<0.002$
p-value C versus B: $^{q}p<0.01$; $^{s}p<0.002$

Peripheral blood cell profiles

The median values in erythrocyte counts, its indices and reticulocyte promillages are given in Table 6. Erythrocyte α-tocopherol content is correlated with mean corpuscular volume ($r=0.56$, $0.05<p<0.10$), mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in the control group (Fig. 2), but not in the diabetic group ($r=-0.04$, $r=-0.15$, $r=-0.14$, respectively).

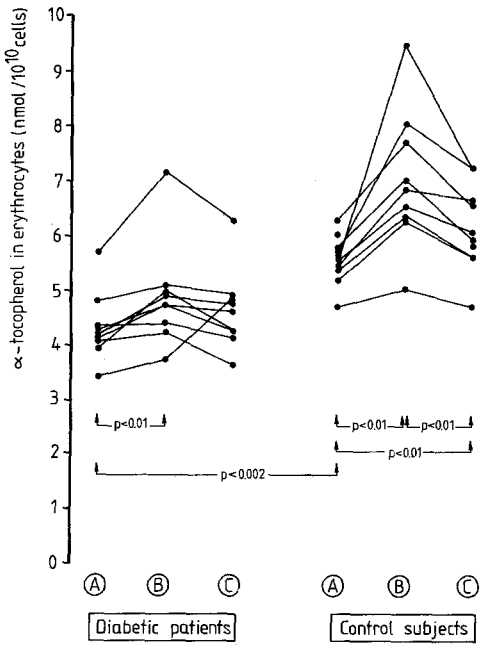

Fig. 1. α-Tocopherol levels in erythrocytes for nine Type 1 (insulin-dependent) diabetic men and ten healthy male control subjects before (A), after evening primrose oil intake during one week (B) and one week thereafter (C). One sample from the group of the control subjects at B is lacking

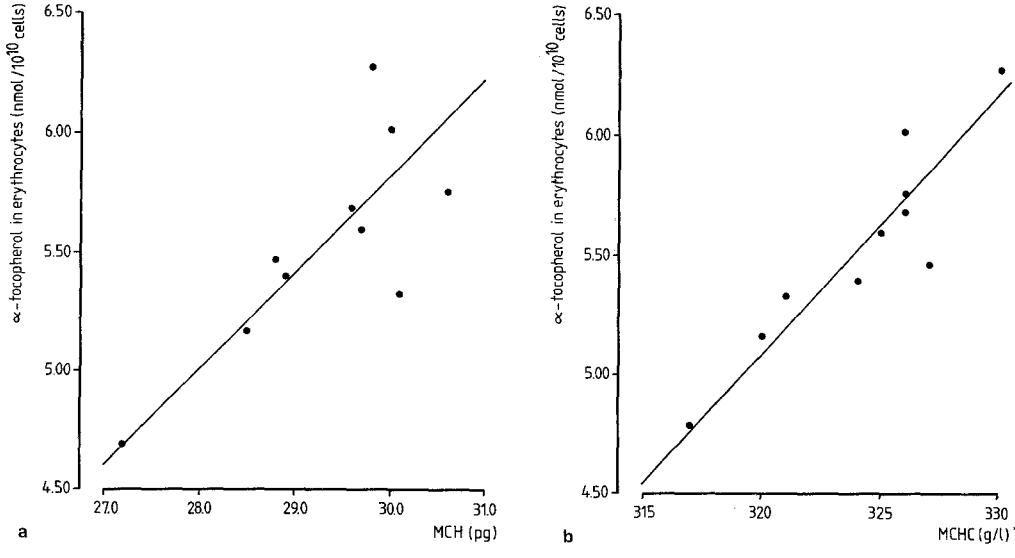

Fig. 2. Correlation between mean corpuscular haemoglobin contents (MCH; **a**) and mean corpuscular haemoglobin concentrations (MCHC; **b**) and α-tocopherol levels in erythrocytes from ten healthy male control subjects at the start of the study. Linear regression lines and correlation coefficients are $y=0.4046x-6.32$ and 0.78 ($p<0.01$) and $y=0.1086x-29.654$ and 0.92 ($p<0.001$), respectively

Table 5. Median values of desaturase and chain-elongation product/precursor ratios and essential fatty acid status parameters in plasma, erythrocytes and platelets from Type 1 diabetic men and healthy male control subjects at the start (A), at the end of a one week period of evening primrose oil intake (B), at one week thereafter (C)

