Biochimica et Biophysica Acta, 1149 (1993) 313–318

BBAMEM 76028

Effects of dietary fatty-acid supplementation on fatty-acid composition and deformability of young and old erythrocytes

David E. Mills [a], William R. Galey [b] and Heather Dixon [a]

[a] *Department of Health Studies, University of Waterloo, Waterloo, Ontario (Canada) and*
[b] *Department of Physiology, University of New Mexico School of Medicine, Albuquerque, NM (USA)*

(Received 22 March 1993)

Key words: ($n-3$) Fatty acid; ($n-6$) Fatty acid; Aging; Deformability; Fish oil; Safflower oil; (Erythrocyte)

The effects of cell age on erythrocyte phospholipid fatty-acid composition and deformability were examined in 20 healthy adults (11 male, 9 female) prior to and following 12 weeks of dietary supplementation with 3.5 g/day of safflower oil (high in $n-6$ fatty acids) or fish oil (high in $n-3$ fatty acids). In the absence of dietary supplementation, old erythrocytes demonstrated an increase in filtration time ($P < 0.001$), an increase in membrane phospholipid total $n-6$ fatty acids ($P < 0.01$), and a decrease in total $n-3$/total $n-6$ ratio ($P < 0.01$) compared to young erythrocytes. Both safflower and fish oil supplementation attenuated age-related differences in membrane phospholipid total $n-6$ and total $n-3$ fatty acids. Fish oil supplementation also increased the proportion of $n-3$ fatty acids ($P < 0.01$) and the $n-3/n-6$ ratio ($P < 0.05$) in the phospholipids of both young and old erythrocytes, and eliminated age-related differences in erythrocyte filtration time by reducing the relative filtration time of the old erythrocytes.

Introduction

Aging of the circulating erythrocyte is known to be accompanied by numerous structural and functional changes, including alterations in membrane fatty-acid composition [1–6] and transport function [7], protein expression [8,9], cell deformability [10] and osmotic fragility [11]. These age-related changes are believed to impede the movement of erythrocytes through the capillary bed, interfere with the delivery of oxygen to peripheral tissues, and ultimately contribute to the removal of the senescent erythrocyte from the circulation [7,8].

It is well-established that the type of polyunsaturated fatty acids consumed in the diet influences erythrocyte membrane phospholipid fatty-acid composition. In recent years the effects of dietary supplementation with $n-6$ and $n-3$ fatty acids on whole blood viscosity, erythrocyte lipid composition and deformability have been examined in both animals and humans [4,12–21]. Most [12–16,20], but not all [17–19], of the studies in humans have demonstrated that dietary supplementation with 20 and 22-carbon (20:5($n-3$) and 22:6($n-3$)) $n-3$ fatty acids increase membrane phospholipid $n-3/n-6$ fatty-acid ratios, decrease whole blood viscosity, and increase cell deformability in humans. In addition, one study in rats suggested that the ability of erythrocytes to incorporate saturated and $n-6$ fatty acids into membrane phospholipids may vary with cell age as a function of variations in transacylase activity [4]. None of the studies to date have examined the effect of cell age on (1) the ability of human erythrocytes to incorporate $n-6$ and $n-3$ fatty acids and (2) the changes in erythrocyte deformability in response to long-chain $n-3$ fatty acids.

The purpose of the present study was to investigate the effects of dietary supplementation with $n-6$ fatty acids (safflower oil) and long-chain $n-3$ fatty acids (fish oil) on phospholipid fatty-acid composition and deformability in young and old human erythrocytes separated by density centrifugation.

Materials and Methods

Subjects. Healthy adult volunteers were recruited from the University of Waterloo student population. After elimination of smokers, individuals taking medication or the oral contraceptives, and those currently under medical treatment, 10 subjects (5 males and 5 females) were enrolled for study 1 and 10 subjects (6 males and 4 females) enrolled for study 2. All participants were between 19–32 years of age and completed

Correspondence to: D.E. Mills, Department of Health Studies, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada.

TABLE I

Fatty-acid composition of dietary supplements

Dietary supplements used in the study were analyzed for fatty-acid composition by gas chromatography, as described in the text.

