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THE EFFECT OF THE SATURATION AND ISOMERIZATION OF DIETARY FATTY ACIDS ON THE OSMOTIC FRAGILITY AND WATER DIFFUSIONAL PERMEABILITY OF RAT ERYTHROCYTES

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Weanling rats were fed semi-purified diets containing 15% by weight of either corn oil, a high oleic acid safflower oil, lard or hydrogenated soybean oil. Significant changes in the fatty acid composition of erythrocytes were induced by these dietary fats. The compositional changes did not effect water diffusional permeability, but did affect their osmotic fragility. An increased fragility appeared to be associated with an increased octadecenoate content of the membranes.

The physical state of membrane lipids influences the functioning of membrane proteins [1–3]. In vitro studies and studies with microorganisms have indicated that the composition of membrane fatty acids is an important factor. Several studies have shown that variations in the composition of dietary fatty acids alter the fatty acid composition of membranes [4]. However, it is not clear whether such changes influence membrane properties. Most studies to date do not permit an unequivocal interpretation of results because of inadequate experimental designs. The level of dietary fat, the type of diet, and the level of essential fatty acids have varied between experimental groups [4]. In addition some diets were deficient in trace elements, which can influence lipid metabolism [5]. It does seem apparent, however, that the dietary fatty acid composition affects glucagon-stimulated adenylate cyclase activity in liver plasma membranes [6] and Ca^{2+}-ATPase activity in rat erythrocytes [7], but does not affect rat heart mitochondrial respiration [8].

The composition of the red cell membrane changes with alterations in the lipid composition of the diet [4]. It therefore is a good model for investigating what influences the dietary fatty acid composition has on membrane properties. One of the important properties of the red cell membrane is the regulation of cell volume. In this report we have examined whether the saturation and isomerization of dietary fatty acids influences the osmotic fragility and the diffusional water permeability of erythrocytes, two properties related to the regulation of red cell volume.

Weanling, male Sprague-Dawley rats were fed purified diets containing 15% fat (w/w) for 6 weeks. The fats used were corn oil, a high oleic acid safflower oil, lard, and a blend of 82% hydrogenated soybean oil and 18% soybean oil. The components of the diets and their fatty acid compositions have been previously described [8]. 8–12 ml of blood were collected by canulation of the

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thoracic aorta in a syringe containing 1 ml of 10 mM EDTA in phosphate-buffered saline (pH 7.4). Erythrocytes were collected by centrifugation at 500 × *g* for 8 min and washed three times with 166 mM NaCl, removing the buffy layer on top of the erythrocytes each time.

The dietary fats induced changes in the fatty acid composition of the erythrocytes as shown in Table I. The major changes were in the 18 carbon unsaturated fatty acids. Only minor changes were detected in the levels of the major fatty acids, palmitic, stearic, and arachidonic acids, except in the hydrogenated soybean oil treatment, where *trans* isomers were apparently incorporated in place of saturated fatty acids. Erythrocytes from rats on the safflower oil diet contained the highest 18 : 1 *cis* level, those on the corn oil diet contained the highest 18 : 2 level, whereas those on the hydrogenated soybean oil diet contained the highest 18 : 1 *trans* level. Erythrocytes from rats fed hydrogenated soybean oil had lower levels of 20 : 4 ($n-6$) and higher levels of 18 : 2 ($n-6$), 22 : 5 ($n-3$), and 22 : 6 ($n-3$). This apparently results from the higher 18 : 3 ($n-3$) content of this diet and the inhibition of 18 : 2 ($n-6$) elongation by ($n-3$) fatty acids [10]. Despite the higher concentration of saturated fatty acids in the lard diet, it did not elevate the level of saturated fatty acids in rat erythrocytes compared to the corn oil and safflower oil diets. These results are similar to those obtained with feeding 10% fat diets [7]. They suggest that regulatory mechanisms function to preserve an almost constant lipid environment in the erythrocyte membrane. This may relate to the crucial properties of membrane lipid in influencing membrane and cellular functions.

