Zeitschrift für
Ernährungswissenschaft Z Ernährungswiss 33:146–158 (1994)

The effects of dietary oils on the fatty acid composition and osmotic fragility of rat erythrocytes

M. Kirchgeßner, G.I. Stangl, A.M. Reichlmayr-Lais and K. Eder

Institut für Ernährungsphysiologie der Technischen Universität München, Freising-Weihenstephan

Wirkung verschiedener Diätöle auf die Fettsäurezusammensetzung und Hämolyseresistenz von Rattenerythrozyten

Summary: The present study was carried out to investigate the effect of different dietary oils representing special fatty acids which varied in chain length, position and number of double bonds on fatty acid composition of erythrocyte membranes and on the osmotic fragility of rat erythrocytes after incubation in NaCl solutions of different concentrations. For this purpose all animals were initially fed a control diet (CO) containing 10 % coconut oil and 0.4 % safflower oil for 28 days. After that 10 groups of 10 animals each were switched to test diets for another 20 days in which 50 % or 100 % of the coconut oil was exchanged for one of the following oils: olive oil (OO 5, OO 10), safflower oil (SFO 5, SFO 10), evening primrose oil (EPO 5, EPO 10), linseed oil (LO 5, LO 10) or salmon oil (SLO 5, SLO 10).

The results show that the fatty acid composition of rat erythrocyte membranes was affected by the fatty acid composition of the dietary fats. Rats fed OO 10, EPO 10, LO 5 and LO 10 had a slightly lower concentration of saturated fatty acids (SFA) in erythrocyte membranes than control rats. Groups fed olive oil showed the highest level of monounsaturated fatty acids (MUFA) in the erythrocyte membrane. This increase in MUFA at the expense of SFA and (n-6) polyunsaturated fatty acids (PUFA) was most pronounced with respect to 18 : 1 and occurred in a dose-dependent fashion. Rats fed SFO, EPO or LO had higher linoleic acid levels in the erythrocyte membrane than control rats. This increase in 18 : 2 (n-6) was mainly at the expense of 18 : 1 and occurred in a dose-dependent fashion. The proportion of 20 : 4 (n-6) did not remarkably change feeding diets with (n-6) PUFA-rich oils. The (n-3) PUFA concentration in the erythrocyte membranes considerably increased, whereas (n-6) PUFA decreased feeding linseed oil or salmon oil rich in (n-3) PUFA. Linseed oil and salmon oil caused similar changes in the membrane, which were more pronounced in rats fed salmon oil than in rats fed linseed oil.

The osmotic fragility of erythrocytes was also influenced by dietary oil, respectively fatty acid pattern of the erythrocytes. In almost all NaCl solutions erythrocytes from rats fed the dietary oils were less resistant to hemolysis than those from control rats. These changes became statistically apparent feeding EPO 5, LO 5, LO 10 and SLO 5.

Zusammenfassung: In der vorliegenden Studie sollte die Wirkung verschiedener Diätöle auf die Fettsäurezusammensetzung der Erythrozytenmembranen und die Hämolyseresistenz von Rattenerythrozyten untersucht werden. Die ausgewählten Diätöle zeichneten sich durch hohe Gehalte an bestimmten Fettsäuren aus, die sich in Zahl und Stellung ihrer Doppelbindungen unterschieden. Dazu erhielten zunächst männliche Sprague-Dawley Ratten eine Kontrolldiät mit 10 % Kokosfett und 0,4 % Distelöl über 28 Tage. Im Anschluß daran wurde bei 10 Gruppen die Diät umgestellt und 50 bzw. 100 % des Kokosfettes durch Olivenöl, Distelöl, Nachtkerzenöl, Leinöl oder Lachsöl ersetzt und weitere 20 Tage an die Tiere verabreicht.

Die Fettsäurezusammensetzung der Erythrozytenmembranen von Ratten wurde durch den Einsatz von Diätölen verschiedener Fettsäurezusammensetzung beeinflußt. Ratten, die OO 10, EPO 10, LO 5 und LO 10 erhielten, wiesen geringfügig niedrigere Konzentrationen an gesättigten Fettsäuren (SFA)

in der Erythrozytenmembran auf als Kontrollratten. Tiere mit Olivenöldiät zeigten den höchsten Anteil an Monoenfettsäuren (MUFA) in der Erythrozytenmembran. Dabei stiegen die MUFA abhängig von der eingesetzten Olivenölkonzentration vornehmlich auf Kosten der SFA und (n-6) PUFA. Ratten, deren Diäten SFO, EPO oder LO enthielten, zeigten eine höhere Konzentration an 18:2 (n-6) in der Erythrozytenmembran als Kontrollratten. Dabei verdrängte 18:2 (n-6), abhängig von der eingesetzten Ölmenge, die 18:1 in der Membran. Die Konzentration der 20:4 (n-6) blieb bei Einsatz der (n-6) PUFA-reichen Öle nahezu unverändert. Bei Zulage von (n-3) PUFA-reichem Lein- oder Lachsöl stieg die Konzentration der (n-3) PUFA in der Membran beträchtlich an, während die Konzentration der (n-6) PUFA abfiel. Die Fettsäurezusammensetzung in der Membran veränderte sich bei Einsatz von Lein- oder Lachsöl in ähnlicher Weise, wobei sich der Einfluß von Lachsöl als stärker erwies.

Die Hämolyseresistenz der Erythrozyten wurde ebenfalls durch die Diätöle, beziehungsweise durch das Fettsäuremuster der Erythrozyten, beeinflußt. In nahezu allen NaCl-Lösungen war die Hämolyserate der Erythrozyten von Ratten, die die Testöle erhielten, höher als die von Kontrollratten. Die Hämolyseresistenz wurde jedoch nur bei Einsatz von EPO 5, LO 5, LO 10 und SLO 5 statistisch signifikant verändert.

Key words: Dietary oils – fatty acids – osmotic fragility – erythrocytes – rat

Schlüsselwörter: Diätöle – Fettsäuren – Hämolyseresistenz – Erythrozyten – Ratte

Introduction

The fatty acid composition of erythrocytes can be modified by diet in both animals and humans, because the mature erythrocyte lacks the ability to synthetize fatty acids (21, 23). Mechanical, physical and functional properties of the erythrocyte membrane are largely determined by their fatty acid pattern. Also, membrane proteins, e.g., receptors, enzymes, and ion channels are highly sensitive to lipid environment (30, 31), moreover, membrane parameters such as shape, deformability, permeability, fluidity and osmotic fragility can be affected by alterations in fatty acyl groups (5, 14, 23).

Although compensatory alterations occur to minimize changes in erythrocyte fluidity (1), an altered fatty acid composition can modify the morphology of erythrocytes and their ability to resist hemolysis (12, 23, 27, 32).

