Fatty Acid Composition of Different Serum Phospholipids in Men and Women

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1. Eight young healthy persons, four men and four women, were maintained for a total of 2 months on a diet in which 40%, 16% and 44% of the total calories were present as fats, proteins and carbohydrates respectively. The ratio of complex to simple carbohydrates in the diet was 1:4. 2. The fatty acids of serum kephalins, lecithins, lysolecithins and sphingomyelins were determined by gas-liquid column chromatography. 3. Lysolecithins in both men and women had the highest content of saturated acids, followed by sphingomyelins, lecithins and kephalins in that order. The degrees of saturation and of polyunsaturation of the fatty acids in the different phospholipid fractions were significantly different, except for the differences in the polyunsaturation of the kephalins and lecithins. 4. No sex difference was found in the fatty acid composition of the different phospholipids.

Changes in dietary fat or carbohydrate have been shown to affect significantly the major classes of serum lipids and their fatty acids (Ahrens *et al.* 1959; Dole, James, Webb, Rizack & Sturman, 1959; Macdonald & Braithwaite, 1964; Antar & Ohlson, 1965); however, most of the fatty acid analyses of the individual phospholipids of human serum or plasma that have been reported (Nelson & Freeman, 1960; Hanahan, Watts & Pappajohn, 1960; Tattrie & Cyr, 1963) have been based on a pooled sample, samples from persons whose dietary history was unknown, or samples drawn after a loading test. In this study, which was part of a larger project designed to test the effect of dietary carbohydrates on serum lipids, the fatty acid composition of the individual phospholipids of young men and women maintained on a prescribed diet is reported.

METHODS

Details of the experimental design have been given previously (Antar & Ohlson, 1965). Briefly, eight healthy persons, four men and four women, aged 20–25 years, participated in the study as out-patients. Their medical histories were taken and each was given a complete physical examination. Three spaced meals were eaten daily in the metabolic ward under the supervision of a dietician. A bedtime snack was also provided. The diet was composed as follows: fats, 40%; proteins, 16%; carbohydrates, 44%, of the total calories. Saturated and polyunsaturated fatty acids (as linoleic acid) comprised respectively 50% and 7%

of the total fat. The cholesterol content was 829 mg./2500 kcal. Animal proteins (92%) and some vegetable proteins (8%) accounted for total protein. About 80% of the carbohydrates was given as simple carbohydrates, consisting mainly of mono- and di-saccharides (refined sugars, syrups, milk and fruits), and 20% given as complex carbohydrates (cereal products, potatoes, legumes and vegetables). The results are for two identical dietary periods of 4 weeks, separated by a 4-week interval when the subjects ate the same diet except that starches replaced simple sugars. The diet was adjusted for each person by a percentage increase or decrease of all nutrients to maintain constant weight throughout the study. Blood samples, each about 25 ml., were taken from an antecubital vein with a silicone-treated needle and syringe after overnight starvation at the end of each dietary period.

Total serum lipids were extracted with chloroform-methanol (2:1, v/v), purified, and separated into phospholipids and non-phospholipids by silicic acid column chromatography. The phospholipids were further fractionated by silicic acid column chromatography (Unisil-Clarkson Chemical Co. Inc., Williamsport, Pa., U.S.A.), under N₂, as described in detail by Antar & Ohlson (1965) by the method of Hanahan, Dittmer & Warashina (1957). The collecting tubes, which were screw-cap vials (16 mm. × 125 mm.) with Teflon liners, were capped as rapidly as possible after filling and stored at 4° until required for analysis. Separation of the fractions containing the main phospholipids was by measuring phosphorus on successive samples according to Bartlett (1959). The mean recovery of total phosphorus in the different fractions was 91.5 ± 1.75% (S.D.) of the phosphorus in the total phospholipids. These compare with the recoveries reported by Hanahan *et al.* (1957) of 88–95%.

The main fractions (kephalins, lecithins, lysolecithins and sphingomyelins) were evaporated under vacuum. Methanolysis was carried out under N₂ in sealed tubes with screw caps and Teflon liners at 70–75° for 5 hr. with freshly

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prepared methanol-H₂SO₄ (95:5, v/v). The methyl esters were extracted with hexane three times; the extracts were washed with distilled water three times and dried with anhydrous Na₂SO₄. Methanol-H₂SO₄ was preferred to methanol-HCl because the former gives quantitative recoveries of the expected methyl esters of sphingomyelins (Nelson, 1962).