TABLE I

THE EFFECT OF DIETARY FATS ON ERYTHROCYTE FATTY ACID COMPOSITION

Lipids were extracted from cells using a modification of the method of Ways and Hanahan [9]. 1 ml of washed erythrocytes was mixed with 5 ml of methanol. 5 ml of $CHCl_3$ were added and the extract was allowed to sit at 22 °C for 30 min, vortexing periodically. After centrifugation at 500 × *g* for 8 min the supernatant was filtered and the residue resuspended in 10 ml of $CHCl_3/CH_3OH$ (1 : 1) containing 0.1% BHT. This suspension was sonicated in a Bransonic 220 bath for 20 min, maintaining the temperature at 22 °C. Following centrifugation and filtration, the combined extracts were dried under N_2. The resultant residue was then reextracted twice with 5 ml of $CHCl_3$ and once with 5 ml of CH_3OH, filtering each extract before pooling. This was then washed with 3 ml of 0.1 M KCl, followed by washing with 5 ml of the upper phase from a 10 : 5 : 3 mixture of $CHCl_3/CH_3OH$/0.1 M KCl. The extract was dried and fatty acid methyl esters were prepared and identified by gas chromatography using a 60-m capillary column as previously described [8]. The data are expressed as the mean percent of total ± S.E. for five samples. CO, corn oil; HSO, 82% partially hydrogenated soybean oil + 18% soybean oil; L, lard; SFO, high oleic acid safflower oil. PUFA, polyunsaturated fatty acid.

Fatty acid	Erythrocyte fatty acid composition			
Diet:	CO	SFO	L	HSO
16 : 0	25.3 ± 0.7	21.8 ± 0.5	23.7 ± 0.7	17.3 ± 0.5
18 : 0	11.9 ± 0.8	12.2 ± 0.8	11.3 ± 0.5	8.3 ± 0.4
18 : 1 *trans*	0.6 ± 0.1	0.1 ± 0.1	0.1 ± 0.03	7.5 ± 0.8
18 : 1 *cis*	6.3 ± 0.1	13.4 ± 0.3	10.8 ± 0.4	11.9 ± 0.6
18 : 2 ($n-6$)	12.5 ± 0.6	7.2 ± 0.3	7.6 ± 0.2	10.3 ± 0.4
20 : 3 ($n-9$)	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.1 ± 0.1
20 : 4 ($n-6$)	31.7 ± 0.5	33.9 ± 1.0	32.6 ± 1.1	29.2 ± 1.1
24 : 1 ($n-9$)	0.3 ± 0.1	1.1 ± 0.2	0.8 ± 0.2	0.3 ± 0.1
22 : 4 ($n-6$)	2.7 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	1.6 ± 0.1
22 : 5 ($n-6$)	2.1 ± 0.1	2.6 ± 0.3	1.7 ± 0.1	0.5 ± 0.1
22 : 5 ($n-3$)	0.6 ± 0.0	0.2 ± 0.1	0.7 ± 0.03	1.5 ± 0.1
22 : 6 ($n-3$)	1.8 ± 0.1	1.1 ± 0.1	2.3 ± 0.1	4.1 ± 0.2
Total saturated	37.5 ± 1.0	34.3 ± 0.9	35.4 ± 0.9	25.9 ± 0.8
Total PUFA	52.4 ± 0.7	48.0 ± 1.0	48.3 ± 1.1	48.2 ± 0.8
Total octadecenoic	6.9 ± 0.1	13.5 ± 0.3	10.9 ± 0.4	19.4 ± 0.7
Total ($n-6$)	49.8 ± 0.7	46.3 ± 1.0	44.8 ± 1.1	42.4 ± 0.9
Total ($n-3$)	2.4 ± 0.1	1.4 ± 0.1	3.0 ± 0.1	5.6 ± 0.2