Compartments	Molar ratios		Diabetic patients ($n=9$)			Control subjects ($n=10$)		
			A	B	C	A	B	C
Plasma	16:1c,ω7/16:0	(1)	0.055[E]	0.052	0.060	0.082	0.063[c]	0.086[r]
	18:1c,ω9/18:0	(1)	2.18	1.85[b]	2.27[q]	2.66	2.14[d]	2.52
	18:1c,ω7/16:0	(2)	0.081	0.078[c]	0.076	0.107	0.100	0.113
	18:3c,ω6/18:2c,ω6	(3)	0.006[I]	0.020[c]	0.009[a, r]	0.015	0.030[d]	0.015[r]
	20:3c,ω6/18:2c,ω6	(4)	0.028[G]	0.037[c]	0.036[c, r]	0.038	0.044[d]	0.043[r]
	20:4c,ω6/20:3c,ω6	(5)	4.63	3.06[c]	4.32[c, r]	4.70	3.88[c]	4.51[r]
	22:5c,ω6/22:4c,ω6	(6)	0.526	0.492	0.538[r]	0.520	0.456[a]	0.541[r]
	22:6c,ω3/22:5c,ω3	(6)	2.67	2.76	2.35	2.51	2.22	2.53
	18:1c,ω7/16:1c,ω7	(7)	1.48	1.51	1.64	1.18	1.40[c]	1.19[q]
	20:3c,ω6/18:3c,ω6	(7)	4.30[H]	1.72[c]	2.98[r]	2.74	1.55[c]	3.05[a, s]
	22:4c,ω6/20:4c,ω6	(7)	0.034	0.032	0.034	0.033	0.029	0.032
	22:5c,ω3/20:5c,ω3	(7)	1.57	2.17	1.55	1.07	1.43	1.11
	20:3c,ω9/20:4c,ω6	(8)	0.025[D]	0.015[b]	0.019	0.032	0.017[c]	0.021[c]
Erythrocytes	18:1c,ω9/18:0	(1)	0.654	0.647[c]	0.648[p]	0.692	0.666[a]	0.713
	18:1c,ω7/16:0	(2)	0.082[E]	0.091	0.070	0.099	0.091	0.079[a]
	20:3c,ω6/18:2c,ω6	(4)	0.124	0.139[c]	0.128[b]	0.137	0.148[c]	0.140[p]
	20:4c,ω6/20:3c,ω6	(5)	8.11[D]	7.22[c]	7.21	9.06	8.85[a]	8.80
	22:5c,ω6/22:4c,ω6	(6)	0.141	0.140	0.140	0.142	0.141[b]	0.144
	22:6c,ω3/22:5c,ω3	(6)	1.35	1.32	1.35[p]	1.57	1.58	1.59[r]
	22:4c,ω6/20:4c,ω6	(7)	0.213	0.216	0.214[p]	0.205	0.209	0.204[c, q]
	22:5c,ω3/20:5c,ω3	(7)	6.26	6.93[b]	6.41	5.10	5.41	5.04
	20:3c,ω9/20:4c,ω6	(8)	0.022	0.022	0.019	0.025	0.020	0.017[b]
Platelets	18:1c,ω9/18:0	(1)	0.759	0.758	0.758	0.793	0.794	0.782
	18:1c,ω7/16:0	(2)	0.093	0.103	0.091	0.094	0.124[d]	0.110[c, r]
	18:3c,ω6/18:2c,ω6	(3)	0.008	0.028[c]	0.019	tr	0.029[c]	0.022
	20:3c,ω6/18:2c,ω6	(4)	0.102	0.164	0.180	0.129	0.134	0.136
	20:4c,ω6/20:3c,ω6	(5)	15.87	11.83[c]	12.46[b, p]	16.84	13.86[d]	16.20[q]
	22:5c,ω6/22:4c,ω6	(6)	0.092	0.093	0.100	0.097	0.098	0.098
	22:6c,ω3/22:5c,ω3	(6)	0.973	0.947	0.964	0.921	0.987	1.000
	20:3c,ω6/18:3c,ω6	(7)	14.50	5.75[c]	8.36	>11.00	4.62[b]	7.73
	22:4c,ω6/20:4c,ω6	(7)	0.093	0.097	0.101	0.091	0.102[d]	0.098[c]
	20:3c,ω9/20:4c,ω6	(8)	0.008	0.009	0.009	0.011	0.007[c]	0.007

tr, trace amount
ratios representing: 1, Δ9 desaturase; 2, Δ9 desaturase + chain-elongation; 3, Δ6 desaturase; 4, Δ6 desaturase + chain-elongation; 5, Δ5 desaturase; 6, Δ4 desaturase; 7, chain-elongation; 8, parameter for essential fatty acid status.
p-value diabetic patients versus controls: [D] $p<0.05$; [E] $p<0.02$; [G] $p<0.005$; [H] $p<0.002$; [I] $p<0.001$
p-value B or C versus A: [a] $p<0.05$; [b] $p<0.02$; [c] $p<0.01$; [d] $p<0.002$
p-value C versus B: [p] $p<0.05$; [q] $p<0.02$; [r] $p<0.01$; [s] $p<0.002$