The present study was carried out to collect data about the changes of the fatty acid composition of erythrocytes in rats fed different dietary oils. This leads also to the question of how osmotic fragility may be directly altered by the modified fatty acid composition of erythrocytes. For this purpose rats were fed olive oil rich in MUFA, safflower oil and evening primrose oil rich in (n-6) PUFA, and linseed oil as well as salmon oil, rich in (n-3) PUFA. This design also allows to examine the effect of γ-linolenic acid by comparing the effect of safflower oil with that of evening primrose oil. In addition, the effects of α-linolenic acid and long-chain (n-3) PUFA on fatty acid pattern and osmotic fragility of erythrocytes can be compared by feeding linseed oil and salmon oil.

Materials and methods

Animals and diets

110 male Sprague-Dawley rats (SAVO GmbH, Kisslegg, FRG) weighing 66 ± 4 g (mean ± SD) were initially fed a semipurified control diet (CO), containing 10 % coconut oil and 0.4 % safflower oil for 28 days, to supply essential fatty acids. After 28 days 10 groups of 10 animals each were fed one of the 10 semipurified test diets for 20 days containing 5 % (w/w) or 10 % (w/w) olive oil (OO 5, OO 10), safflower oil (SFO 5,

SFO 10), evening primrose oil (EPO 5, EPO 10), linseed oil (LO 5, LO 10) or salmon oil (SLO 5, SLO 10) for exchange of coconut oil. The control group was fed the coconut oil diet (CO) for another 20 days. The composition of the diets is given in Table 1 and their fatty acid composition in Table 2. The different oils were selected on the basis of fatty acid chain length as well as number and position of double bonds. Olive oil (Henry Lamotte, Bremen, FRG) was comprised largely (72 %) of oleic acid (18 : 1). Safflower oil (Maurel, Hanweiler, FRG) contained 78 % linoleic acid (18 : 2 n-6) and evening primrose oil additional 9 % γ-linolenic acid (18 : 3 n-6); linseed oil (Wasserfuhr, Bonn, FRG) consisted of 56 % α-linolenic acids (18 : 3 n-3) and salmon oil (Caesar & Loretz, Hilden, FRG) of 30 % long chain (n-3) polyunsaturated acids, namely, eicosapentaenoic acid (20 : 5 n-3), docosapentaenoic acid (22 : 5 n-3) and docosahexaenoic acid (22 : 6 n-3). The last two oils represented oils rich in (n-3) polyunsaturated fatty acids. The other components of the diet were kept constant.

All diets were fortified with recommended amounts of vitamins and minerals. The diets were stabilized with 0.01 % (of the triglyceride content) BHT (butylated hydroxytoluene) as antioxidant. Oils and base mixtures were stored at 4 °C until preparation of the diets. All fish oil diets were prepared weekly to minimize oxidative deterioration.

The rats were housed in Macrolon cages with 2 rats to a cage in a room maintained at 23 °C with a humidity of 60 % and were given free access to food and water. All animals were kept under conditions of controlled lighting with alternative dark (1800–0600 h) and light (0600–1800 h) cycles. At the end of the experimental period of 48 d, rats were food-deprived for 12 h, anesthesized with diethyl ether and killed by decapitation. Blood was collected from neck into heparinized tubes for determination of the fatty acid composition and osmotic fragility of the erythrocytes.

Table 1. Ingredients of the fat-modified experimental diets

Ingredient	Amount (%)		
	Control diet	test diet with 5 % oil	test diet with 10 % oil
Casein, fat-free	20	20	20
Corn starch	30.4	30.4	30.4
Sucrose	30	30	30
Coconut oil	10	5	–
Safflower oil	0.4	0.4	0.4
Test lipid[3]	–	5	10
Fiber (cellulose)	3	3	3
Vitamin mixture[1]	2	2	2
Mineral mixture[2]	4	4	4
DL-methionine	0.2	0.2	0.2

[1] Vitamins per kg diet: 5000 IU all-trans-retinol; 300 IU cholecalciferol; 300 mg all-rac-α-tocopherol; 5 mg menadione sodium bisulfite; 5 mg thiamine · HCl; 10 mg riboflavin; 6 mg pyridoxine · HCl; 50 mg Ca pantothenate; 20 mg nicotinic acid; 1000 mg choline chlorid; 0.2 mg folic acid; 0.025 mg cyanocobalamin; corn starch and sucrose ad 20 g

[2] Minerals per kg diet: $Na_2HPO_4 \cdot 2H_2O$ 8.80 g; KH_2PO_4 8.20 g, KCl 6.00 g; $MgCl_2 \cdot 6H_2O$ 3.40 g; $CaCO_3$ 13.6 g; $FeSO_4 \cdot 7H_2O$ 248.8 mg; $ZnSO_4 \cdot 7H_2O$ 219.9 mg; $CuSO_4 \cdot 7H_2O$ 47.2 mg; $MnSO_4 \cdot 5H_2O$ 123.1 mg; KJ 9.0 mg; $NiSO_4 \cdot 6H_2O$ 4.48 mg; $Na_2MoO_4 \cdot 2H_2O$ 0.50 mg; $SnCl_2 \cdot 2H_2O$ 0.57 mg; $Na_2SeO_3 \cdot 5H_2O$ 0.67 mg; $CrCl_3 \cdot 6H_2O$ 0.51 mg; NH_4VO_3 0.23 mg; $Na_2SiO_3 \cdot 5H_2O$ 1.51 mg

[3] Test lipid: coconut oil, olive oil, safflower oil, evening primrose oil, linseed oil, salmon oil, stabilized with 0.01 % BHT of the oil concentration

Table 2. Fatty acid composition of the experimental diets

Fatty acid	CO[1]	OO 5	OO 10	SFO 5	SFO 10	EPO 5	EPO 10	LO 5	LO 10	SLO 5	SLO 10
	g/100 g fatty acid methyl esters										
SFA											
6:0	0.8	0.4	–[2]	0.3	–	0.4	–	0.4	–	0.5	–
8:0	7.4	3.6	–	3.2	–	3.4	–	3.3	–	5.1	–
10:0	5.3	2.6	–	2.5	–	2.6	–	2.6	–	3.5	–
12:0	41.6	20.3	–	20.1	–	20.4	–	20.8	–	26.8	0.5
14:0	16.9	8.4	–	8.5	–	8.6	–	8.6	–	10.8	6.9
16:0	9.6	10.8	11.8	8.4	7.0	8.2	6.6	7.5	5.4	11.6	17.7
18:0	4.3	3.7	3.0	3.6	2.7	3.0	1.8	3.9	3.5	3.3	2.7
20:0	0.1	0.3	0.4	0.2	0.3	0.2	0.3	0.1	–	–	–
MUFA											
14:1	–	–	–	–	–	–	–	–	–	0.2	0.5
16:1	–	0.5	1.0	–	0.1	–	–	–	–	4.5	8.8
18:1	8.0	38.8	68.7	11.0	12.9	9.8	11.7	13.6	19.1	11.6	20.0
n-6 PUFA											
18:2	5.9	9.8	13.5	41.8	76.3	38.8	70.6	11.9	17.8	5.4	7.3
18:3	–	–	–	–	–	4.3	8.4	0.1	0.2	0.1	0.2
20:2	–	0.3	0.5	–	–	–	–	–	–	–	–
20:4	–	–	–	–	–	–	–	–	–	1.8	3.7
n-3 PUFA											
18:3	–	0.7	1.1	0.4	0.7	0.4	0.6	27.1	54.0	3.6	6.7
20:5	–	–	–	–	–	–	–	–	–	6.0	13.5
22:5	–	–	–	–	–	–	–	–	–	1.5	3.1
22:6	–	–	–	–	–	–	–	–	–	3.9	8.6