The fatty acid mixtures were analyzed by gas-liquid column chromatography on a Barber-Colman model 10 gas chromatograph equipped with an argon ionization detector. A polyethylene glycol succinate coating on a Chromasorb W column was maintained at 185–190° and 14 lb./in.² argon inlet pressure. Weighed mixtures of known fatty acids obtained from the National Institutes of Health Metabolism Study Section (Standard Mixtures A, B, C and D) and the Hormel Institute (Standard Mixtures 1, 3, 4 and 5) were used as standards. Areas under the chromatogram curves proportional to the amounts of different fatty acids were measured with a polar planimeter. The percentages of the main fatty acids detected are recorded separately. Fatty acids for which no standards were available are reported as unidentified fatty acids. When the percentage of fatty acid was less than 0.5, it is recorded as a trace.

RESULTS

Table 1 gives the mean fatty acid composition of the different fractions of serum phospholipids during two periods on the same diet. Each fraction had a characteristic fatty acid pattern. Lysolecithins

in both men and women had the highest content of saturated acids, followed by sphingomyelins, lecithins and cephalins, in that order.

Analysis of variance indicated that these differences in fatty acid pattern were significant. The *t* test showed that differences in the percentages of saturated, monounsaturated and polyunsaturated fatty acids in each pair of fractions were statistically significant ($P < 0.01$), except for the difference in the polyunsaturated fatty acids of cephalins and lecithins, and the difference in the monounsaturated fatty acids of lecithins and sphingomyelins. Such differences suggest selective incorporation or hydrolysis of fatty acid residues in different phospholipids. There was no significant sex or subject difference.

Fatty acids of chain length less than 16 carbon atoms constituted a small proportion of the total, except for the fatty acids from the cephalins, which showed many small unidentified peaks. Some fractions contained a trace of linolenic acid.

DISCUSSION

Differences in fatty acid composition of individual fractions of phospholipids have been previously reported (Nelson & Freeman, 1960; Hanahan *et al.*

Table 1. *Fatty acid composition of serum phospholipids in men and women*

The amount of each fatty acid is expressed as a percentage of the total present in the fraction $\pm$ S.D. Each value is the mean of eight analyses (two on each individual). M, Men; W, women.

Fatty acids	Sex	Kephalins	Lecithins	Lysolecithins	Sphingomyelins
12:0	M	0.8 $\pm$ 0.29	Trace	Trace	0.6 $\pm$ 0.52
	W	0.8 $\pm$ 0.46	Trace	Trace	1.8 $\pm$ 1.62
14:0	M	2.3 $\pm$ 0.94	0.7 $\pm$ 0.35	1.8 $\pm$ 0.89	1.5 $\pm$ 0.82
	W	2.2 $\pm$ 0.71	0.9 $\pm$ 0.37	1.4 $\pm$ 0.60	1.4 $\pm$ 0.73
16:0	M	27.2 $\pm$ 5.85	43.2 $\pm$ 2.97	62.2 $\pm$ 2.55	47.5 $\pm$ 6.32
	W	26.0 $\pm$ 4.53	42.3 $\pm$ 3.47	63.6 $\pm$ 8.11	48.0 $\pm$ 3.22
18:0	M	18.6 $\pm$ 3.39	13.3 $\pm$ 1.19	13.1 $\pm$ 2.35	14.4 $\pm$ 2.26
	W	20.5 $\pm$ 4.55	13.3 $\pm$ 1.31	13.3 $\pm$ 2.67	13.7 $\pm$ 2.24
20:0	M	1.6 $\pm$ 1.15	0.8 $\pm$ 0.78	4.4 $\pm$ 1.66	Trace
	W	1.3 $\pm$ 1.15	0.7 $\pm$ 0.66	3.3 $\pm$ 1.10	Trace
16:1	M	2.3 $\pm$ 0.78	1.0 $\pm$ 0.79	0.8 $\pm$ 0.77	1.8 $\pm$ 0.83
	W	2.1 $\pm$ 0.88	1.0 $\pm$ 0.51	0.6 $\pm$ 0.35	1.2 $\pm$ 0.20
18:1	M	14.4 $\pm$ 3.28	11.1 $\pm$ 1.10	4.4 $\pm$ 1.57	10.6 $\pm$ 2.40
	W	13.1 $\pm$ 2.21	12.2 $\pm$ 1.62	4.8 $\pm$ 2.64	11.5 $\pm$ 1.83
18:2	M	10.3 $\pm$ 4.28	16.4 $\pm$ 0.61	2.6 $\pm$ 1.57	11.0 $\pm$ 4.57
	W	8.5 $\pm$ 3.64	17.4 $\pm$ 1.03	3.6 $\pm$ 1.81	12.6 $\pm$ 1.86
20:4	M	13.9 $\pm$ 3.15	9.2 $\pm$ 0.70	8.0 $\pm$ 3.11	7.7 $\pm$ 3.03
	W	16.0 $\pm$ 3.82	9.1 $\pm$ 0.58	6.1 $\pm$ 3.13	6.4 $\pm$ 3.01
Unidentified	M	8.5 $\pm$ 4.27	4.3 $\pm$ 0.96	2.5 $\pm$ 0.83	3.9 $\pm$ 3.48
	W	9.6 $\pm$ 2.53	2.8 $\pm$ 0.85	3.0 $\pm$ 2.27	3.3 $\pm$ 1.94
Total saturated	M	50.8 $\pm$ 4.16	58.0 $\pm$ 2.09	81.7 $\pm$ 5.51	65.0 $\pm$ 6.42
	W	50.5 $\pm$ 1.72	57.4 $\pm$ 2.96	82.1 $\pm$ 6.55	65.0 $\pm$ 3.46
Total monounsaturated	M	16.7 $\pm$ 2.77	12.1 $\pm$ 1.53	5.2 $\pm$ 2.05	12.4 $\pm$ 3.78
	W	15.2 $\pm$ 2.58	13.2 $\pm$ 1.87	5.4 $\pm$ 2.91	12.7 $\pm$ 1.62
Total polyunsaturated	M	24.2 $\pm$ 3.61	25.6 $\pm$ 0.75	10.6 $\pm$ 4.16	17.9 $\pm$ 5.64
	W	24.4 $\pm$ 3.50	26.5 $\pm$ 1.08	9.6 $\pm$ 4.31	19.0 $\pm$ 3.69