In the diabetic group, mean platelet volume increased after one week EPO use and decreased thereafter (median values: 9.0 to 9.3 ($p<0.05$) to 9.1 fl ($p<0.05$). In the control group, polymorphonuclear leukocyte count was increased at one week after EPO intake (median values: 3.0 to 3.6 to 3.4×10^9/l ($p<0.02$ versus at the start)). No other changes were found for platelet counts, its indices, total leukocyte counts and differentiations.

Platelet function

Platelet factor 4 level in plasma was decreased after one week EPO use and returned to base-line level at one week thereafter (median values for diabetic patients: 18.6 to 8.9 ($p<0.02$) to 16.5 ng/l ($p<0.01$), and for control subjects: 16.1 to 9.3 ($0.05<p<0.10$) to 13.7 ng/l). Plasma β-thromboglobulin demonstrated a similar pattern. Its changes were only significant in the diabetic group (median values for patients: 55.3 to 34.4 ($p<0.05$) to 51.6 ng/l ($p<0.01$) and for control subjects: 52.9 to 40.1 to 48.2 ng/l). At the start of the study, no differences were found in these two platelet function parameters between the diabetic patients and the control subjects.

Parameters of diabetic control

Parameters of diabetic control are given as median values in Table 6.

Other laboratory tests

At the start of the study, differences between the diabetic and the control group were demonstrated for the

Table 6. Median values of some haematological tests and parameters of diabetic control from Type 1 diabetic men and healthy male control subjects at the start (A), at the end of a one week period of evening primrose oil intake (B) and at one week thereafter (C)

	Diabetic patients ($n=9$)			Control subjects ($n=10$)		
	A	B	C	A	B	C
ery count (10^{12}/l)	4.82	4.90[a]	4.97[b]	4.76	4.95[c]	4.86[c]
Hb (g/l)	140	146[c]	146[c, p]	139	145[c]	145[d]
MCV (fl)	89.0	88.8	88.7	90.5	90.4	90.8
MCH (pg)	28.8	28.9	29.4[c, r]	29.7	29.6	30.0[c, r]
MCHC (g/l)	324	322	331[c, r]	326	325	333[d, r]
reticulocytes (‰)	6	6	6	3	12	5
GsHb (%)	8.3[I]	8.3	7.6[c]	6.1	6.0[a]	5.8[a]
fructosamine (mmol/l)	2.55[I]	2.42	2.47	2.10	2.05	2.02[a]
glucose (mmol/l) (1)	10.3[G]	13.7	11.4	5.1	4.8	4.6
glucose (mmol/l) (2)	8.89	8.42	8.27	ND	ND	ND

ery, erythrocyte; Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; GsHb, glycosylated haemoglobin. 1, venous blood glucose; 2, mean capillary blood glucose calculated from 24 h profiles on the day before venous blood sampling; ND, not done.
p-value diabetic patients versus control subjects: [G] $p<0.005$; [I] $p<0.001$
p-value B or C versus A: [a] $p<0.05$; [b] $p<0.02$; [c] $p<0.01$; [d] $p<0.002$
p-value C versus B: [p] $p<0.05$, [r] $p<0.01$

serum concentrations of urea (patients: 4.9–7.8 mmol/l, median 6.3 mmol/l; control subjects: 4.2–6.8 mmol/l, median 5.3 mmol/l, $p<0.05$), creatinine (patients: 68–89 μmol/l, median: 78 μmol/l; control subjects: 78–100 μmol/l, median: 89 μmol/l, $p<0.005$), total protein (patients: 62–70 g/l, median: 64 g/l; control subjects: 64–76 g/l, median: 69 g/l, $p<0.05$), albumin (patients: 41–44 g/l, median 42 g/l; control subjects: 41–49 g/l, median 45 g/l, $p<0.02$), alkaline phosphatase (patients: 61–78 U/l, median 77 U/l; control subjects: 40–73 U/l, median 56 U/l, $p<0.02$) and free T_4 (patients: 10.1–16.4 pmol/l, median: 13.2 pmol/l; control subjects: 12.9–16.9 pmol/l, median: 15.8 pmol/l, $p<0.02$). The serum concentration of thyroid stimulating hormone did not differ between both groups. From one week of EPO use to one week thereafter a slight decrease in serum levels was observed for total protein in both groups (median value in the group of the patients from 66 to 65 g/l, $p<0.02$, and in that of the control subjects from 71 to 69 g/l, $p<0.05$), and for albumin and alkaline phosphatase in the diabetic patients (median from 43 to 42 g/l, $p<0.05$, and from 80 to 73 U/l, $p<0.02$, respectively).