Fatty acid	Safflower oil (% total fatty acids)	Fish oil (% total fatty acids)
14:0	–	7.6
16:0	6.6	16.4
16:1(*n*-7)	0.1	9.0
18:0	2.3	3.0
18:1(*n*-9)	11.1	12.6
18:2(*n*-6)	72.3	3.7
18:3(*n*-3)	0.4	–
20:4(*n*-6)	–	0.6
20:5(*n*-3)	–	18.6
22:5(*n*-3)	–	2.4
22:6(*n*-3)	–	12.7
Σ(*n*-6)	72.3	4.3
Σ(*n*-3)	0.4	33.7

an informed consent form prior to the study. The volunteers for study 2 were then randomized within gender to one of 2 dietary intervention groups (3 males and 2 females/diet group).

Protocol.

Study 1. After an overnight fast, a 60-ml sample of venous blood was drawn into a heparinized tube from an antecubital vein for analysis of erythrocyte phospholipid fatty-acid composition and deformability of the youngest and oldest cells, as described below.

Study 2. This experiment was conducted under double-blind conditions. Prior to and following 12 weeks dietary supplementation with 3.0 g/day of either safflower oil (SAF, high in 18:2($n-6$)) or fish oil (Polepa, high in 20:5($n-3$) and 22:6($n-3$)) in 0.5 ml gelatin capsules, 60 ml venous blood was drawn, as described above. The fatty-acid compositions of the dietary supplements used are shown in Table I. This supplementation resulted in an addition to the usual diet of 2.2 g/day 18:2($n-6$) in the SAF group, and 0.56 and 0.38 g/day of 20:5($n-3$) and 22:6($n-3$), respectively, in the fish oil group. Dietary compliance was assessed via periodic pill counts at intervals throughout the study.

Preparation and age separation of erythrocytes. Following the removal of plasma and buffy coat, packed erythrocytes were washed twice with 0.05 M phosphate-buffered saline ((pH 7.4), 294 ± 2 mosm/l). Cells were separated by age according to the method of Murphy [22]. 12-ml aliquots of packed cells, adjusted to a hematocrit of 85–90%, were centrifuged for 60 min at 27000 × g in a fixed angle rotor at 27°C. After discarding the supernatant, the top and bottom 0.8 ml of erythrocytes were aspirated from the tubes. Of these fractions, 0.5 ml of each was resuspended in buffer for deformability measures and 0.3 ml was frozen under N_2 at −80°C for subsequent lipid analysis. This centrifugation method has been demonstrated to isolate the oldest cells in the most dense fraction (see Ref. 7). Following density separation of the youngest and oldest erythrocyte fractions, phospholipid fatty-acid composition and cell deformability were assessed for each fraction, as described below. In addition, an additional 0.2 ml of young and old erythrocytes were counted, and then weighed before and after 24 h drying in a 90°C oven to assess cell water content.

Measurement of erythrocyte deformability. Erythrocyte deformability of the young and old fractions was determined by measuring the time required for a fixed volume of erythrocytes at a known hematocrit to pass through a micropore filter, as described by Reid et al. [23]. In this method, the filtration time of the cells is inversely proportional to their deformability (flexibility). Following reconstitution of each packed erythrocyte sample to 5% hematocrit in phosphate-buffered saline at 21–23°C, the time required for the passage of 2.0 ml each of phosphate buffer and erythrocyte solution through a filter of pore size 5.0 μm (Hemafil, Nucleopore) was measured. Erythrocytes and buffer were filtered under gravity at a constant hydrostatic pressure. Deformability results were expressed as the Relative Filtration Time = erythrocyte filtration time/buffer filtration time.

Measurement of erythrocyte phospholipid fatty-acid composition. Lipids were extracted from 0.5 ml packed erythrocytes with chloroform/methanol, as previously described [24]. The phospholipid fraction was then obtained by thin-layer chromatography, and subsequently transmethylated with BF_3 [25]. The fatty-acid methyl esters were then analyzed on a Perkin-Elmer gas chromatograph (model 8420) equipped with a flame ionization detector and a 30-m capillary column (Supelcowax 10, Supelco). A temperature-ramped program was used, consisting of 2 min at 180°C, followed by a 2 C°/min increase to 220°C, and then by 2 min at 220°C. Fatty acids were identified by comparing their retention time with those of known standards.