1960; Rowe, 1960; Nelson, 1962; Tattrie & Cyr, 1963; Sweeley, 1963). There are discrepancies within these reports that may be due in part to differences in the previous dietary intake of the subjects from whom the blood was withdrawn. In the present study a diet containing a high proportion of simple carbohydrates was given under defined conditions, and our data indicate that the kephalins were the most unsaturated phospholipids of serum in both men and women; lecithins, sphingomyelins and lysolecithins showed less unsaturation, in that order. In general, this is in agreement with the data of Nelson & Freeman (1960), Hanahan *et al.* (1960), Rowe (1960), Nelson (1962) and Tattrie & Cyr (1963), but no dietary histories of their subjects were reported. Hanahan *et al.* (1960) found, as in the present study, that sphingomyelins contained fewer unsaturated fatty acids than lecithins, but their values for the unsaturated fatty acids of sphingomyelins were less than the values reported here and than those of Nelson (1962).

Sweeley (1963), using pooled samples of human plasma, found seven major fatty acids in sphingomyelins: 16:0, 18:0, 18:1, 20:0, 22:0, 23:0 and 24:1. Of these, palmitic acid comprised about 41.7% of the fatty acids in comparison with 47.5–48.0% in the present study. He found little evidence for the presence of linoleic acid or arachidonic acid, which together comprised 7–29% of the total in the

studies of other investigators (Nelson & Freeman, 1960; Hanahan *et al.* 1960; Nelson, 1962) and in the present study. These discrepancies cannot be explained at present.

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REFERENCES

- Ahrens, E. H., jun., Insull, W., Hirsch, J., Stoffel, W., Peterson, M. L., Farquhar, J. W., Miller, T. & Thomasson, H. J. (1959). *Lancet*, i, 115.
Antar, M. A. & Ohlson, M. A. (1965). *J. Nutr.* **85**, 329.
Bartlett, G. R. (1959). *J. biol. Chem.* **234**, 466.
Dole, V. P., James, A. T., Webb, J. P. W., Rizack, M. A. & Sturman, M. F. (1959). *J. clin. Invest.* **38**, 1544.
Hanahan, D. J., Dittmer, J. C. & Warashina, E. (1957). *J. biol. Chem.* **228**, 685.
Hanahan, D. J., Watts, M. R. & Pappajohn, D. (1960). *J. Lipid Res.* **1**, 421.
Macdonald, I. & Braithwaite, D. M. (1964). *Clin. Sci.* **27**, 23.
Nelson, G. J. (1962). *J. Lipid Res.* **3**, 71.
Nelson, G. J. & Freeman, N. K. (1960). *J. biol. Chem.* **235**, 578.
Rowe, C. E. (1960). *Biochem. J.* **76**, 471.
Sweeley, C. C. (1963). *J. Lipid Res.* **4**, 402.
Tattrie, N. H. & Cyr, R. (1963). *Biachim. biophys. Acta*, **70**, 693.