Discussion

This study shows that Type 1 diabetic men differ from healthy male control subjects for plasma and cellular fatty acid patterns and erythrocyte α-tocopherol levels. Furthermore, it describes the effects of short-term intake of a considerable amount of α-tocopherol enriched EPO on a variety of biochemical tests and on parameters for erythropoiesis, platelet function and diabetic control in both groups.

Fatty acids

Before EPO supplementation, the group of Type 1 diabetic patients had abnormal fatty acid patterns in plasma, erythrocytes and platelets (Table 2), consistent with those reported in the literature [7–9]. These abnormal profiles as well as the abnormal desaturase and chain-elongation product/precursor ratios (Table 5) may reflect diminished activity of the $\Delta 9$, $\Delta 6$ and $\Delta 5$ desaturase enzymes due to insulin deficiency, as has been shown in in vitro and in vivo studies in diabetic rats [11, 12, 23, 24]. The elevated 18:2c,ω6 content in all three compartments might be due to the impaired activity of $\Delta 6$ desaturase by hormonal derangement. However, the lower plasma 20:3c,ω9/20:4c,ω6 ratio, that is considered to be a reliable parameter of the essential fatty acid status under normal circumstances [22], but not in certain pathological conditions [25–27], points to a possibly higher dietary 18:2c,ω6 intake in the diabetic group. In contrast to others [8], we found a negative correlation between erythrocyte 18:2c,ω6 contents and glycosylated haemoglobin levels, for which we have no explanation.

Evening primrose oil contains a high amount of 18:2c,ω6 (72.40 mol/100 mol) and some 18:3c,ω6 (8.74 mol/100 mol). The latter is normally a quantitatively minor dietary constituent. Roughly estimated, the total dietary intake of 18:2c,ω6 was doubled by the quantity of the EPO supplementation, used in our study. Conceivably, the intake of this oil led to an increase of both fatty acids and total ω6 fatty acids, and to a fall in the 20:3c,ω9/20:4c,ω6 ratios. The level of 20:3c,ω6 increased in all compartments of both groups, suggesting partial metabolisation of the supplemented 18:3c,ω6 by chain-elongation, as has also been found in other studies in man [28–30]. However,

an increase of 20:4c,ω6 was only found in the plasma compartment of the control group. This finding, along with the decrease in the chain-elongation product/precursor ratio 20:3c,ω6/18:3c,ω6 and the Δ5 desaturase product/precursor ratio 20:4c,ω6/20:3c,ω6, found in all compartments in both diabetic patients and control subjects, points to the probable existence of regulatory mechanisms, that maintain tissue 20:4c, ω6 content constantly. This hypothesis is consistent with data from studies in animals with essential fatty acid deficiency [31], in man during different feeding regimens [32–34] and in several human pathological conditions [27, 35]. Apparently, the lowered 20:4c,ω6 level in plasma and erythrocytes in diabetic patients cannot be corrected by EPO administration. Likewise, supplementation by 6 g EPO daily during 8 weeks in Type 2 (non-insulin-dependent) diabetic patients, leading to an even larger increase of 20:3c,ω6 in erythrocytes and platelets than was seen in our study, did not augment the 20:4c,ω6 level in these cells [29]. The refractoriness of 20:4c,ω6 to oral supplementation with its ω6 precursors in diabetes mellitus has also been shown by an experiment in which daily intake of 1 g 20:3c,ω6 for 3 weeks enhanced 20:4c,ω6 content in erythrocytes from healthy volunteers, but not in those from Type 1 diabetic patients [36]. However, 20:4c,ω6 deficiency in tissue lipids of genetically diabetic mice was shown to be corrected by dietary EPO supplementation [37]. Therefore, it seems important to study the effect of long-term supplementation with 18:3c,ω6 in high doses, for which administration either as a crude oil with a complex fatty acid composition or in a highly purified form, may theoretically differ [38]. As 20:4c,ω6 only increased in the plasma compartment of the control group, it would also be interesting to determine the fatty acid compositions of the individual lipid fractions, such as phospholipids and cholesterylesters. It is noteworthy that improvement of diabetic control results in a 20:4c,ω6 increase in plasma [7, 39], erythrocytes [7, 39] and platelets [40]. Therefore, an attempt to correct low 20:4c,ω6 levels by administration of 18:3c,ω6 might only be useful for those patients in whom optimal diabetic control appears impossible.