Statistics. Data comparing deformability and lipid composition of young and old cells (experiment 1) were compared using Student's t-test. Deformability and fatty-acid data prior to and following dietary interventions (experiment 2) were analyzed using a two-way analysis of variance (treatment × time) on repeated measures. Where a significance of $P < 0.05$ was achieved, specific comparisons were then made using Tukey's HSD test.

Results

Study 1

The phospholipid fatty-acid composition of density-separated erythrocytes is shown in Table II. Relative to the least dense cells (youngest), the most dense cells

(oldest) had significantly higher levels of 18:1($n-9$) ($P<0.01$), 18:1($n-7$) ($P<0.02$), 18:2($n-6$) ($P<0.01$) and 22:4($n-6$) ($P<0.05$), and lower levels of 20:5($n-3$) ($P<0.02$) and 22:5($n-6$) ($P<0.05$). In addition, while the sum of phospholipid $n-3$ fatty acids did not differ between fractions, the sum of $n-6$ fatty acids in the oldest cells increased ($P<0.01$) and the $n-3/n-6$ ratio decreased ($P<0.02$), relative to the youngest cells. No change was observed in the ratio of polyunsaturated/saturated fatty acids (P/S). Relative filtration time (Fig. 1a), an indicator of erythrocyte deformability, was significantly higher in the older cells as compared to the younger cells ($P<0.001$), demonstrating a decrease in deformability with increasing cell age.

Study 2

Phospholipid fatty-acid composition prior to and following dietary supplementation with SAF or fish oils in density separated cells is shown in Table III. Prior to dietary supplementation, phospholipid fatty-acid differences between young and old cells in both treatment groups were similar to those observed in study 1. Supplementation with $n-6$-rich SAF had no effect on the young erythrocytes, as compared to the pre-supplementation measure. In contrast, in the old erythrocytes, SAF supplementation significantly increased 16:1($n-7$) ($P<0.01$), 20:5($n-3$) ($P<0.05$) and 22:5($n-6$) ($P<0.01$) and decreased 18:1($n-9$), 18:2($n-6$) ($P<0.05$), 20:3($n-6$), 20:4($n-6$), 22:4($n-6$) and 22:5($n-3$) ($P<0.01$) in comparison to the pre-supplementation values. These changes were accompanied by decreases in the P/S ratio and total $n-6$ fatty-acid composition ($P<0.01$).

