Title
FATTY ACID COMPOSITION OF MOUSE LIPIDS AND LIPOPROTEINS

Authors
Rehnborg, Carl S.
Nichols, Alex V.
Ashikava, James K.

Publication Date
1961

UCRL-9521

UNIVERSITY OF CALIFORNIA

Ernest O. Lawrence

Radiation Laboratory

BERKELEY, CALIFORNIA

DISCLAIMER

UCRL-9521

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

FATTY ACID COMPOSITION OF MOUSE LIPIDS AND LIPOPROTEINS

Carl S. Rehnborg, Alex V. Nichols, and James K. Ashikawa

January 1961

Recent advances in gas chromatographic techniques have made possible the determination of the fatty acid composition of small quantities of biological lipids. The sensitivity of these techniques allows measurement not only of whole plasma fatty acids, but of fatty acids present in the various plasma lipid esters as well as in isolated lipoprotein fractions. Thus, Dole et al (1) have reported on the plasma lipid fatty acid composition for man and a number of animal species. Lindgren et al (2) have determined the fatty acid distribution in lipid esters of the major human serum lipoprotein fractions. The wide use of mice in biological experimentation both in this and other laboratories has prompted the present report on the fatty acid composition of plasma lipids in normal untreated mice. Included in this report is a comparison of mouse plasma fatty acid composition data with those obtained for humans.

Methods

Samples of plasma were obtained from 31 ten-week-old male Swiss white mice, weighing approximately 26 grams, and existing exclusively on Simonsen Laboratory white diet and water ad libitum. Blood was drawn by heart puncture and the separated plasma was used in subsequent lipid analyses. Plasma lipoprotein fractions were isolated by the method of successive preparative ultracentrifugation previously reported (3). Three centrifugal fractions were obtained: (1) all low density lipoproteins having a density less than 1.063 g/ml., (2) high density lipoproteins (HDL) ranging in density between 1.063 to 1.218 g/ml., and (3) a sedimenting protein fraction of density greater than 1.218 g/ml. The latter fraction contains the major part of the plasma non-esterified fatty acids (NEFA) which are bound to albumin. Lipid extraction was accomplished by a modified method of Sperry et al (4). NEFA extraction was carried out by the procedure of Dole (5). The extracted lipids were separated by silicic acid chromatography (6) into three chemical fractions: (1) cholesteryl esters, (2) glycerides, cholesterol, and NEFA, and (3) phospholipids. The amounts of lipid in these fractions were determined by infrared spectroscopy. Lipid fractions were transmethylated according to the procedure of Stoffel et al (7). Methyl esters were subjected to gas-liquid chromatography using the apparatus and

procedures described by Upham et al (8). A 52 inch glass column (6mm. inner diameter) was packed with 48-65 mesh Chromosorb, coated with 30% by weight succinic acid diethylene glycol polyester (LAC-2R-728)*. The resulting chromatograms were evaluated by a punched card technique reported by Tandy et al (9). The major fatty acids are reported according to the nomenclature proposed by Dole et al (1). Minor and unidentified constituents, amounting to approximately 15% of the total methyl ester weight, are designated as in a previous report (2) by: Pre 16:0, 16:0 - 18:0, 18:2 - 20:4, and Post 20:4. These designations refer to the elution position of such esters relative to the more abundant identified esters on the succinic acid diethylene glycol polyester column coating.

Results

The fatty acid composition of the individual lipid compounds in mouse plasma are presented in Table I. These values show significant differences in fatty acid composition. The cholesteryl esters contain a high percentage of polyunsaturated acids, linoleic and arachidonic, with all other acids markedly low. The fraction containing both glycerides and NEFA shows palmitic, oleic, and linoleic as the principal acids. In the phospholipids, palmitic, stearic, and linoleic predominate while the lower oleic content is comparable to that of arachidonic acid.

Comparison of the mouse data can be made with normal human lipid fatty acid values (2) shown in Table II. The percentage of arachidonic acid in mouse cholesteryl esters is significantly higher than in the human. On the other hand the human cholesteryl esters have a higher percentage of palmitic and oleic acids than mouse esters. In the glyceride and NEFA fraction of the mouse the percentage of linoleic acid is approximately double that in the human. However, oleic acid in human glycerides is higher than in the mouse. The phospholipid fractions of both the mouse and human show a general similarity in fatty acid composition. The content of linoleic acid is greater in the mouse than in human phospholipids. In general this

*Cambridge Industries, 101 Potter Street, Cambridge 42 Mass.

comparison shows a significantly higher content of polyunsaturated fatty acids in mouse lipids than in the human.