EPO intake induced changes in fatty acid profiles which differ between erythrocytes and platelets. At one week after discontinuation of EPO use, most fatty acids in platelets were returning to their base-line values, whereas those in erythrocytes continued to alter. This difference is most probably explained by the higher turnover rate of platelets compared with erythrocytes (mean survival rate normally about 10 days and 120 days, respectively), and the relatively slow equilibration of about 4–6 weeks [32] between dietary fatty acids and erythrocytes fatty acids as effected by continuous exchange of lipids between plasma and erythrocytes [41]. Furthermore, the cell species differ in their fatty acid response, especially for 14:0, 16:0, total saturated fatty acids, 22:4c,ω6, 22:5c,ω3, and total ω3 fatty acids in the control group. Such a difference in response was not noted in human studies with long-term administration of cod-liver oil [42–44].

Except for 22:0 and 22:4c,ω6 in erythrocytes, the fatty acid compositions of the diabetic and the control group changed in the same direction, notwithstanding the fact that insulin deficiency causes profound disturbances in fatty acid metabolism in animals, such as a decline in *de novo* synthesis [23, 45] and desaturation of fatty acids [11, 12, 23, 24], an acceleration of their chain-elongation and the promotion of lipolysis and fatty acid oxidation [46].

It should be noted that blood samples were taken 1–3 h after breakfast. The data on the plasma fatty acid composition and serum lipids should therefore be interpreted with caution.

α-tocopherol

In the diabetic group, the α-tocopherol level in erythrocytes was found to be abnormally low (Fig. 1), whereas that in plasma appeared to be normal. A similar discrepancy has been shown between platelets and plasma [13]. The reason for the decreased intracellular α-tocopherol levels is unknown. Theoretically, two explanations are possible. The delivery of α-tocopherol from the plasma compartment to the cells might be disturbed and/or intracellular α-tocopherol consumption might be enhanced. It is notable that in plasma of diabetic patients free radical activity seems to be increased [47], while plasma α-tocopherol is protected against oxidation by protein binding [48].

It is tempting to speculate, that the lowered 22:6c, ω3 level in erythrocytes of the diabetic group, as also found in a previous study [7], may be caused by increased lipid peroxidation and/or hampered Δ4 desaturation due to an α-tocopherol deficit [49].

Erythropoiesis

The supplementation with EPO, which corresponded with an approximately 40-fold higher α-tocopherol intake than the estimated Dutch consumption [50], induced an increase in erythrocyte count, haemoglobin level, mean corpuscular haemoglobin (MHC) and mean corpuscular haemoglobin concentration (MCHC) (Table 6), suggesting an augmentation of erythrocyte production as well as haemoglobin synthesis. The assumption of enhanced erythropoiesis concurs with the finding of a decline in glycosylated haemoglobin percentage in both groups [51], which took place without apparent changes in other parameters of diabetic control (blood glucose levels in patients and control subjects, plasma fructosamine levels in patients; see Table 6).

It is a matter of speculation, which compound(s) in the oil induced these changes. MCH and MCHC appeared to be strongly correlated with erythrocyte α-

tocopherol content in the normal group (Fig. 2). One of the possible reasons for this relation could be the stimulatory function of vitamin E in the regulation of haem biosynthesis, presumably by controlling the mechanisms of induction and repression of δ-aminolevulinic acid synthetase [52]. The existence of an erythropoietic effect of α-tocopherol is, however, disputed [52, 53].

Platelet function

β-Thromboglobulin and platelet factor 4 are constituents of the platelet α-granule contents. Their concentrations in plasma are regarded as useful indicators of in vivo platelet activation and release reaction [54]. The plasma levels are influenced by dietary fatty acids [55–57]. EPO intake caused a decrease in the plasma levels of both compounds, which was most pronounced in the diabetic group. This result confirms the finding of another study in which a daily intake of 4.8 g EPO during 6 weeks by Type 1 diabetic patients also led to a decrease in β-thromboglobulin [56]. As herein no effects were seen on either prostaglandin E_1 or thromboxane B_2 generation by platelets, the observed change in platelet function is rather believed to result from alterations in membrane fatty acid composition, and therefore in membrane functioning, than from modifications in prostaglandin biosynthesis [56]. Some effect from α-tocopherol cannot be ruled out as vitamin E supplementation in Type 1 diabetic patients diminishes platelet aggregation in vitro [58].