[1] Abbreviations used for the diets: CO: diet containing 10 % coconut oil; OO 5: diet containing 5 % olive oil; OO 10: diet containing 10 % olive oil; SFO 5: diet containing 5 % safflower oil; SFO 10: diet containing 10 % safflower oil; EPO 5: diet containing 5 % evening primrose oil; EPO 10: diet containing 10 % evening primrose oil; LO 5: diet containing 5 % linseed oil; LO 10: diet containing 10 % linseed oil; SLO 5: diet containing 5 % salmon oil; SLO 10: diet containing 10 % salmon oil

[2] – fatty acid not detectable

Analytical methods

Erythrocyte membranes were prepared by the method of Hanahan and Ekholm (8). Erythrocytes were washed three times with isotonic saline and hemolyzed by adding distilled water. The erythrocyte membranes were washed three times with Tris buffer (ph 7.6). Erythrocyte membrane lipids were extracted 4 h with isopropanol according to the method of Eder et al. (4). For that 40 ml isopropanol (with 10 ml/l BHT as antioxidant) were added to 1 g membranes. After 4 h the lipid extracts were filtered and the lipids were dried by evaporating the solvent. Fatty acids of lipids were converted to methyl esters by transesterification with boron trifluoride in methanol as described by Morrison and Smith (17). Fatty acid methyl esters were separated and quantified using a capillary column gas chromatographic system (SICHROMAT 2–8, Siemens, Karlsruhe, FRG) fitted with a programmed temperature vaporizer (PTV), a CP-Sil 88 WCOT fused silica column (50 m * 0.25 mm I.D., film thickness 0.2 µm, Chrompack, Middleburg, The Netherlands), a flame ionization detector (FID) and an integrator

(D-2500, Merck-Hitachi, Darmstadt, FRG). Conditions of chromatographic separation were as described by Eder et al. (3). Fatty acid methyl esters were identified by comparison with individual purified standards and quantified using heptadecanoic acid methyl ester as internal standard.

Osmotic fragility of erythrocytes was determined according to the method of O'Dell et al. (20). For every assay 25 µl blood were added to 2.5 ml of 0.00 %, 0.30 %, 0.32 %, 0.34 %, 0.36 %, 0.38 %, 0.40 %, 0.42 %, 0.44 %, 0.46 %, 0.48 % and 0.85 % sodium chloride solutions (in 5 mM phosphate buffer, pH 7.4). The release of hemoglobin was used to assess fragility of erythrocytes. The suspensions were incubated at room temperature for 15 min, centrifugated at 500 g for 10 min. Absorbance of the supernates was measured at 540 nm. Degree of hemolysis in a 0.0 % NaCl solution was defined 100 %, degree of hemolysis in a 0.85 % NaCl solution was defined 0 %.

Statistical analysis

All data presented are mean values ± SD. The effect of dietary fat supplements was analyzed by a two-way analysis of variance (ANOVA). Comparisons between mean values were made using the Student-Newman-Keul's test (11). Statistically different means ($p < 0.05$) are marked with different superscript letters.

Results

Food intake and weight gain

All groups showed normal growth. The daily mean food intake within the first 28 days was 17 ± 2 g and the total body-weight gain was 224 ± 19 g. From d 29 until 48 control rats fed CO diet had the highest daily food intake (Table 3). Rats fed EPO 10 or SLO 10 had a significantly lower food intake than control rats, which is perhaps caused by off-

Table 3. Daily food intake (g) and body weight gain (g) from rats fed diets containing 5 % and 10 % different oils from day 29 until 48[1), 2)]

Parameter	Amount of test oil in the diet	CO[3)]	OO	SFO	EPO	LO	SLO
Food intake[4)]	5 %	–	22.0 ± 1.0^{abc}	23.6 ± 1.0^{bc}	22.8 ± 2.8^{abc}	21.2 ± 2.2^{abc}	21.8 ± 1.1^{abc}
	10 %	24.2 ± 1.0^{c}	22.1 ± 1.9^{abc}	22.1 ± 1.4^{abc}	20.1 ± 1.2^{a}	21.2 ± 1.8^{abc}	20.6 ± 1.5^{ab}
Body weight gain[5)]	5 %	–	112.0 ± 15.8^{abc}	124.6 ± 10.3^{c}	125.3 ± 20.7^{c}	108.3 ± 15.8^{abc}	119.6 ± 11.1^{bc}
	10 %	119.5 ± 8.6^{bc}	113.4 ± 13.6^{abc}	119.9 ± 11.5^{bc}	98.5 ± 12.5^{a}	115.2 ± 16.9^{abc}	102.7 ± 18.4^{ab}

1) Data are means ± SD; n = 10 for all groups

2) Means were compared by Student-Newman-Keuls test; means not sharing the same superscript letter differ significantly ($p < 0.05$)

3) Abbreviations used for the diets: CO, coconut oil diet; OO, olive oil diet; SFO, safflower oil diet; EPO, evening primrose oil diet; LO, linseed oil diet; SLO, salmon oil diet
Significance of factors:

4) Type of dietary oil: N.S., amount of test oil: $p < 0.05$

5) Type of dietary oil: N.S., amount of test oil: $p < 0.01$
N.S. = not significant

Table 4. Proportions of saturated fatty acids in the erythrocyte membranes (%, by weight) of rats receiving diets with different oils[1), 2)]

