Proceedings
of the
Society
for
Experimental Biology and Medicine

Vol. 103 APRIL, 1960 No. 4

SECTION MEETINGS

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Fatty Acid Composition of Tissue Cholesterol Esters in Elderly Humans with Atherosclerosis.* (25624)

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The current interest in the fatty acid composition of the cholesterol esters in human tissues is due to the suggested role of cholesterol esters in essential fatty acid transport and metabolism(1) and the hypothesis that in atherosclerosis there is a replacement of unsaturated fatty acids by saturated or nonessential unsaturated fatty acid in tissue cholesterol esters(2). In several recent studies (3-7) a comparison of serum and plaque cholesterol ester fatty acid (CEFA) was made in relation to atherosclerosis. In the summary of these studies(6) it was concluded that "at present the evidence is too discrepant to lend support to a theory of atherogenesis based specifically on differences in CEFA in plasma and atheroma." These investigators used the alkali isomerization technic to measure polyunsaturated fatty acids with saturated and monoenoic acids being obtained by difference or by gas-liquid chromatography. While gas-liquid chromatography yields more definitive data, the published studies(4,7) so far have described limited groups or pooled samples, or separation of saturated and unsaturated fatty acids of the same chain length was not achieved. A recent study(8) on deposition and turnover of cholesterol, using tracer doses of cholesterol-4-C^{14} in elderly male humans, indicated that a large portion of the cholesterol in the normal intima and thickened intima (early plaques) of the aorta was derived from the plasma. The present study was undertaken to obtain further information concerning the nature of cholesterol deposition in atherosclerosis. The CEFA of

* This study supported by grants from PHS and Am. Heart Assn.

TABLE I. Retention Volumes of Fatty Acid Methyl Esters Relative to Methyl Myristate.

Fatty acid* Chain length carbons	No. double bonds	Apparent retention vol;† methyl myristate = 1.00
8	0	.20
10	0	.35
12	0	.58
12	1	.74
14	0	1.00
14	1	1.23
16	0	1.68
16	1	2.00
16	2	2.55
18	0	2.81
18	1	3.29
18	2	4.13
20	0	4.82
18	3	5.43
22	0	8.01
20	4	9.20

* Stationary phase was succinate polyester of diethylene glycol; temp. 209°C; 2 M column; helium flow rate 82 ml/min.

† Fatty acids listed in order of elution; calculated from retention time (peak emergence time relative to air peak).

the free-lying plaque material of advanced atheromatous lesions was compared with those of the media and liver of the original subjects and with the serum of 6 patients with occlusive atherosclerosis who were in good nutritional status.

Methods and materials. The tissues, other than serum, were obtained at autopsy from 6 hospitalized male patients (aged 61-74 years). Five died of chronic disease and one died suddenly of a recurrent acute myocardial infarction. The subjects were in good nutritional status except for a short terminal period. Autopsies were performed within 6 hours after death and portions of the abdominal aorta and liver obtained. The aorta was freed of adherent tissue and its layers carefully dissected along their cleavage planes into adventitia, media and intima. The intima were partitioned, as far as their occurrence permitted, into normal appearing portions, thickened portions or plaques, and free-lying lipid containing material (either solid or granular) from far-advanced plaques. Data on the aorta reported here are on the media and the free-lying lipid material. Fasting serum was obtained from 6 elderly male patients with occlusive atherosclerosis, in good nutritional status. Lateral roentgenograms of the abdomen were obtained for evaluation of degree of atherosclerosis by observation of extent of calcification in the abdominal aorta. Lipid extracts of the tissues and serum were prepared as described earlier(9). Cholesterol esters were separated from other lipid components by chromatography on silicic acid (10). Quantitative recovery of sterol esters from the silicic acid column in the 1% ethyl ether-petroleum ether fraction was verified with the aid of labeled cholesterol-4-C^{14} oleate. No overlap with the triglyceride fraction was noted as checked with triolein-1-C^{14}. The isolated cholesterol esters were interesterified in HCl-methanol and the methyl esters sublimed according to the method of Stoffel *et al.* (11). Gas-liquid chromatography was carried out in a Perkin-Elmer Model 154-C Vapor Fractometer using a hot wire detector. The column, 2 M in length, was maintained at a constant temperature of 209°C. The carrier gas was helium; a flow rate of 82 ml/min was used for elution. The inert support was celite 545 and the stationary phase was a succinate polyester of diethylene glycol(12). With this stationary phase it was possible to separate the individual components of mixtures of saturated and unsaturated fatty acids ranging from C_6 to C_{20} within 30 minutes. The apparent retention volumes relative to methyl myristate are shown in Table I. Methyl myristate had a retention time of 3.1 minutes relative to air peak. The areas under the peaks were obtained by triangulation and fatty acid composition calculated from those values(13). Table II shows recovery of a mixture of pure methyl esters.