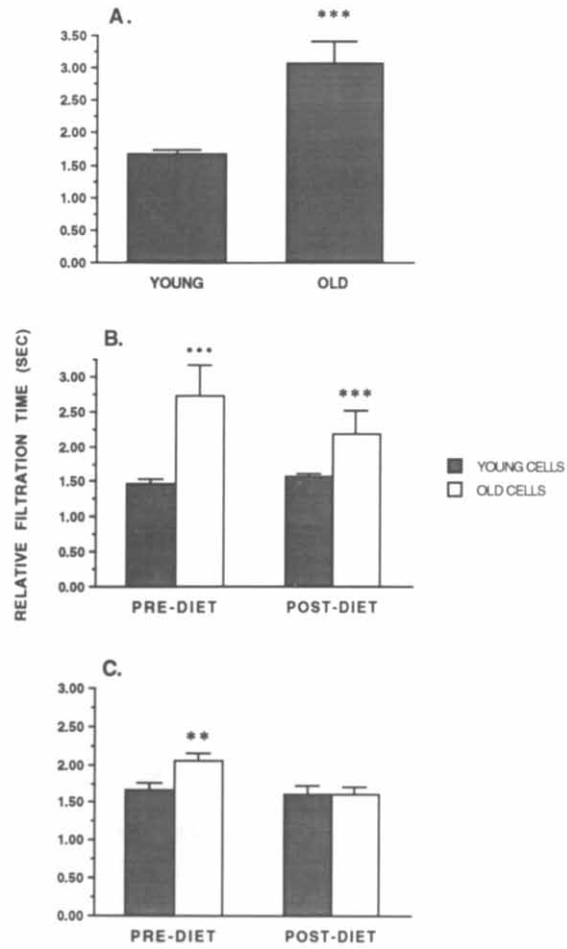

Fig. 1. Relative filtration time of least dense (young) and most dense (old) erythrocytes from 10 healthy subjects (a) and from subjects ($n=5$/group) before and following 12 weeks dietary supplementation with 3.0 g/day of safflower oil (b) and fish oil (c). Values represent mean ± S.E. ** $P<0.01$ vs. young *** $P<0.001$ vs. young.

TABLE II

Phospholipid fatty-acid composition of density-separated erythrocytes

Washed erythrocytes were separated by density centrifugation. Phospholipid composition (% total fatty acids) was determined in lipid extract of the top 7% and bottom 7% of the cells (youngest and oldest cells, respectively) as described in the text. Values represent mean ± S.E. ($n=10$/group)

Fatty acid	Least dense (youngest)	Most dense (oldest)	P value
14:0	0.1 ±0.01	–	
16:0	18.9 ±1.4	20.2 ±0.6	
16:1($n-7$)	1.3 ±0.2	1.4 ±0.6	
18:0	12.8 ±1.0	14.0 ±0.6	
18:1($n-9$)	10.2 ±1.0	13.8 ±0.6	<0.01
18:2($n-6$)	9.7 ±1.0	12.7 ±0.5	<0.02
18:3($n-3$)	0.2 ±0.1	–	
20:3($n-6$)	1.4 ±0.3	1.5 ±0.1	
20:4($n-6$)	11.3 ±1.0	13.2 ±0.7	
20:5($n-3$)	1.8 ±0.3	1.0 ±0.2	<0.02
22:4($n-6$)	2.4 ±0.3	3.5 ±0.3	<0.05
22:5($n-6$)	0.5 ±0.2	0.1 ±0.1	<0.05
22:5($n-3$)	1.3 ±0.2	1.9 ±0.2	<0.05
22:6($n-3$)	2.5 ±0.4	2.7 ±0.3	
P/S ratio	1.0 ±0.05	1.1 ±0.04	
$\Sigma(n-3)$	5.7 ±0.5	5.7 ±0.3	
$\Sigma(n-6)$	22.9 ±1.8	30.9 ±1.4	<0.01
($n-3/n-6$)	0.23±0.01	0.19±0.02	<0.01

Supplementation with fish significantly increased levels of 18:1($n-9$) ($P<0.05$), 22:5($n-3$) and 22:6($n-3$) ($P<0.01$), as well as the $n-3/n-6$ ratio in young erythrocytes vs. pre-supplementation values. In old erythrocytes, fish-oil supplementation increased 16:0 ($P<0.01$) and 22:6($n-3$) ($P<0.05$) and decreased 18:2($n-6$), 20:3($n-6$) ($P<0.05$), and 22:4($n-6$) ($P<0.01$) vs. pre-supplementation values. In addition, fish reduced the total $n-6$ fatty acids and P/S ratio ($P<0.05$), and increased the $n-3/n-6$ ratio ($P<0.05$) in old cells vs. pre-treatment.

Prior to dietary supplementation, in both treatment groups the relative filtration time was significantly higher in the older erythrocytes than in the younger erythrocytes, as was observed in study 1. Supplementa-

tion with SAF had no significant effect on the relative filtration times of either erythrocyte fraction (Fig. 1b). In contrast, following supplementation with fish oil, the relative filtration time of the older cells was significantly reduced to where it was identical to that of the younger cells (Fig. 1c). The relative filtration time of the younger cells was unaffected by fish-oil supplementation. Cell water content was significantly reduced in old cells vs. young cells (1.63 ± 0.04 l/kg vs. 1.81 ± 0.04 l/kg, $P < 0.001$) and was unaffected by dietary intervention (data not shown).