This study proposes that Type 1 diabetic patients have abnormal fatty acid patterns, suggesting an impaired $\Delta 9$, $\Delta 6$ and $\Delta 5$ desaturation and an enhanced chain-elongation. When compared with healthy control subjects, they have lower α-tocopherol levels in erythrocytes, which warrants further investigation. A short-term high dose EPO intake increased 20:3c,ω6 in both diabetic patients and control subjects, but 20:4c,ω6 only in control subjects. This indicates a possible refractoriness of 20:4c,ω6 in diabetes mellitus to dietary intervention. EPO use lowered plasma levels of the platelet release parameters β-thromboglobulin and platelet factor 4, especially in the diabetic group.

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References

1. Kamada T, Yamashita T, Baba Y, Kai M, Setoyamo S, Chuman Y, Otsuji S (1986) Dietary sardine oil increases erythrocyte membrane fluidity in diabetic patients. Diabetes 35: 604–611
2. Schimke E, Hildebrandt R, Beitz J, Semmler S, Honigmann G, Mest H-J, Schliack V (1984) Influence of a cod liver oil diet in diabetics type I on fatty acid patterns and platelet aggregation. Biomed Biochim Acta 43: S351–S353
3. Fleischman AI, Bierenbaum ML, Stier A, Somol H, Watson PB (1976) In vivo platelet function in diabetes mellitus. Thromb Res 9: 467–471
4. Houtsmuller AJ, van Hal-Ferwerda J, Zahn KJ, Henkes HE (1981) Favorable influences of linoleic acid on the progression of diabetic micro- and macroangiopathy in adult onset diabetes mellitus. Progr Lipid Res 20: 377–386
5. Howard-Williams J, Patel P, Jelfs R, Carter RD, Awdry P, Bron A, Mann JI, Hockaday TDR (1985) Polyunsaturated fatty acids and diabetic retinopathy. Br J Ophthalmol 69: 15–18
6. Jamel GA, Carmichael H, Weir AI (1986) Gamma-linolenic acid in diabetic neuropathy. Lancet I: 1098 (Letter)
7. Van Doormaal JJ, Muskiet FAJ, van Ballegooie E, Sluiter WJ, Doorenbos H (1984) The plasma and erythrocyte fatty acid composition of poorly controlled, insulin-dependent (type I) diabetic patients and the effect of improved metabolic control. Clin Chim Acta 144: 203–212
8. Tilvis RS, Miettinen TA (1985) Fatty acid compositions of serum lipids, erythrocytes, and platelets in insulin-dependent diabetic women. J Clin Endocrinol Metab 61: 741–745
9. Jones DB, Carter RD, Mann JI (1986) Indirect evidence of impairment of platelet desaturase enzymes in diabetes mellitus. Horm Metabol Res 18: 341–344
10. Lepšanović J, Verešbaranji I, Lepšanović L, Ivković T, Daničić B, Kovač T (1984) Characteristics of the adipose tissue fatty acid composition in diabetes mellitus. Diab Croat 13: 97–109
11. Faas FH, Carter WJ (1980) Altered fatty acid desaturation and microsomal fatty acid composition in the streptozotocin diabetic rat. Lipids 15: 953–961
12. Poisson J-P (1985) Comparative in vivo and in vitro study of the influence of experimental diabetes on rat liver linoleic acid $\Delta 6$- and $\Delta 5$-desaturation. Enzyme 34: 1–14
13. Karpen CW, Cataland S, O'Dorisio TM, Panganamala RV (1985) Production of 12-hydroxyeicosatetraenoic acid and vitamin E status in platelets from type I human diabetic subjects. Diabetes 34: 526–531
14. Van der Lelie J, von dem Borne AEGKr (1985) The clinical significance of platelet volume analysis. Neth J Med 28: 457–462
15. Muskiet FAJ, van Doormaal JJ, Martini IA, Wolthers BG, van der Slik W (1983) Capillary gas chromatographic profiling of total long chain fatty acids and cholesterol in biological materials. J Chromatogr Biomed Appl 278: 231–244
16. Nilsson B, Johansson B, Jansson L, Holmberg L (1978) Determination of plasma alpha-tocopherol by high-performance liquid chromatography. J Chromatogr 145: 169–172
17. Chiu D, Lubin B (1979) Abnormal vitamin E and glutathione peroxidase levels in sickle cell anemia. Evidence for increased susceptibility to lipid peroxidation in vivo. J Lab Clin Med 94: 542–548
18. Stump DD, Roth Jr EF, Gilbert HS (1984) Simultaneous determination by high-performance liquid chromatography of tocopherol isomers, α-tocopheryl quinone, and cholesterol in erythrocytes and plasma. J Chromatogr Biomed Appl 306: 371–376
19. Johnson RN, Metcalf PA, Baker JR (1982) Fructosamine, a new approach to the estimation of serum glycosylprotein. An index of diabetic control. Clin Chim Acta 127: 87–95
20. Flückiger R, Winterhalter KH (1976) In vitro synthesis of hemoglobin A_{1C}. FEBS Lett 71: 356–360
21. Mandel J (1964) The statistical analysis of experimental data. Interscience Publishers, New York, pp 272–312
22. Holman RT (1978) How essential are essential fatty acids? J Am Oil Chem Soc 55: 774A–781A
23. Worcester NA, Bruckdorfer KR, Hallinan T, Wilkins AJ, Mann JA (1979) The influence of diet and diabetes on stearoyl coenzyme A desaturase (EC 1.14.99.5) activity and fatty acid composition in rat tissues. Br J Nutr 41: 239–252
24. Poisson J-P, Blond J-P (1985) Effect of streptozotocin diabetes upon the in vivo conversion of 14C γ-linolenic and dihomo-γ-linolenic acids into arachidonic acid in kidneys and whole rat. Diab Metab 11: 289–294
25. Lundberg WO (1980) The significance of cis, cis, cis 5,8,11 eicos-