Fatty acid	Amount of test oil in the diet	CO[3)]	OO	SFO	EPO	LO	SLO
		g/100 g fatty acid methyl esters					
SFA							
12:0[4)]	5 %	–	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$
	10 %	0.3 ± 0.1^{b}	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$
14:0[5)]	5 %	–	1.1 ± 0.4^{d}	0.8 ± 0.3^{bc}	1.0 ± 0.2^{cd}	0.6 ± 0.2^{ab}	0.8 ± 0.1^{bc}
	10 %	1.8 ± 0.6^{e}	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.6 ± 0.1^{ab}
16:0[6)]	5 %	–	28.3 ± 0.7^{ab}	28.1 ± 0.8^{ab}	28.9 ± 0.8^{ab}	28.6 ± 1.7^{ab}	32.2 ± 1.4^{c}
	10 %	27.5 ± 1.0^{ab}	28.1 ± 0.9^{ab}	29.3 ± 1.7^{b}	28.6 ± 1.6^{ab}	27.2 ± 0.9^{a}	33.0 ± 2.6^{c}
18:0[7)]	5 %	–	14.1 ± 0.7^{cd}	14.9 ± 1.0^{de}	14.3 ± 0.6^{cd}	13.2 ± 0.6^{bc}	10.6 ± 0.9^{a}
	10 %	14.8 ± 0.6^{de}	13.0 ± 1.2^{b}	15.5 ± 0.8^{e}	13.4 ± 1.3^{bc}	13.4 ± 0.8^{bc}	11.3 ± 1.0^{a}
22:0[8)]	5 %	–	0.6 ± 0.1^{bc}	0.4 ± 0.1^{ab}	0.7 ± 0.2^{cd}	0.4 ± 0.2^{abc}	0.7 ± 0.3^{cd}
	10 %	0.9 ± 0.3^{de}	0.6 ± 0.1^{cd}	0.3 ± 0.0^{a}	0.4 ± 0.2^{ab}	1.0 ± 0.4^{e}	0.3 ± 0.1^{a}
24:0[9)]	5 %	–	1.4 ± 0.3^{a}	2.1 ± 0.4^{bc}	1.8 ± 0.3^{abc}	2.1 ± 0.4^{bc}	2.2 ± 0.5^{c}
	10 %	1.9 ± 0.2^{bc}	1.4 ± 0.2^{a}	1.7 ± 0.3^{ab}	2.2 ± 0.4^{bc}	2.8 ± 0.6^{d}	1.9 ± 0.4^{bc}
Total[10)]	5 %	–	45.5 ± 1.0^{bcd}	46.2 ± 1.1^{bcd}	46.7 ± 0.7^{cd}	45.0 ± 2.2^{abc}	46.6 ± 1.4^{cd}
	10 %	47.1 ± 0.7^{d}	43.6 ± 1.3^{a}	47.2 ± 1.5^{d}	45.0 ± 1.2^{abc}	44.7 ± 1.0^{ab}	47.1 ± 2.2^{d}

1) Data are means ± SD; n = 10 for all groups

2) Means were compared by Student-Newman-Keuls test; means not sharing the same superscript letter differ significantly ($p < 0.05$)

3) Abbreviations used for the diets: CO: diet containing 10 % coconut oil; OO 5: diet containing 5 % olive oil; OO 10: diet containing 10 % olive oil; SFO 5: diet containing 5 % safflower oil; SFO 10: diet containing 10 % safflower oil; EPO 5: diet containing 5 % evening primrose oil; EPO 10: diet containing 10 % evening primrose oil; LO 5: diet containing 5 % linseed oil; LO 10: diet containing 10 % linseed oil; SLO 5: diet containing 5 % salmon oil; SLO 10: diet containing 10 % salmon oil
Significance of factors:

4) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.01$

5) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.01$

6) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.

7) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.

8) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.

9) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.

10) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.

N.S. = not significant

flavoring components in the dietary oils. The lower food intake consequently resulted in reduced body-weight gains compared to CO group, but reached only significance in rats fed EPO 10 diet.

Fatty acid composition of erythrocyte membranes

The lipids of the rat erythrocyte membrane were remarkable for its high levels of palmitic acid (16 : 0), stearic acid (18 : 0), oleic acid (18 : 1) and arachidonic acid (20 : 4 n-6). The erythrocyte membrane showed only small concentrations of 12 : 0 and 14 : 0, even in rats fed coconut oil, which contained a lot of these fatty acids. Erythrocyte membranes from rats had SFA concentrations between 43 % and 47 % (Table 4). Rats

Table 5. Proportions of monounsaturated fatty acids in the erythrocyte membranes (%, by weight) of rats receiving diets with different oils[1), 2)]

Fatty acid	Amount of test oil in the diet	CO[3)]	OO	SFO	EPO	LO	SLO
			g/100 g fatty acid methyl esters				
MUFA							
14:1[4)]	5 %	–	0.3 ± 0.1^{a}	0.8 ± 0.2^{b}	0.4 ± 0.1^{a}	1.1 ± 0.3^{c}	0.4 ± 0.0^{a}
	10 %	0.7 ± 0.2^{b}	0.3 ± 0.1^{a}	0.8 ± 0.3^{b}	1.1 ± 0.2^{c}	0.8 ± 0.3^{b}	0.4 ± 0.1^{a}
16:1[5)]	5 %	–	1.0 ± 0.2^{b}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}	1.0 ± 0.2^{b}	1.5 ± 0.2^{c}
	10 %	1.0 ± 0.2^{b}	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}	0.7 ± 0.1^{a}	0.8 ± 0.2^{a}	1.5 ± 0.2^{c}
18:1[6)]	5 %	–	17.9 ± 1.6^{e}	12.8 ± 1.4^{a}	12.9 ± 1.2^{a}	15.0 ± 1.1^{bc}	16.3 ± 1.8^{cd}
	10 %	14.4 ± 1.2^{b}	19.8 ± 1.6^{f}	11.5 ± 1.1^{a}	12.9 ± 1.1^{a}	15.2 ± 0.6^{bc}	16.8 ± 1.1^{de}
24:1[7)]	5 %	–	2.1 ± 0.3^{d}	0.9 ± 0.1^{a}	3.0 ± 0.3^{e}	1.1 ± 0.2^{ab}	1.7 ± 0.2^{c}
	10 %	1.7 ± 0.2^{c}	2.1 ± 0.3^{d}	1.4 ± 0.2^{b}	1.1 ± 0.2^{ab}	1.3 ± 0.2^{b}	1.9 ± 0.4^{cd}
Total[8)]	5 %	–	21.3 ± 1.6^{f}	15.2 ± 1.6^{ab}	17.0 ± 1.3^{cd}	18.2 ± 1.2^{d}	19.8 ± 1.8^{e}
	10 %	17.9 ± 1.2^{d}	22.9 ± 1.6^{g}	14.3 ± 1.1^{a}	15.8 ± 1.4^{bc}	18.1 ± 0.8^{d}	20.5 ± 1.3^{ef}

