Human Erythrocyte Phosphoglycerides. II. Diet and Lecithin Structure

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Abstract

Lecithins (separated on basic silicic acid columns) were obtained from humans fed three different diets: either *ad-libitum* or diets containing 40% of calories from linoleic acid (as corn oil) or from oleic acid (as triolein). Four lecithin subfractions were studied from each dietary group. Lecithin fractions eluting earliest (and apparently the least polar) contained the highest molar ratios of unsaturated fatty acids and the highest proportion of C-20 to C-22 polyunsaturated fatty acids. A slight increase in proportions of diunsaturated molecules occurred in corn oil and triolein groups. However, over 90% of lecithins of each dietary group were maintained as the *monosaturated - monounsaturated* type. Therefore, in contrast to human adipose tissue triglycerides, the saturated/unsaturated fatty acid ratio of lecithins of the erythrocyte membrane is largely unaffected by immense increases in dietary unsaturated fatty acid. Major shifts of oleic and linoleic acid occurred but proportions were unaltered of longer chain length (> C-18) polyunsaturated fatty acids. The relevance of these findings to membrane structure and function and to glycerophosphatide biosynthesis is discussed.

Introduction

PREVIOUSLY THE EFFECTS of alterations of dietary fatty acids on the various phospholipids of human erythrocytes were described (1). This report extends the earlier studies to detail the differences in fatty acid composition induced by diet in *sub*-fractions of a single class of red cell phosphatide, the choline phosphoglycerides. In human erythrocytes only 8% of this lipid class is in the form of alkenyl, alkylglycerylphosphorylcholine (choline plasmalogen) (2); the remainder is in the diacyl form (lecithin) (2). Therefore calculated molar ratios of unsaturated to saturated fatty acids of the total mixed choline phosphoglycerides will allow estimates of the most common internal molecular arrangement of fatty acids in these molecules.

Methods

Erythrocytes were obtained from thrice-washed blood samples placed into EDTA (1 mg EDTA per ml blood) after withdrawal from 10 human subjects. Eight subjects were normal controls and were ambulatory. Two subjects were hospitalized in a metabolic ward and their dietary intake controlled, body weights were stable during this period. The subjects consumed different diets: the erythrocytes obtained from the subjects will be identified by the nature of the diet. First diet period: *Ad-libitum,* a diet of unknown but usual nature, consumed habitually by the 8 "normal" subjetcs. Equal amts of washed erythrocytes (by volume) from each of these subjects were pooled for subsequent study. Second diet period: *Triolein,* a liquid formula diet (3) containing 40% of calories from triolein, 15% from protein and 45% from carbohydrate. Fatty acid content of the fed triolein was > 99% oleic acid when analyzed by gas-liquid chromatography following transesterification with methonal (4). This was consumed for 15 weeks by a subject (a 35-year-old white female with familial hypercholesterolemia) before the blood was obtained for study. Third diet period: *Corn Oil,* a liquid formula diet containing 70% of calories from corn oil, 15% from protein and 15% from carbohydrate. Fatty acid content of the corn oil was approx 56% linoleic acid, therefore 40% of calories were derived from this fatty acid. Other fatty acids in corn oil were: 18% oleic acid, and 23% saturated fatty acids (principally palmitic acid). This was consumed for 8 weeks by a subject (a 54-year-old white male with arteriosclerotic heart disease and hypercholesterolemia) before blood was obtained for this study.

An almost complete recovery (> 97%) of the erythrocyte lecithin of the three categories was obtained by methods previously reported (2). These lecithins were then separated, by differences in net polarity, into four separate and almost equal fractions, by chromatography on basic silicic acid columns (5). Molar fatty acid compositions of each fraction were determined by methods previously described (4,6).

Results and Discussion

A minimal frequency of occurrence of *disaturated,* of *monounsaturated-monosaturated,* and of *diunsaturated* lecithins was calculated for each of the four lecithin fractions of erythrocytes representing the three different dietary conditions. The calculations, Table I, were made by use of a binomial expansion, substituting the frequency of saturated fatty acids (S) and of unsaturated acids (U) in each lecithin fraction into: $(S + U)^2$. Results are expressed as the sum of minimum frequency of occurrence of *SS, US* and *UU* molecules and are contrasted to the frequency

TABLE I

Human Erythrocyte Lecithins: Deviations of Structure from Random Distribution of Fatty Acids

Dietary state	*Diunsaturated*		*Monosat-Monounsat*		*Disaturated*	
	Theory (Random)	Found	Theory (Random)	Found	Theory (Random)	Found
Ad libitum	25%	2.9%	50%	93.6%	25%	3.5%
Triolein	34%	8.8%	49%	91.2%	17%	0
Corn Oil	27%	4.4%	50%	95.6%	23%	0

TABLE II

Human Erythrocyte Choline Phosphoglycerides: Dietary Effects on Fatty Acid Distributions

	Ad libitum	Corn Oil	Triolein
16:0	39	36	38
18:0	11	12	5
18:1	21	16	45
18:2	20	30	6
20:un	8	6	6
22:un			
Sat [a]	50	48	42
Unsat	50	52	58

[a] Includes aldehydrogenic chains.