atrienoic acid in essential fatty acid deficiency. Nutr Rev 38: 233–235
26. Johnson SB, Gordon E, McClain C, Low G, Holman RT (1985) Abnormal polyunsaturated fatty acid patterns of serum lipids in alcoholism and cirrhosis: arachidonic acid deficiency in cirrhosis. Proc Natl Acad Sci USA 82: 1815–1818
27. Van Doormaal JJ, Muskiet FAJ, Martini IA, Doorenbos H (1986) Changes in fatty acid profiles of plasma, erythrocytes and polymorphonuclear leukocytes in induced hypothyroidism in man: indirect evidence for altered $\Delta 6$ desaturase activity. Clin Chim Acta 156: 299–314
28. Darcet Ph, Driss F, Mendy F, Delhaye N (1980) Exploration du métabolisme des acides gras des lipides totaux plasmatiques et de l'agrégation plaquettaire d'une population d'hommes agés à l'aide d'une alimentation enrichie en acide γ linolénique. Ann Nutr Alim 34: 277–290
29. Peterson DB, Fisher K, Carter RD, Gerring S, Mann JI (1986) Changes in dietary fatty acids favourably influence platelet composition in Type 2 (non-insulin-dependent) diabetes. Diabetologia 29: 582A (Abstract)
30. Boberg M, Vessby B, Selinus I (1986) Effects of dietary supplementation with n-6 and n-3 long-chain polyunsaturated fatty acids on serum lipoproteins and platelet function in hypertriglyceridaemic patients. Acta Med Scand 220: 153–160
31. Lefkowith JB, Flippo V, Sprecher M, Needleman P (1985) Paradoxical conservation of cardiac and renal arachidonate content in essential fatty acid deficiency. J Biol Chem 260: 15736–15744
32. Farquhar JW, Ahrens Jr EH (1963) Effects of dietary fats on human erythrocytes fatty acid patterns. J Clin Invest 5: 675–685
33. Wene JD, Connor WE, DenBesten L (1975) The development of essential fatty acid deficiency in healthy men fed fat-free diets intravenously and orally. J Clin Invest 56: 127–134
34. Van Doormaal JJ, Muskiet FAJ, Martini IA, Doorenbos H (1987) Rapid changes in serum, plasma and erythrocyte lipid compositions and serum transaminase levels during continuous enteral hyperalimentation by carbohydrates alone. Metabolism 36: 1132–1140
35. Muskiet MD, Muskiet FAJ (1984) Lipids, fatty acids and trace elements in plasma and erythrocytes of pediatric patients with homozygous sickle cell disease. Clin Chim Acta 142: 1–10
36. Mikhailidis DP, Kirtland SJ, Barradas MA, Mahadeviah S, Dandona P (1986) The effect of dihomogammalinolenic acid on platelet aggregation and prostaglandin release, erythrocyte membrane fatty acids and serum lipids: evidence for defects in PGE_1 synthesis and Δ^5-desaturase activity in insulin-dependent diabetics. Diabetes Res 3: 7–12
37. Cunnane SC, Manku MS, Horrobin DF (1985) Abnormal essential fatty acid composition of tissue lipids in genetically diabetic mice is partially corrected by dietary linoleic and γ-linolenic acids. Br J Nutr 53: 449–458
38. Brenner RR (1981) Nutritional and hormonal factors influencing desaturation of essential fatty acid. Progr Lipid Res 20: 41–47
39. Tilvis RS, Helve E, Miettinen TA (1986) Improvement of diabetic control by continuous subcutaneous insulin infusion therapy changes fatty acid composition of serum lipids and erythrocytes in Type 1 (insulin-dependent) diabetes. Diabetologia 29: 690–694
40. Jones DB, Carter RD, Mann JI (1986) Increased arachidonic acid level in diabetic platelets following improvement in diabetic control. Diab Metab 12: 65–67
41. Shohet SB (1972) Hemolysis and changes in erythrocyte membrane lipids (first of two parts). N Engl J Med 286: 577–583