1) Data are means ± SD; n = 10 for all groups
2) Means were compared by Student-Newman-Keuls test; means not sharing the same superscript letter differ significantly ($p < 0.05$)
3) Abbreviations used for the diets: CO: diet containing 10 % coconut oil; OO 5: diet containing 5 % olive oil; OO 10: diet containing 10 % olive oil; SFO 5: diet containing 5 % safflower oil; SFO 10: diet containing 10 % safflower oil; EPO 5: diet containing 5 % evening primrose oil; EPO 10: diet containing 10 % evening primrose oil; LO 5: diet containing 5 % linseed oil; LO 10: diet containing 10 % linseed oil; SLO 5: diet containing 5 % salmon oil; SLO 10: diet containing 10 % salmon oil
Significance of factors:
4) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.05$
5) Type of dietary oil: $p < 0.01$, amount of test oil: < 0.01
6) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.
7) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.01$
8) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.
N.S. = not significant

fed OO 10, EPO 10, LO 5 and LO 10 had lower erythrocyte SFA levels than CO-fed control rats. Groups fed olive oil showed the highest level of MUFA in the erythrocyte membrane (Table 5). This increase in MUFA at the expense of SFA and (n-6) PUFA was most pronounced with respect to oleic acid (18 : 1) and occurred in a dose-dependent fashion. The concentrations of (n-6) and (n-3) PUFA in erythrocyte membranes enormously differed feeding the various test diets (Table 6 and 7). Rats fed diets with SFO, EPO or LO had higher 18 : 2 (n-6) levels in the erythrocyte membrane than CO-fed rats. In the case of SFO and EPO this increase in 18 : 2 (n-6) was dose-dependent. This increase in 18 : 2 (n-6) was mainly at the expense of 18 : 1. The proportion of 20 : 4 (n-6) did not remarkably change feeding diets with (n-6) PUFA-rich oils. There was not any consistent difference in fatty acid composition of erythrocyte membranes of rats fed SFO or EPO.

The percentages of 20 : 5 (n-3) and 18 : 3 (n-3) in the erythrocyte membranes were at very low levels or not detected at all using diets without added linseed oil or salmon oil. The (n-3) PUFA concentration in the erythrocyte membrane increased, whereas (n-6)

Table 6. Proportions of (n-6) polyunsaturated fatty acids in the erythrocyte membranes (%, by weight) of rats receiving diets with different oils[1), 2)]

Fatty acid	Amount of test oil in the diet	CO[3)]	OO	SFO	EPO	LO	SLO
			g/100 g fatty acid methyl esters				
n-6 PUFA							
18:2[4)]	5 %	–	5.7 ± 0.3^{b}	8.6 ± 1.0^{de}	7.8 ± 0.3^{c}	8.0 ± 0.7^{cd}	5.3 ± 0.5^{b}
	10 %	5.7 ± 0.3^{b}	5.6 ± 0.6^{b}	9.8 ± 0.5^{f}	8.8 ± 0.8^{e}	8.4 ± 0.9^{cde}	4.3 ± 0.6^{a}
18:3[5)]	5 %	–	0.2 ± 0.1^{ab}	0.2 ± 0.0^{a}	0.3 ± 0.1^{c}	0.3 ± 0.1^{c}	0.3 ± 0.1^{bc}
	10 %	0.2 ± 0.1^{ab}	0.2 ± 0.0^{a}	0.3 ± 0.1^{bc}	0.2 ± 0.1^{ab}	0.3 ± 0.1^{c}	0.2 ± 0.1^{ab}
18:4[6)]	5 %	–	0.7 ± 0.1^{ab}	1.3 ± 0.2^{d}	0.7 ± 0.1^{ab}	0.9 ± 0.3^{bc}	0.7 ± 0.2^{a}
	10 %	1.7 ± 0.2^{e}	0.6 ± 0.1^{a}	1.1 ± 0.3^{c}	0.9 ± 0.2^{bc}	0.7 ± 0.1^{ab}	0.7 ± 0.2^{a}
20:4[7)]	5 %	–	22.2 ± 1.1^{d}	22.5 ± 1.3^{d}	24.3 ± 1.2^{e}	16.3 ± 0.9^{c}	13.5 ± 0.9^{a}
	10 %	23.0 ± 1.4^{de}	22.2 ± 1.0^{d}	23.0 ± 1.6^{de}	23.3 ± 1.4^{de}	14.7 ± 0.8^{b}	13.1 ± 1.4^{a}
22:4[8)]	5 %	–	2.6 ± 0.5^{de}	4.0 ± 0.6^{g}	1.9 ± 0.2^{bc}	2.0 ± 0.5^{bc}	1.5 ± 0.3^{ab}
	10 %	2.7 ± 0.5^{e}	3.1 ± 0.5^{ef}	3.3 ± 0.4^{f}	4.7 ± 1.0^{h}	2.2 ± 0.4^{cd}	1.3 ± 0.4^{a}
Total[9)]	5 %	–	31.4 ± 1.1^{e}	36.6 ± 1.3^{h}	35.0 ± 1.1^{g}	27.6 ± 1.2^{d}	21.2 ± 1.1^{b}
	10 %	33.3 ± 1.2^{f}	31.6 ± 1.6^{e}	37.4 ± 1.4^{h}	37.9 ± 1.8^{h}	26.3 ± 1.6^{c}	19.5 ± 1.7^{a}

1) Data are means ± SD; n = 10 for all groups

2) Means were compared by Student-Newman-Keuls test; means not sharing the same superscript letter differ significantly ($p < 0.05$)

3) Abbreviations used for the diets: CO: diet containing 10 % coconut oil; OO 5: diet containing 5 % olive oil; OO 10: diet containing 10 % olive oil; SFO 5: diet containing 5 % safflower oil; SFO 10: diet containing 10 % safflower oil; EPO 5: diet containing 5 % evening primrose oil; EPO 10: diet containing 10 % evening primrose oil; LO 5: diet containing 5 % linseed oil; LO 10: diet containing 10 % linseed oil; SLO 5: diet containing 5 % salmon oil; SLO 10: diet containing 10 % salmon oil
Significance of factors:

4) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.05$

5) Type of dietary oil: N.S., amount of test oil: $p < 0.01$

6) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.05$

7) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.05$

8) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.01$

9) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.
N.S. = not significant

PUFA decreased feeding linseed oil or salmon oil rich in (n-3) PUFA. These increases in 20 : 5 (n-3), 22 : 5 (n-3) and 22 : 6 (n-3) were entirely at the expense of 20 : 4 (n-6) and 22 : 4 (n-6). Concentrations of 18 : 3 (n-3) and 20 : 5 (n-3) in the erythrocyte membrane were higher and 20:4 (n-6) Concentration was lower in membranes of rats fed 10 % linseed oil than in rats fed 5 % linseed oil whereas 5 % salmon oil in the diet produced already a maximal fall in 20 : 4 (n-6) in the membrane. Linseed oil and salmon oil caused similar changes in the membrane, which were more pronounced in rats fed salmon oil than in rats fed linseed oil.