Discussion

Numerous studies have reported an increase in 18:2($n-6$) [1,3,5,26,27] and a decrease in 20:4($n-6$) [3,5] in human erythrocyte phospholipids with increasing cell age. In addition, Phillips et al. reported increases in 16:0 plus 16:1($n-7$) and 18:1($n-9$) in old vs. young erythrocytes, as well as decreases in 20:3($n-6$) + 22:0, 20:4($n-6$) + 22:1, and 22:4($n-6$) [5]. In the present study, the previously reported increases in 18:1($n-9$) and 18:2($n-6$) with age were observed, but also increases in 18:1($n-7$) and 22:4($n-6$) were seen. Furthermore, in contrast to earlier reports in the literature, no age-related decreases in 20:3($n-6$) or 20:4($n-6$) were observed presently, whereas decreases in 20:5($n-3$) and 22:5($n-6$) were noted.

The results of the present study agree with the principal findings of the previous studies; however, there are discrepancies among certain of the fatty-acid changes reported. Some of these discrepancies may be due to the reporting of combinations of fatty acids in several of the earlier studies vs. individual fatty acids in the present study. However, it is also possible that individual and dietary variations among the subjects in the various studies could account for these differences, as animal studies have shown that dietary fatty-acid intake can influence the fatty-acid composition of both young and old erythrocytes over a relatively short (8–12 days) period of time [4]. The previous studies cited were conducted on or before 1970. Since that time, total dietary fat intake, as well as P/S ratios, have changed significantly in North America (for review, see Ref. 28).

It is well-documented that dietary supplementation with 20 and 22-carbon $n-3$ fatty acids, in the form of fish oil or ethyl ester concentrates of $n-3$ fatty acids, in humans for periods from 2–16 weeks significantly alters erythrocyte phospholipid fatty-acid composition and increases the phospholipid double-bond index, membrane fluidity and deformability, as well as reducing whole blood viscosity [12–20,29]. While these

TABLE III

Phospholipid fatty-acid composition of least dense and most dense erythrocytes in safflower- and fish oil-supplemented individuals

Washed cells were separated by density centrifugation prior to and following 12 weeks of dietary supplementation with 3.0 g/day of safflower oil or fish oil. Phospholipid fatty-acid composition of least dense and most dense cells was determined by gas chromatography. Values represent mean ± S.E. ($n = 5$/group). * $P < 0.05$ vs. pre-diet; ** $P < 0.01$ vs. pre-diet; † $P < 0.05$ vs. safflower oil; †† $P < 0.01$ vs. safflower oil.