Since all mouse plasma lipids are transported bound either to lipoproteins or to albumin the fatty acid composition of such macromolecular complexes was determined. In Table III are presented the data on fatty acids in three mouse plasma macromolecular fractions. Comparison of these data indicates the following points: (1) the low density lipoprotein fatty acid composition is very similar to the albumin-bound NEFA composition, (2) the percentage of arachidonic and stearic acid is highest in the HDL fraction, and (3) the percentage of oleate is highest in the low density lipoprotein fraction. From data obtained in this laboratory, it is known that approximately two thirds of the lipid present in mouse plasma is generally carried in the HDL lipoproteins. Thus, the fatty acid composition of mouse plasma is in great part determined by the fatty acid distribution in HDL lipoproteins.

In the course of these analyses it was found that mice normally transport a significantly higher amount of albumin-bound NEFA than humans. Values ranging from approximately 1330 to 2000 uEq./L. are obtained in the mouse while in humans the values usually encountered are in the range of approximately 400 to 600 uEq./L. Since salt addition in the ultracentrifugal procedures used is known to displace a small amount of fatty acids from the albumin the above values are probably even somewhat less than actually present on mouse albumin.

Summary

1. Mouse plasma lipid and lipoprotein fatty acids were evaluated by gas chromatography.

2. The plasma cholesteryl ester fraction contained a high percentage of polyunsaturated acids, linoleic (50.1%) and arachidonic (29.9%).

3. Mouse fatty acid data were compared with human values.

4. The concentration of albumin-bound NEFA in mouse plasma was found to be significantly higher than normally present in humans.

TABLE I

Fatty Acid Composition of Mouse Plasma Lipids.
Values expressed as percentage of total fatty acid methyl esters

Fatty Acid	Cholesteryl Esters	Glycerides plus NEFA	Phospholipids
Pre 16:0	0.4	0.8	0.4
Palmitic 16:0	5.0	24.8	29.9
Palmit oleic 16:1	1.5	2.8	0.5
16:1 - 18:0	0.1	0.8	0.9
Stearic 18:0	1.2	3.1	17.7
Oleic 18:1	6.0	27.2	8.2
Linoleic 18:2	50.1	33.4	30.0
18:2 - 20:4	3.4	4.1	3.7
Arachidonic 20:4	29.9	2.4	8.3
Post 20:4	2.4	0.7	0.6

TABLE II

Fatty Acid Composition of Human Serum Lipids
Values expressed as percentage of total fatty acid methyl esters

Fatty Acid	Cholesteryl Esters	Glycerides plus NEFA	Phospholipids
Pre 16:0	3.0	3.5	2.0
Palmitic 16:0	10.0	29.8	33.2
Palmit[illegible] oleic 16:1	3.2	3.7	1.1
16:1 - 18:0	1.1	1.1	0.9
Stearic 18:0	1.2	4.6	14.3
Oleic 18:1	17.8	39.1	11.9
Linoleic 18:2	55.3	15.7	21.9
18:2 - 20:4	2.1	1.2	3.3
Arachidonic 20:4	5.6	1.3	9.3
Post 20:4	0.6	-	2.0

TABLE III

Fatty Acid Composition of Lipoprotein Fractions of Mouse Plasma
Values expressed as percentage of total fatty acid methyl esters

Fatty Acid	Low Density Lipoproteins	High Density Lipoproteins	Albumin-bound NEFA
Pre 16:0	0.8	0.4	1.2
Palmitic 16:0	25.0	18.1	28.0
Palmit oleic 16:1	2.3	1.0	2.9
16:1 - 18:0	0.9	0.9	1.3
Stearic 18:0	5.0	10.7	5.9
Oleic 18:1	24.1	8.9	19.8
Linoleic 18:2	33.8	39.3	32.5
18:2 - 20:4	3.9	3.4	4.0
Arachidonic 20:4	3.6	16.4	3.4
Post 20:4	0.8	1.0	1.2

1. Dole, V., James, A., Webb, J., Rizack, M., Sturman, M., J. Clin. Invest., 1959, V38, 1544.

2. Lindgren, F.T., Nichols, A.V., Wills, R.D., J. Clin. Nutrition, in press.

3. Lindgren, F.T., Nichols, A.V., Freeman, N.K., J. Phys. Chem., 1955, V59, 930.

4. Sperry, W.M., Brand, F.C., J. Biol. Chem., 1955, V213, 69.

5. Dole, V.P., J. Clin. Invest., 1956, V35, 150.

6. Freeman, N.K., Lindgren, F.T., Ng, Y., Nichols, A.V., J. Biol. Chem., 1957, V 227, 449.

7. Stoffel, W., Chu, F., Ahrens, E., Jr., Anal. Chem. 1959, V31, 307.

8. Upham, F., Lindgren, F.T., Nichols, A.V., University of California Radiation Laboratory Report No. 8988, December 1959.

9. Tandy, R.K., Lindgren, F.T., Martin, W.H., Wills, R.D., ibid., No. 9472, November, 1960.