The osmotic fragility of erythrocytes was also influenced by dietary oils. Table 8 shows the rate of hemolyzed erythrocytes with application of the different diets. Erythrocytes from rats fed olive oil or safflower oil diet were statistically as resistant to hemolysis as from animals on coconut oil diet. In the range between 0.42 % till 0.48 %

Table 7. Proportions of (n-3) polyunsaturated fatty acids in the erythrocyte membranes (%, by weight) of rats receiving diets with different oils[1), 2)]

Fatty acid	Amount of test oil in the diet	CO[3)]	OO	SFO	EPO	LO	SLO
		g/100 g fatty acid methyl esters					
n-3 PUFA							
18:3[4)]	5 %	–	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	1.1 ± 0.3^{d}	0.7 ± 0.2^{b}
	10 %	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	1.7 ± 0.4^{e}	0.9 ± 0.3^{c}
20:5[5)]	5 %	–	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	3.1 ± 0.9^{b}	5.5 ± 0.6^{d}
	10 %	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	$<0.1^{a}$	4.3 ± 0.9^{c}	6.5 ± 1.1^{e}
22:5[6)]	5 %	–	0.5 ± 0.1^{a}	0.5 ± 0.3^{a}	0.5 ± 0.1^{a}	2.9 ± 0.6^{c}	3.1 ± 0.6^{c}
	10 %	0.5 ± 0.2^{a}	0.6 ± 0.1^{a}	0.3 ± 0.2^{a}	0.4 ± 0.1^{a}	2.9 ± 0.3^{c}	2.5 ± 0.5^{b}
22:6[7)]	5 %	–	1.3 ± 0.3^{bc}	1.5 ± 0.5^{c}	0.9 ± 0.2^{ab}	2.1 ± 0.6^{d}	3.1 ± 0.5^{e}
	10 %	1.2 ± 0.2^{bc}	1.4 ± 0.3^{c}	0.8 ± 0.1^{a}	0.9 ± 0.1^{ab}	1.9 ± 0.4^{d}	3.1 ± 0.5^{e}
Total[8)]	5 %	–	1.8 ± 0.3^{a}	2.0 ± 0.6^{a}	1.4 ± 0.3^{a}	9.2 ± 1.6^{b}	12.4 ± 1.3^{d}
	10 %	1.7 ± 0.3^{a}	2.0 ± 0.4^{a}	1.1 ± 0.2^{a}	1.2 ± 0.2^{a}	10.9 ± 0.6^{c}	12.9 ± 1.2^{d}

1) Data are means ± SD; n = 10 for all groups
2) Means were compared by Student-Newman-Keuls test; means not sharing the same superscript letter differ significantly ($p < 0.05$)
3) Abbreviations used for the diets: CO: diet containing 10 % coconut oil; OO 5: diet containing 5 % olive oil; OO 10: diet containing 10 % olive oil; SFO 5: diet containing 5 % safflower oil; SFO 10: diet containing 10 % safflower oil; EPO 5: diet containing 5 % evening primrose oil; EPO 10: diet containing 10 % evening primrose oil; LO 5: diet containing 5 % linseed oil; LO 10: diet containing 10 % linseed oil; SLO 5: diet containing 5 % salmon oil; SLO 10: diet containing 10 % salmon oil
Significance of factors:
4) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.01$
5) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.01$
6) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.05$
7) Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.05$
8) Type of dietary oil: $p < 0.01$, amount of test oil: N.S.
N.S. = not significant

NaCl solution erythrocytes from rats fed EPO 5, LO 5, and LO 10 had a statistically higher rate of hemolysis than erythrocytes from control rats. Erythrocytes from rats on SLO 5 diet were statistically less resistant to hemolysis in NaCl solutions of 0.36 %, 0.38 %, and 0.48 %. Using EPO 10 or SLO 10 diet changes in osmotic fragility of erythrocytes became not statistically apparent compared to erythrocytes from control rats. In almost all NaCl solutions erythrocytes from rats fed the dietary test oils were slightly less resistant to hemolysis than those from control rats.

Discussion

From the experiment described above it is quite evident that the fatty acid composition of the erythrocyte membrane lipids can be altered by dietary fatty acids within 20 days. The relative amount of SFA in erythrocyte membranes was only slightly affected by diet, whereas the relative amount of MUFA, (n-6) and (n-3) PUFA markedly changed with the diet. There was a clear relationship especially between the concentration of

Table 8. Rate of hemolyzed erythrocytes from rats receiving diets with different oils[1), 2)]

NaCl solution	Amount of test oil in the diet	CO[3)]	OO	SFO	EPO	LO	SLO
				%			
0.30 %[4)]	5 %	–	95 ± 14^{ab}	90 ± 17^{ab}	72 ± 16^{a}	84 ± 16^{ab}	85 ± 14^{ab}
	10 %	79 ± 15^{ab}	85 ± 19^{ab}	100 ± 18^{b}	102 ± 15^{b}	87 ± 16^{ab}	82 ± 17^{ab}
0.32 %[5)]	5 %	–	82 ± 21	71 ± 20	62 ± 17	79 ± 20	76 ± 10
	10 %	66 ± 17	67 ± 19	85 ± 20	89 ± 21	76 ± 17	71 ± 22
0.34 %[6)]	5 %	–	61 ± 13^{bcd}	48 ± 15^{ab}	52 ± 19^{abc}	55 ± 16^{abcd}	68 ± 19^{d}
	10 %	53 ± 18^{abcd}	44 ± 20^{a}	66 ± 22^{cd}	68 ± 22^{d}	66 ± 8^{cd}	45 ± 15^{ab}
0.36 %[7)]	5 %	–	33 ± 11^{abc}	24 ± 9^{ab}	30 ± 17^{abc}	37 ± 12^{abc}	47 ± 16^{c}
	10 %	23 ± 9^{ab}	19 ± 7^{a}	38 ± 18^{bc}	42 ± 17^{bc}	34 ± 6^{abc}	30 ± 17^{abc}
0.38 %[8)]	5 %	–	17 ± 7^{ab}	7.8 ± 1.0^{a}	14 ± 3^{ab}	18 ± 9^{ab}	25 ± 8^{b}
	10 %	10 ± 3^{a}	8.9 ± 2.9^{a}	18 ± 10^{ab}	20 ± 8^{ab}	16 ± 6^{ab}	16 ± 12^{ab}
0.40 %[9)]	5 %	–	7.0 ± 2.5	4.1 ± 2.8	7.2 ± 2.0	8.4 ± 4.5	8.9 ± 3.0
	10 %	5.3 ± 2.8	3.7 ± 2.5	8.0 ± 5.3	6.7 ± 3.3	7.1 ± 3.4	8.5 ± 9.7
0.42 %[10)]	5 %	–	2.4 ± 0.8^{a}	1.9 ± 1.7^{a}	5.5 ± 2.6^{bc}	5.8 ± 4.2^{c}	4.1 ± 1.9^{abc}
	10 %	1.4 ± 1.0^{a}	1.7 ± 0.7^{a}	2.3 ± 1.4^{a}	3.1 ± 1.5^{ab}	5.1 ± 2.4^{bc}	1.9 ± 2.4^{a}
0.44 %[11)]	5 %	–	1.2 ± 1.1^{abc}	1.3 ± 0.9^{abc}	2.9 ± 2.0^{cd}	4.1 ± 2.5^{d}	1.5 ± 1.1^{abc}
	10 %	0.5 ± 1.5^{a}	0.6 ± 1.7^{ab}	0.5 ± 0.9^{ab}	0.9 ± 1.4^{abc}	2.7 ± 1.6^{bcd}	1.5 ± 1.4^{abc}
0.46 %[12)]	5 %	–	0.2 ± 1.0^{a}	0.3 ± 1.9^{a}	2.1 ± 1.4^{c}	1.9 ± 1.4^{bc}	0.6 ± 1.1^{a}
	10 %	-0.3 ± 0.8^{a}	0.0 ± 0.8^{a}	0.0 ± 0.9^{a}	0.5 ± 1.2^{a}	2.3 ± 1.6^{c}	0.3 ± 1.2^{ab}
0.48 %[13)]	5 %	–	0.1 ± 1.6^{abc}	0.3 ± 0.8^{abc}	1.7 ± 1.3^{c}	1.2 ± 0.9^{bc}	0.8 ± 0.9^{bc}
	10 %	-1.1 ± 2.2^{a}	-0.3 ± 1.2^{ab}	0.3 ± 1.3^{abc}	0.1 ± 1.4^{abc}	1.4 ± 0.6^{bc}	0.5 ± 1.0^{abc}