	Safflower oil (% total fatty acids)				Fish oil (% total fatty acids)			
	Pre-diet		Post-diet		Pre-diet		Post-diet	
	Young	Old	Young	Old	Young	Old	Young	Old
14:0	0.1 ±0.1	–	0.2 ±0.1	0.2 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1
16:0	21.0 ±0.7	20.7 ±0.6	25.0 ±2.4	23.8 ±0.9	16.8 ±2.5	19.6 ±1.0	21.9 ±0.5	23.1 ±1.3 **
16:1($n-7$)	1.1 ±0.1	0.4 ±0.4	0.9 ±0.1	3.8 ±0.8 **	1.6 ±0.4	2.3 ±1.0	0.9 ±0.1	1.5 ±0.6
18:0	13.8 ±0.7	14.3 ±0.9	14.7 ±0.4	14.4 ±0.8	11.7 ±1.7	13.7 ±1.0	14.3 ±0.5	13.9 ±0.8
18:1($n-9$)	12.3 ±0.7	14.9 ±0.5	13.9 ±0.7	12.3 ±0.5 *	8.1 ±1.4 †	12.6 ±0.9 †	13.8 ±0.6 *	13.3 ±0.7
18:1($n-7$)	2.5 ±0.4	3.5 ±0.3	2.6 ±0.6	3.1 ±0.1	2.3 ±0.4	2.8 ±0.2	3.2 ±0.2	3.2 ±0.2
18:2($n-6$)	11.3 ±0.8	13.5 ±0.4	10.5 ±0.4	10.0 ±0.5 *	8.1 ±1.5	11.9 ±0.8	10.6 ±0.77	9.9 ±0.7 *
18:3($n-3$)	0.2 ±0.1	–	0.1 ±0.1	0.1 ±0.01	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.2 ±0.1
20:3($n-6$)	1.5 ±0.3	1.7 ±0.2	1.4 ±0.2	1.4 ±0.1 **	1.3 ±0.5	1.3 ±0.1 †	1.0 ±0.1	1.0 ±0.1 *
20:4($n-6$)	12.3 ±1.0	13.9 ±0.5	11.7 ±0.7	10.4 ±0.5 **	10.3 ±1.7	12.5 ±1.3	10.7 ±0.4	9.7 ±0.6
20:5($n-3$)	1.4 ±0.3	0.8 ±0.4	1.5 ±0.2	1.5 ±0.2 *	2.2 ±0.4	1.3 ±0.2	2.2 ±0.6	1.5 ±0.2
22:4($n-6$)	2.3 ±0.2	4.3 ±0.4	2.3 ±0.2	2.3 ±0.1 **	2.5 ±0.5	2.6 ±0.2 ††	1.7 ±0.2	1.7 ±0.2 **
22:5($n-6$)	0.6 ±0.3	–	0.6 ±0.2	0.7 ±0.1 **	0.4 ±0.3	0.2 ±0.1	0.3 ±0.1	0.2 ±0.1
22:5($n-3$)	1.5 ±0.1	1.9 ±0.1	1.4 ±0.1	1.4 ±0.1 **	1.0 ±0.3	1.9 ±0.4	2.1 ±0.1 **	2.0 ±0.1
22:6($n-3$)	2.7 ±0.7	2.9 ±0.5	2.2 ±0.3	2.0 ±0.1	2.3 ±0.5	2.5 ±0.3	3.9 ±0.3 **	3.6 ±0.1 *
P/S	0.8 ±0.1	1.1 ±0.1	0.8 ±0.1	0.7 ±0.1 **	0.9 ±0.1	1.0 ±0.1	0.8 ±0.1	0.8 ±0.1 *
$\Sigma(n-3)$	5.8 ±0.7	5.6 ±0.5	5.2 ±0.5	5.1 ±0.1	5.6 ±0.7	5.7 ±0.5	8.3 ±0.2	7.3 ±0.3
$\Sigma(n-6)$	28.0 ±1.9	33.4 ±1.3	26.5 ±1.2	24.7 ±0.9 **	22.6 ±0.3	28.4 ±2.0	24.2 ±0.9	22.5 ±0.9 *
($n-3/n-6$)	0.21±0.02	0.17±0.02	0.19±0.02	0.21±0.01	0.25±0.02	0.21±0.02	0.35±0.02 *	0.33±0.02 *

changes have most often been reported with supplementary doses on the order of 2–3 g $n-3$/day [12–19,29], they have also been observed with doses as low as 0.46 g $n-3$/day [20]. However, none of these studies have described the relationship between erythrocyte age (density) and fatty-acid incorporation or cell function, and only two have compared $n-3$ intake with a calorically-matched control oil (olive) [18,19].

In the present study, the phospholipid fatty-acid composition of young erythrocytes was unaffected by dietary supplementation with SAF. In contrast, in old cells SAF supplementation reduced the phospholipid content of most $n-6$ fatty acids vs. pre-treatment, and increased that of $20:5(n-3)$. This led to a reduction of P/S and total $n-6$ fatty acids relative to pre-treatment. It has been reported that dietary supplementation with $18:2(n-6)$ can inhibit desaturation and elongation of $n-6$ fatty acids [30,31]. It is possible that such a mechanism may have reduced the availability of metabolic products of $18:2(n-6)$ for incorporation into the erythrocyte. It does not explain, however, why $18:2(n-6)$ itself was reduced in the old cells post-supplementation. Unlike SAF, supplementation with fish oil altered the fatty-acid composition of both young and old erythrocytes. In young cells, fatty-acid changes in response to supplementation with long chain $n-3$ fatty acids primarily increased their proportion in the phospholipids (along with the $n-3/n-6$ ratio). In old cells, in addition to increasing incorporation of $n-3$ fatty acids and raising the $n-3/n-6$ ratio, there was a significant reduction in most of the $n-6$ fatty acids. The reduction of $n-6$ fatty acids in the phospholipids of fish oil-supplemented individuals may reflect a suppression of $n-6$ fatty-acid metabolism by increased $n-3$ intake [32] or a competition between the $n-6$ and $n-3$ fatty acids for transacylases [33].