Results. Plaque material (Table III).

TABLE II. Analysis of a Standard Mixture of Fatty Acid Methyl Esters.

Methyl ester	Weighed, mg	Found, mg	Recovery, %
Laurate	20	21.3	106.5
Myristate	50	47.7	95.4
Palmitate	100	104.7	104.7
Stearate	100	101.9	101.9
Oleate	100	95.3	95.3
Linolenate	100	96.4	96.4
Erucate	100	95.3	95.3
Total	570	562.6	98.7

TABLE III. Cholesterol Ester Fatty Acid Composition of Isolated Lipid Material from Far-Advanced Atherosclerotic Plaques in Elderly Subjects.

Fatty acid* Chain length carbons	No. double bonds	% of fatty acids in subjects 1	2	3	4	5	6	Avg
6 to 12		1.3	.8	.3	1.0	.7	1.3	.9 ± .4†
14	0	.7	1.5	.6	.3	.3	1.0	.7 ± .5
14	1	1.3	.3	.3	.2	.2	.3	.4 ± .4
16	0	26.4	25.2	16.0	36.0	19.2	27.7	25.1 ± 7.0
16	1	5.2	8.8	7.3	1.5	8.0	10.5	6.7 ± 3.2
16	2	.2	.3	.4	.2	.4	.4	.3 ± .1
18	0	.2	.7	.7	.5	.6	1.5	.8 ± .4
18	1	39.5	39.5	36.0	36.0	37.2	37.7	37.8 ± 1.6
18	2	21.9	22.3	32.7	23.4	32.7	16.9	25.1 ± 6.4
18	3	.9	.3	.5	.3	.2	.4	.4 ± .2
20	4	2.4	.3	5.2	.6	.5	1.3	1.8 ± 1.9

* These represent major acids found; very small amounts of 18:4, 20:0, 20:3 and others were also detected. Values were calculated as % of total fatty acids determined.
† Stand. dev.

TABLE IV. Cholesterol Ester Fatty Acid Composition of Aortic Media in Elderly Subjects.

Fatty acid* Chain length carbons	No. double bonds	% of fatty acids in subjects 1	2	3	4	5	6	Avg
6 to 12		1.3	2.8	2.7	1.8	2.8	2.1	2.3 ± .6
14	0	.9	1.7	1.5	1.8	1.6	1.7	1.5 ± .3
14	1	.4	.5	1.1	.4	.5	.6	.6 ± .3
16	0	16.1	23.4	18.3	25.8	17.7	15.1	19.4 ± 4.4
16	1	2.8	5.1	3.1	5.9	3.5	1.7	3.7 ± 1.5
16	2	.1	.1	.3	.3	.2	.1	.2 ± .1
18	0	.1	.5	.9	.3	.4	.6	.5 ± .3
18	1	25.9	32.6	23.0	31.0	21.0	28.0	26.9 ± 2.9
18	2	45.3	28.2	40.7	30.5	44.6	47.3	39.4 ± 8.1
18	3	1.3	.2	.3	.4	.2	.3	.5 ± .4
20	4	5.8	4.9	8.1	1.8	7.5	2.5	5.1 ± 2.2

* See footnotes to Table III.

Three fatty acids† (palmitic, oleic and linoleic) comprised 88% of total CEFA of the far-advanced plaques. The major acid was oleic acid and total monoenoic fatty acids accounted for 45% of the CEFA. The polyunsaturated fatty acids (linoleic, linolenic and arachidonic) comprised only 27% of total fatty acids with linoleic acid the major acid in this group. Arachidonic acid was low in plaque material. Small amounts of short chain acids and stearic acid were detected in all samples.

Media (Table IV). As with plaque material, major CEFA's of the aortic media were palmitic, oleic and linoleic (86% of the total), with linoleic present in greatest amount (39% of the total). The monoenoic fraction accounted for 31% of total fatty acids and polyunsaturated fatty acids 45%. Short chain fatty acids accounted for more than 4% of the total fatty acids. Very little stearic acid was present.