A key property of erythrocytes is the regulation of cell volume by diffusional water flow through the membrane. This is believed to occur through a protein-formed channel in the membrane. The protein nature of the channel is evident in the inhibition of water transport by sulphydryl reagents such as *p*-chloromercuribenzene sulphonate and fluorescein mercuric acetate [11] and the sensitivity of the *p*-chloromercuribenzene sulphonate binding site to papain digestion [12]. The exact identification of the protein or proteins forming the channel is not known. There is some evidence using sulphydryl inhibitors and proteases that band 3 is involved [12,13]. Such a channel must span the membrane and its sensitivity to the physical state of the membrane lipids in its environment is evident form an Arrhenius plot of water transport versus temperature. A discrete breakpoint has been detected at 26 °C in human erythrocytes [14]. For measuring water permeability by an NMR technique, erythrocytes were suspended in 166 mM NaCl containing 0.1% glucose and 1% defatted bovine serum albumin, at a hematocrit of 50%, and 0.2 ml of this suspension was mixed with 0.1 ml doping solution (40 mM $MnCl_2$, 100 mM NaCl). The water proton relaxation time of the erythrocytes (T'_{2a}) was measured by the spin-echo method as previously described [15,16]. The T'_{2a} is dominated by the exchange process through the membrane and the water exchange time through erythrocyte membranes is inversely related to the water permeability of erythrocytes as shown by Conlon and Outhred [17]. Measurements were performed with an Aremi-78 spectrometer (Institute of Physics and Nuclear Engineering, Bucharest-Magurele, Romania). A variable temperature unit attached to the spectrometer maintained the temperature at 37 ± 0.2 °C by air flow over an electrical resistance.

There were no significant differences in the water permeability of erythrocytes from rats fed the four diets as reflected in the measured water proton relaxation times (T'_{2a}). Mean values ± S.D. in ms were: corn oil, 3.66 ± 0.22, ($n = 7$); safflower oil, 3.55 ± 0.29 ($n = 7$); lard, 3.64 ± 0.32 ($n = 8$); hydrogenated soybean oil, 3.55 ± 0.25 ($n = 8$). These results indicate that if the lipid environment containing the water channel is altered by changes in the dietary fatty acid composition, such changes do not affect diffusional water transport. These observations are consistent with the report by Sha'afi [18] that varying the cholesterol content in the membrane from 0.84 to 1.87 mg/ml cells did not change the permeability coefficient of the human red cell membrane to water.

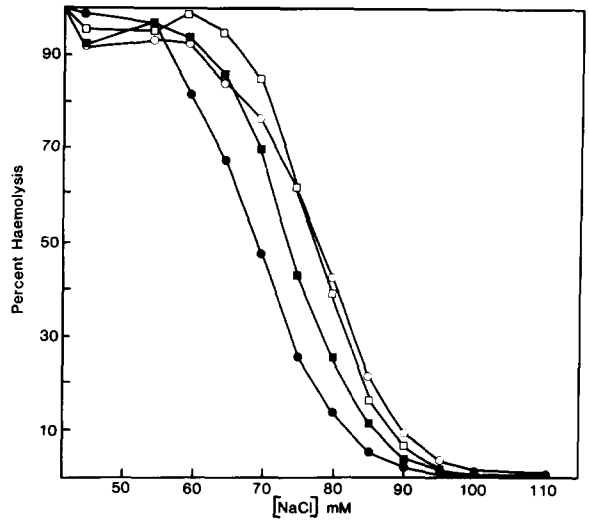

Fig. 1. Effect of dietary fatty acids on the haemolysis of rat erythrocytes. The method described by Ehrstrom et al. [19] was used to measure the osmotic fragility of erythrocytes from rats fed either corn oil (●), safflower oil (○), lard (■), and hydrogenated soybean oil (□). The results represent the mean values of five animals.