While the present study demonstrated fatty-acid changes over a 12-week period of supplementation, it is not clear that such a long period is required for such changes to occur. In addition to fatty-acid incorporation into maturing cells in the bone marrow, circulating erythrocytes actively exchange phospholipids and fatty-acids with those present in plasma lipoproteins [4,34,35], Studies in both animals and humans have demonstrated significant transfer in vivo in as little as 10–14 days via this mechanism [4,13,36,37]. The differential effects of supplementation on young vs. old erythrocytes in the present study suggests that this transfer may be qualitatively different among cells of different ages, and supports the contention of Walker et al. that erythrocyte transacylase activities change with cell age [4].

The decrease in erythrocyte deformability observed in old cells in the present study is consistent with previous reports from human and animal studies [10]. Similarly, the increase in erythrocyte deformability presently observed following dietary supplementation with long-chain $n-3$ fatty acids has been reported by several investigators [12,14,15]. Terano et al. administered $20:5(n-3)$ as a 75% ethyl ester concentrate and noted a significant correlation between membrane deformability and phospholipid $20:5(n-3)$ [12]. In the present study, deformability was altered only in the old erythrocytes, and was independent of the level of any particular fatty acid in the total phospholipids. It is possible that either the fatty-acid composition of one of the phospholipid subfractions may better predict cell deformability than that of total phospholipids, or that the relationship observed by Terano et al. [12] was not causal in nature.

In the present study, SAF supplementation was without effect on erythrocyte deformability. Whether this was due to the (1) shorter chain length of SAF fatty acids; (2) lower level of unsaturation of SAF fatty acids, or (3) predominance of $n-6$ fatty acids relative to fish oil cannot be elucidated from the present study. However, as dietary supplementation with perilla oil (high in $18:3(n-3)$) for 12 weeks did not alter erythrocyte deformability in rats [38], the different effects of SAF and fish oil are probably related to chain length and/or degree of unsaturation.

These findings further support the concept that dietary fish oil might be of some benefit in the treatment of diseases in which there are changes in the rheological properties of blood, such as cerebrovascular and cardiovascular disease, and diabetes mellitus [39,40]. They also suggest that the effects of fish oil on the erythrocyte vary with cell age.

Acknowledgements

This study was conducted with the support of grants from the Heart and Stroke Foundation of Ontario and the Natural Sciences and Engineering Research Council of Canada to D. Mills. and from the American Heart Association (GNM-04-90) to W. Galey.

References

1 Munn, J.I. (1958) Br. J. Haematol. 4, 34–349.
2 Westerman, M., Pierce, L.E. and Jensen, J. (1963) J. Lab. Clin. Med. 62, 394–400.
3 Van Gastel, C., Van den Berg, D., De Gier, J. and Van Deenen, L.L.M. (1965) Br. J. Haematol. 11, 193–199.
4 Walker, B.L. and Yurkowski, M. (1967) Biochem. J. 108, 218–224.
5 Phillips, G.B., Dodge, J.T. and Howe, C. (1970) Lipids 4, 544–549.
6 Kunimoto, M., Kaya, K and Miura, T. (1984) Lipids 19, 443–448.
7 Zanner, M.A. and Galey, W.R. (1985) Biochim. Biophys. Acta 818, 310–315.
8 Kay, M.M.B. (1981) Nature 289, 491–494.
9 Pfeffer, S.R. and Swislocki, N.I. (1976) Arch. Biochem. Biophys. 177, 117–122.
10 Weed, R.I., La Celle, L. and Merrill, E.W. (1969) J. Clin. Invest. 48, 795–809.