42. Sanders TAB, Vickers M, Haines AP (1981) Effect on blood lipids and haemostasis of a supplement of cod-liver oil, rich in eicosapentaenoic and docosahexaenoic acids, in healthy young men. Clin Sci 61: 317–324
43. Lorenz R, Spengler U, Fischer S, Duhm J, Weber PC (1983) Platelet function, thromboxane formation and blood pressure control during supplementation of the western diet with cod liver oil. Circulation 67: 504–511
44. Von Schacky C, Fischer S, Weber PC (1985) Long-term effects of dietary marine ω-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J Clin Invest 76: 1626–1631
45. Tanaka Y, Ando S (1981) Increased stearate formation by chain elongation in streptozotocin diabetic mice: compensation for decreased de novo fatty acid synthesis. Biomed Res 2: 404–412
46. Chorcáthová V, Ondreicka R, Ozdin L, Dzurik R (1981) Metabolism of palmitate-1-^{14}C in the tissues of rats with streptozotocin diabetes. Physiol Bohemoslov 30: 259–266
47. Wolff SP (1987) The potential role of oxidative stress in diabetes and its complications: implications for theory and therapy. In: Crabbe MJC (ed) Diabetic complications. Scientific and clinical aspects. Churchill Livingstone, Edinburgh London Melbourne New York, pp 167–220
48. Baitella-Eberle G, Tuchschmid P, Duc G (1986) Vitamin E is exposed to oxidation in red cell membranes but is protected during transport in plasma. Pediatr Res 20: 1048 (Abstract)
49. Infante JP (1986) Vitamin E and selenium participation in fatty acid desaturation. A proposal for an enzymatic function of these nutrients. Mol Cell Biochem 69: 93–108
50. Den Hartog C, Hautvast JGAJ, Den Hartog AP (1980) Nieuwe Voedingsleer. Het Spectrum BV, Utrecht, p 204
51. Fitzgibbons JF, Koler RD, Jones RT (1976) Red cell age-related changes of haemoglobins A_{Ia+b} and A_{Ic} in normal and diabetic subjects. J Clin Invest 58: 820–824
52. McCay PB, King MM (1980) Vitamin E: its role as a biological free radical scavenger and its relationship to the microsomal mixed-function oxidase system. In: Machlin LJ (ed) Vitamin E. A comprehensive treatise. Dekker, New York, pp 289–317
53. Horwitt MK (1980) Interpretation of human requirements for vitamin E. In: Machlin LJ (ed) Vitamin E. A comprehensive treatise. Dekker, New York, pp 621–636
54. Kaplan KL, Owen J (1981) Plasma levels of β-thromboglobulin and platelet factor 4 as indices of platelet activation in vivo. Blood 57: 199–202
55. Jakubowski JA, Ardlie NG, Morgan FJ, Chesterman CN (1982) Further observations on the effects of dietary fatty acid composition on platelet reactivity and blood coagulation in man and the influence of methodology on findings. Atherosclerosis 41: 285–294
56. Chaintreuil J, Monnier L, Colette C, Crastes de Paulet P, Orsetti A, Spielmann D, Mendy F, Crastes de Paulet A (1984) Effects of dietary γ-linolenate supplementation on serum lipids and platelet function in insulin-dependent diabetic patients. Hum Nutr Clin Nutr 38C: 121–130
57. Hay CRM, Durber AP, Saynor R (1982) Effect of fish oil on platelet kinetics in patients with ischaemic heart disease. Lancet I: 1269–1272
58. Monnier LH, Colette C, Perdriset G, Cartry E, Blotman MJ (1986) Effects of high doses of vitamin E on platelet function and lipid metabolism of Type 1 (insulin-dependent) diabetic patients. Diabetologia 29: 573A (Abstract)

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