The osmotic fragility of erythrocytes is a property depending on the movement of water into the cells. An increased osmotic fragility is of clinical significance because it has been linked to several diseases that result in haemolytic anemia [19]. This change in osmotic sensitivity has been associated in most cases with changes in the intracellular content of monovalent cations, the major, osmotically active species within the erythrocyte.

The osmotic fragilities of erythrocytes from rats fed the various diets are illustrated in Fig. 1. Fifty percent haemolysis was obtained at 68.9 mM NaCl for erythrocytes from rats on the corn oil diet, 84.0 ± 4.3 mM NaCl on the lard diet, 77.7 ± 3.2 mM NaCl on the safflower oil diet, and 77.9 ± 3.4 on the hydrogenated soybean oil diet. The effect of corn oil was significantly different from both the effect of safflower or hydrogenated oils ($P < 0.05$), using the Student's *t*-test, but was not different from that of lard.

These results suggest that there is a relationship between *cis*-octadecenoic acid levels in the membrane and osmotic fragility, as shown in Fig. 2. The erythrocytes from the rats fed the hydrogenated soybean oil did not follow this trend, presumably because of their high content of *trans*-isomers. The relationship appeared strongest with the total *cis*-octadecenoic acid level. Oleic and vaccenic acids were resolved on the capillary column and the relationship observed was weaker with the level of oleic acid, and was not at all related to the level of *cis*-vaccenic acid. *cis*-Vaccenic acid was 1.3% of the total fatty acids with the corn and safflower oil diets and 2.4% with the lard diet. This suggests that the position of the double bond is probably not important in this relationship at least with respect to the $(n-7)$ and $(n-9)$ positions. The reasons for the relationship between an increased content of *cis*-octadecenoate in the membrane and an increased osmotic fragility are not known. The content of these fatty acids also appears to be important in determining the extent of glucagon-stimulated adenylate cyclase activity in rat liver plasma membranes [6]. It is possible that changes in the *cis*-octadecenoate content of phospholipids alters the partitioning of these phospholipids into fluid- or gel-like lipid domains in the membrane.

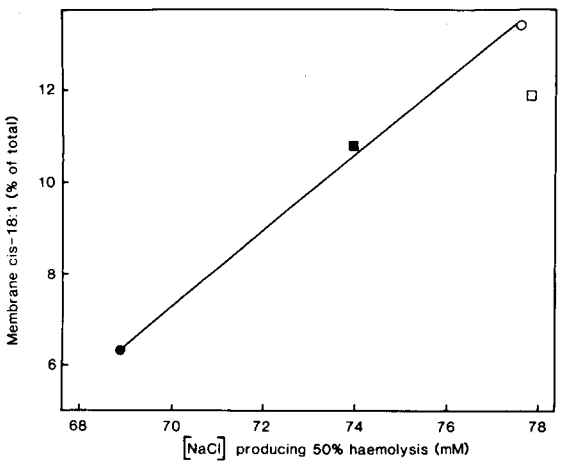

Fig. 2. The relationship of octadecenoate levels to the osmotic fragility of erythrocytes. Symbols are the same as in Fig. 1.

The factors which determine the osmotic fragility of erythrocytes are complicated and include cation content [19], cellular age [21], individual age [22], and a change in cell shape [23]. The changes we have observed suggest that the type of dietary fat ingested and the membrane lipid composition are other factors that have to be considered in understanding the osmotic fragility of erythrocytes. The possible interaction of these factors is evident in a change in the lipid composition of erythrocytes with aging [24]. It is interesting to note that an increase in fragility with the aging of human erythrocytes [21] is associated with an increased 18 : 1 *cis* fatty acid content [24]. Our results also indicate that it is not possible to determine whether in essential fatty acid deficiency in rats, a lack of essential fatty acids or their derivatives causes the increased osmotic fragility of erythrocytes or whether the increased membrane level of *cis*-octadecenoic acid produces the effect [20].

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