11 Marks, P.A. and Johnson, A.B. (1958) J. Clin. Invest. 37, 1542–1548.
12 Terano, T., Hirai, A., Hamazaki, T., Kobayashi, S., Fujita, T., Tamura, Y. and Kumagai, A. (1983) Atherosclerosis 46, 321–331.
13 Popp-Snijders, C., Schouten, J.A., De Jong, A.P. and Van der Veen, E.A. (1984) Scand. J. Lab. Clin. Invest. 44, 39–46.
14 Tamura, Y., Hirai, A., Terano, T., Tahara, K. Yoshida, S. and Kumagai, A. (1984) in Nutritional Prevention of Cardiovascular Disease, pp. 323–330, Academic Press, New York.
15 Cartwright, I.J., Pockley, A.G., Galloway, J.H., Greaves, M. and Preston, F.E. (1985) Atherosclerosis 55, 267–281.
16 Popp-Snijders, C., Schouten, J.A., Van der Meer, J. and Van der Veen, E.A. (1986) Scand. J. Clin. Lab. Invest. 46, 253–258.
17 Popp-Snijders, C., Schouten, J.A., Van Blitterswijk, W.J. and Van der Veen, E.A. (1986) Biochim. Biophys. Acta 854, 31–37.
18 Rogers, S., James, K.S., Butland, B.K., Etherington, M.D., O'Brien, J.R. and Jones, J.G. (1987) Atherosclerosis 63, 137–143.
19 Bruckner, G., Webb, P., Greenwell, L., Show, C. and Richardson, D. (1987) Atherosclerosis 66, 237–245.
20 Driss, F., Darcet, Ph., Delhaye, N. and Mendy, F. (1988) Clin. Hemorheol. 8, 679–685.
21 Sakai, K., Okuyama, H., Kon, K., Maeda, N., Sekiya, M., Shiga, T. and Reitz, R. (1990) Lipids 25, 793–797.
22 Murphy, J.R. (1973) J. Lab. Clin. Med. 82, 334–341.
23 Reid, H.L., Barnes, A.J., Lock, P.J., Dormandy, J.A. and Dormandy, T.L. (1976) J. Clin. Path. 29, 855–858.
24 Mills, D.E., Huang, Y-S. and Ward, R. (1990) Nutr. Res. 10, 663–674.
25 Mills, D.E., Ward, R.P. and Young, C. (1988) Nutr. Res. 8, 273–286.
26 De Gier, J., Van Deenen, L.L.M., Verloop, M.C. and Van Gastel, C. (1964) Br. J. Haematol. 10, 246–256.
27 Ways, P., Reed, C.F. and Hanahan, D.J. (1963) J. Clin. Invest. 42, 1248–1260.
28 Ernst, N.D. (1991) in Health Effects of Dietary Fatty Acids (Nelson, G.J., ed), pp. 1–11, American Oil Chemists' Society, Champaign.
29 Sanders, T.A.B., Vickers, M. and Haines, A.P. (1981) Clin. Sci. 61, 317–324.
30 Brenner, R.R. (1969) Lipids 4, 621–624.
31 Kurata, N. and Privett, O.S. (1980) Lipids 15, 512–515.
32 De Schrijver, R. and Privett, O.S. (1982) J. Nutr. 112, 619–623.
33 Lands, W.E.M., Inoue, M., Suguira, Y. and Okuyama, H. (1982) J. Biol. Chem. 257, 14968–14972.
34 Renooij, W., Van Golde, L.M.G., Zwaal, R.F.A. and Van Deenen, L.L.M. (1976) Eur. J. Biochem. 61, 53–58.
35 Owen, J.S. and Gillett, M.P.T. (1983) Biochem. Soc. Trans. 11, 336–339.
36 Innis, S.M. and Clandinin, M.T. (1981) Biochem. J. 193, 155–158.
37 Innis, S.M. and Clandinin, M.T. (1981) Biochem. J. 198, 167–171.
38 Saiko, K., Okuyama, H., Kon, K., Maeda, N., Sekiya, M., Shiga, T. and Reitz, R.C. (1990) Lipids 25, 793–797.
39 Boisseau, M.R., Lorient, M.F. and Baricaud, H. (1979) Thromb. Haemost. 42, 107–110.
40 Juhan, I., Vague, Ph., Buonocore, M., Moulin, J.P., Jouve, R. and Vialettes, B. (1982) Lancet i, 535–536.