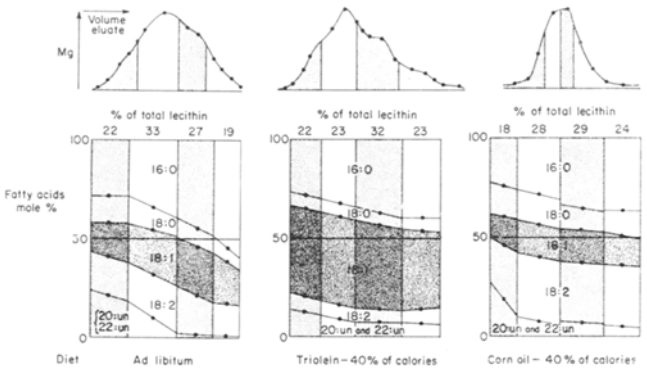

FIG. 1. Human erythrocyte lecithins, isolated by silicic acid chromatography.

expected if all fatty acids were distributed randomly.

It is clear from Table I and Figure 1 that the predominant molecule in erythrocyte lecithin of all three dietary states is monounsaturated, monosaturated. This indeed is expected since lecithins of a variety of animal tissues are predominantly of this type (7–9). These calculations (Table I) are, of course, estimates: analysis of narrower segments of the eluting lecithin would increase slightly the proportions of diunsaturated and disaturated molecules. It is also clear that molecules separate according to their fatty acid composition, the more unsaturated molecules eluting first. A similar behavior has been noted (10) on elution of two fractions of rat liver lecithins on untreated (neutral) silicic acid columns.

Figure 1 shows that the major alterations produced by triolein or corn oil diets occurred in substitution of oleic and linoleic acids, as a function of the total amts provided in the diet. It is also evident from Table I that a modest increase (from 2.9% to 8.8% and 4.4% in dietary periods 1, 2 and 3) in diunsaturated molecules occurred in the triolein and corn oil products. Some minor variations also are evident, such as an apparent decrease in stearic acid following triolein but not following corn oil (Table II). The cause for this is not clear but would occur if, for example, oleic acid but not linoleic acid was "permitted" to occupy the α-position of lecithin. Also, (Table II) the molar proportion of unsaturated fatty acids rose from 50% to 58% and 52% in the triolein and corn oil periods. However, these changes (Table II) are minor in comparison to the extensive changes in fatty acids of tissue triglycerides observed in many subjects ingesting these diets for period up to three years (1,11).

To explain the differences in degree of "imprinting" of dietary fatty acids into triglycerides and phospholipids, one could suggest that the diglyceride precursors in the supposed common pathway of biosynthesis of these glycerol-lipids (12) are selected with greater specificity for diversion into glycerophospholipids. Alternatively, this specificity could well result instead from selectivity of a reacylase enzyme (13–16) responsible for reconstitution of the original glycerophosphatide molecules following their cleavage by phospholipase(s) to α-lysolecithins (16,17). Further study will be required to determine which of these alternatives is responsible, not only for the dietary induced alteration of fatty acids reported, but for the distribution of fatty acids in phospholipids of other membrane structures. Currently there is little direct evidence to allow one to choose between these alternatives. However, recent studies of Stein et al. (18) on lipid synthesis in rabbit aorta provide indirect evidence in support of the thesis that many tissue phospholipids are extensively reorganized by the sequence of phospholipase and lysolecithin reacylase mechanisms proposed by Lands et al. (17,19).

It is of interest to ask if important changes in function of structural phospholipids will occur secondary to environmentally induced variation in their fatty acid structures. Some data exist relevant to this question. Two examples are: the absolute requirement for lecithins containing unsaturated fatty acids for activity of a mitochondrial dehydrogenase (20) and the different swelling rates of mitochondria of varying fatty acid composition (21). Further studies of these issues are clearly needed.

ACKNOWLEDGMENT

Assistance from former colleagues at the Rockefeller Institute, and from E. H. Ahrens, Jr., in whose laboratories this work was begun. Triolein supplied by Dr. F. Mattson, Miami Valley Laboratories, Cincinnati, Ohio.

This work was supported in part by USPHS grants AM 05972, HE-06222, and 5-K3-HE-6003 from the National Institutes of Health.

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