[1)] Data are means ± SD; n = 10 for all groups

[2)] Means were compared by Student-Newman-Keuls test; means not sharing the same superscript letter differ significantly ($p < 0.05$)

[3)] Abbreviations used for the diets: CO: diet containing 10 % coconut oil; OO 5: diet containing 5 % olive oil; OO 10: diet containing 10 % olive oil; SFO 5: diet containing 5 % safflower oil; SFO 10: diet containing 10 % safflower oil; EPO 5: diet containing 5 % evening primrose oil; EPO 10: diet containing 10 % evening primrose oil; LO 5: diet containing 5 % linseed oil; LO 10: diet containing 10 % linseed oil; SLO 5: diet containing 5 % salmon oil; SLO 10: diet containing 10 % salmon oil
Significance of factors:

[4)] Type of dietary oil: N.S., amount of test oil: N.S.

[5)] Type of dietary oil: N.S., amount of test oil: N.S.

[6)] Type of dietary oil: N.S., amount of test oil: N.S.

[7)] Type of dietary oil: $p < 0.05$, amount of test oil: N.S.

[8)] Type of dietary oil: $p < 0.05$, amount of test oil: N.S.

[9)] Type of dietary oil: N.S., amount of test oil: N.S.

[10)] Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.05$

[11)] Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.01$

[12)] Type of dietary oil: $p < 0.01$, amount of test oil: N.S.

[13)] Type of dietary oil: $p < 0.01$, amount of test oil: $p < 0.05$

N.S. = not significant

18 : 2 (n-6) or 20 : 5 (n-3) in the diet and in the erythrocyte membrane. Kuypers et al. (13) have also found that SFA in membrane kept relatively constant, whereas the alteration in erythrocyte PUFA was strongly correlated with the amount of PUFA in the diet. It has been found that the proportion of 20 : 5 (n-3) in the erythrocyte membrane of humans is a marker for the intake of diets rich in (n-3) PUFA or fish oil (2). The present study shows that feeding oils rich in (n-3) PUFA elevated (n-3) PUFA at the expense of (n-6) PUFA. In the case of linseed oil and salmon oil long chain (n-6) and (n-3) PUFA replaced each other, leaving other fatty acid concentrations relatively unchanged. 20 : 5 (n-3) especially replaced 20 : 4 (n-6) in the erythrocyte membrane. Definite changes in concentrations of essential fatty acids in the erythrocyte lipids were observed, and direct incorporation of dietary fatty acids could be seen in all groups receiving different dietary oils. Some fatty acids like 18 : 1, 18 : 2 (n-6) and 20 : 5 (n-3) in the erythrocyte membrane changed continuously with increasing dietary intake, but other fatty acids kept constant. It is also possible that (n-3) PUFA inhibited the incorporation of (n-6) PUFA into the erythrocyte membrane. The (n-3) PUFA incorporation pattern with a decreasing content of 20 : 4 (n-6) observed in this study is similar to that shown in other studies with erythrocytes from rats (34), from humans (22, 26, 33), from monkeys (6), and rabbits (9).

The rat mature erythrocyte has virtually no capacity for the synthesis of fatty acids and membrane lipids (18, 19, 24, 25, 28). The erythrocyte membrane depends on the transition of fatty acids from blood. Plasma lipids originated directly from the diet or from the liver. However, a previous report (29) has shown that the fatty acid composition of the liver was enormously altered by dietary fatty acids and the changes were greater than in the erythrocyte membranes of the present study. Those differences possibly occur on a controlled incorporation of plasma fatty acids into the erythrocyte membrane. The lack of medium chain fatty acids and the only small proportions of long chain fatty acids with 12 and 14 C-atoms in the erythrocyte membrane, in spite of their occurrence in the diet, prompt the conclusion that these fatty acids were β-oxidized in liver and other organs or were scarcely incorporated in the erythrocyte membrane. Greenberg et al. (7) and Leyton et al. (15) showed that medium chain fatty acids and long chain fatty acids with 12 and 14 C-atoms were more preferentially oxidized than other fatty acids. Other studies supplied evidence (16, 30) that the fatty acid composition of phospholipids in the erythrocyte membrane were genetically controlled with the purpose to minimize changes in membrane fluidity (1). In spite of that mechanism, which may serve to maintain specific membrane fluidity in the erythrocyte membrane, it seems likely that less resistance to hemolysis is in turn related to changes in the fatty acid composition of erythrocyte membrane lipids. Also, the fluidity of erythrocyte membranes could be altered by changes in the fatty acid composition (10, 23, 27, 32). This study demonstrates that in rats provided with sufficient linoleic acid, highly unsaturated dietary fatty acids can impair the resistance to hemolysis of erythrocytes. Especially evening primrose oil, linseed oil, and salmon oil affect the resistance to hemolysis. The slight, but not statistically significant effect of EPO 10 and SLO 10 on osmotic fragility of erythrocytes might result from the low food intake of these two groups. The changes in osmotic fragility might not be based on a special fatty acid, but perhaps on the high unsaturation of the fatty acids in the erythrocyte membrane. The point in question is whether or not the altered fatty acid composition affects the osmotic fragility of erythrocytes also in physiological sodium chloride solutions, with consequences to erythrocyte mechanical, physical and functional properties.

References

1. Beynen AC, Schouten JA, Popp-Snijders C (1984) Compensatory changes in the lipid composition of the erythrocyte membrane. Trends Biochem Sci 9:474
2. Brown AJ, Roberts DCK (1990) Erythrocyte EPA as a marker for intake of fish and fish oil. Eur J Clin Nutr 54:668–673
3. Eder K, Reichlmayr-Lais AM, Kirchgeßner M (1991) Gas chromatographic analysis of fatty acid methyl esters: avoiding discrimination by programmed temperature vaporizing injection. J Chromatogr 588:265–272
4. Eder K, Reichlmayr-Lais AM, Kirchgeßner M (1993) Studies on the extraction of phospholipids from erythrocyte membranes in the rat. Clin Chim Acta 219:93–104
5. Ehrström M, Harms-Ringdahl M, Alling C (1981) Osmotic fragility and fluidity of erythrocyte membranes from rat raised on an essential fatty acid deficient diet. Biochim Biophys Acta 644:175–182
6. Gibson RA, Neumann MA, Burnard SL, Rinaldi JA, Patten GS, McMurchie EJ (1992) The effect of dietary supplementation with eicosapentaenoic acid on the phospholipid and fatty acid composition of erythrocytes of marmoset. Lipids 27:169–176
7. Greenberg NJ, Rodfers JB, Isselbacher KJ (1965) Metabolism of 1-C^{14} octanoic and 1-C^{14} palmitic acid by rat intestinal slices. Proc Soc Exp Biol Med 120:468–472
8. Hanahan DJ, Ekholm JE (1974) The preparation of red cell ghosts (membranes). Methods Enzymol 31:168–172
9. Hearn JA, Sgoutas DS, Robinson KA, King SB, Siegel RJ, Roubin GS (1989) Marine lipid concentrate and atherosclerosis in the rabbit model. Atherosclerosis 75:39–47
10. Heath JS, Donaldson WE (1984) Dietary manipulation of membrane fatty acid content and erythrocyte fragility in chicks. Nutr Rep Int 29:1061–1069
11. Keuls M (1952) The use of the "studentized range" in connection with analysis of variance. Euphytica 1:112
12. Kobayashi S, Hirai A, Terano T, Hamazaki T, Tamura Y, Kumager A (1981) Reduction in blood viscosity by eicosapentaenoic acid. Lancet 2:197
13. Kuypers FA, Abraham S, Lubin B, Chiu D (1988) Diet-induced asymmetry of the phosphatidylcholine fatty acyl composition in rat erythrocyte membranes. J Lab Clin Med 111:529–536
14. Kuypers FA, Roelofsen B, Op Den Kamp JAF, Van Deenen LLM (1984) The membrane of intact human erythrocytes tolerates only limited changes in the fatty acid composition of its phosphatidylcholine. Biochim Biophys Acta 769:337–347
15. Leyton J, Drury PJ, Crawford MA (1987) Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. Br J Nutr 57:383–393
16. McMurchie EJ (1988) Dietary lipids and the regulation of membrane fluidity and function. In: Alan R (ed) Physiological regulation of membrane fluidity, Liss. Inc., New York, pp 189–237
17. Morrison WR, Smith LM (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 5:600–608
18. Mulder E, Van Deenen LLM (1965a) Metabolism of red cell lipids: I. Incorporation in vitro of fatty acids into phospholipids from mature erythrocytes. Biochim Biophys Acta 106:106–117
19. Mulder E, Van Deenen LLM (1965b) Metabolism of red cell lipids: III. Pathways for phospholipid renewal. Biochim Biophys Acta 106:348–356
20. O'Dell BL, Browning JD, Reevers PG (1987) Zinc deficiency increases the osmotic fragility of rat erythrocytes. J Nutr 117:1883–1889
21. Popp-Snijders C, Schouten JA, De Jong AP, Van Der Veen EA (1984) Effect of dietary cod-liver oil on the lipid composition of human erythrocyte membranes. Scand J Clin Lab Invest 44:39–46
22. Popp-Snijders C, Schouten JA, Van Der Meer J, Van Der Veen EA (1986) Fatty fish-induced changes in membrane lipid composition and viscosity of human erythrocyte suspensions. Scand J Clin Lab Invest 46:253–258
23. Rao GA, Siler K, Larkin EC (1979) Diet induced alterations in discoid shape and phospholipid fatty acid composition of rat erythrocytes. Lipids 14:30–38
24. Reed CF, Murphy M, Roberts G (1968) Phospholipid exchange between plasma and erythrocytes in man and the dog. J Clin Invest 47:749–760
25. Renooij W, Van Golde LMG, Zwaal RFA, Van Deenen LLM (1976) Topological asymmetry of phospholipid metabolism in rat erythrocyte membranes. Evidence for flip-flop of lecithin. Eur J Biochem 61:53–58

26. Sanders TAB, Vickers M, Haines AP (1981) Effect of blood lipids and haemostasis of a supplement of cod-liver oil, rich in eicosapentaenoic and docosahexaenoic acids, in healthy young man. Clin Sci 61:317–324
27. Shand JH, Noble RC (1981) The maternal diet and its effect on the lipid composition and osmotic fragility of neonatal ovine erythrocytes. Biol Neonate 40:150–159
28. Sohet SB, Nathan DG (1970) Incorporation of phosphatide precursors from serum into erythrocytes. Biochim Biophys Acta 202:202–205
29. Stangl GI, Reichlmayr-Lais AM, Eder K, Kirchgeßner M (1993) Effect of different dietary oils on liver fatty acid composition of rats. J Anim Physiol a Anim Nutr
30. Stubbs CD, Smith AD (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim Biophys Acta 779:89–137
31. Stubbs CD, Smith AD (1990) Essential fatty acids in membrane: physical properties and function. Biochem Soc Trans 18:779–781
32. Terano T, Hirai A, Hammazaki T, Kobayashi S, Fujita T, Tamura Y, Kumagai A (1983) Effect of oral administration of highly purified eicosapentaenoic acid on platelet function, blood viscosity, and red cell deformability in healthy human subjects. Atherosclerosis 46:321–331
33. Von Schacky C, Fisher S, Weber PC (1985) Long-term effects of dietary marine w-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J Clin Invest 76:1626–1631
34. Witting LA, Harvey CC, Century B, Horwitt MK (1961) Dietary alterations of fatty acids of erythrocytes and mitochondria of brain and liver. J Lipid Res 2:412–418

Received September 1, 1993
accepted December 23, 1993

Authors' address:

Prof. Dr. Dr. h.c. mult. M. Kirchgeßner, Institut für Ernährungsphysiologie, Technische Universität München-Weihenstephan, 85350 Freising