Liver (Table V). In liver, polyunsaturated fatty acids made up only 20% of CEFA. The major acid was oleic acid (37%); monoenoics accounted for 45% of the fatty acids. A considerable amount of stearic acid was also present (8.4%) and saturated acids constituted 35% of total acids. Short chain fatty acids accounted for 7% of total fatty acids.

Serum (Table VI). In serum the major CEFA's were linoleic acid (43.3%), oleic acid (25.8%), and palmitic acid (13.7%). Polyunsaturated fatty acids comprised 52% of total fatty acids. Saturated fatty acids ac-

† For simplicity the acids designated in Tables by chainlength and degree of saturation are referred to in the text by their common names.

TABLE V. Cholesterol Ester Fatty Acid Composition of Livers in Elderly Subjects.

Fatty acid* Chain length carbons	No. double bonds	% of fatty acids in subjects 1	2	3	4	5	6	Avg
6 to 12		.8	1.5	3.7	2.9	3.4	2.9	2.5 ± 1.1
14	0	1.3	1.2	5.9	2.5	3.1	2.9	2.8 ± 1.7
14	1	.5	.9	4.6	1.3	1.9	2.0	1.9 ± 1.3
16	0	24.0	36.3	25.9	16.5	23.1	18.8	24.1 ± 7.7
16	1	3.6	3.9	10.7	2.9	6.6	5.3	5.5 ± 2.9
18	0	8.4	6.0	5.9	9.7	10.3	9.9	8.4 ± 1.6
18	1	40.1	36.5	38.1	35.7	33.8	39.6	37.3 ± 2.4
18	2	17.2	10.8	3.9	26.0	12.8	27.0	16.3 ± 9.1
18	3	.2	.3	Tr	Tr	Tr	Tr	.2 ± .2
20	4	3.7	2.4	1.5	2.5	5.0	2.9	3.0 ± 1.2

* See footnotes to Table III.

counted for only about 16% of the total. Short chain fatty acids were also detected. In all sera studied small amounts of C_{20} saturated and C_{20} trienoic acids were also found.

Discussion. For evaluation and comparison of our data with those in the literature (3-7), the following points are significant. (a) Data on the portions of the aorta involved a careful separation by dissection and analysis of the different portions from each individual. (b) Average values were derived from separate analyses of individual specimens. Consequently, as presented, the data provide information on variability of CEFA between several subjects. The consistency of CEFA values in each group of determinations is noteworthy. (c) Serum data were from a separate series of patients with occlusive atherosclerosis, of the same age and sex as the subjects from which the tissues were obtained, and in good nutritional status at time blood samples were obtained. This eliminated possible changes in blood composition due to poor nutrition immediately prior to death.

There were striking differences between CEFA composition of the free-lying plaque material and the serum (Table VI). Plaque material had significantly ($P<.01$) greater amounts of palmitic and monoenoic acids (chiefly oleic with smaller amounts of palmitoleic) and significantly less ($P<.01$) polyunsaturated fatty acids (linoleic, arachidonic) than the serum.

The differences between CEFA compositions of plaque material and of those of the media were the same as those differences between plaque material and serum. CEFA compositions of serum and of media had a very notable similarity. This suggests that cholesterol esters were deposited in the media

TABLE VI. Comparison of the Cholesterol Ester Fatty Acid Composition of Tissues and Serum from Elderly Subjects.

Fatty acid Chain length carbons	No. double bonds	% of fatty acids in subjects (avg) Plaque material	Media	Liver	Serum*
6 to 12		.9 ± .4	2.3 ± .6	2.5 ± 1.1	1.0 ± .2
14	0	.7 ± .5	1.5 ± .3	2.8 ± 1.7	.9 ± .2
14	1	.4 ± .4	.6 ± .3	1.9 ± 1.3	.3 ± .1
16	0	25.1 ± 7.0	19.4 ± 4.4	24.1 ± 7.7	13.7 ± 1.4
16	1	6.7 ± 3.2	3.7 ± 1.5	5.5 ± 2.9	4.1 ± 1.1
16	2	.3 ± .1	.2 ± .1	Tr	.4 ± .1
18	0	.8 ± .4	.5 ± .3	8.4 ± 1.6	1.3 ± .3
18	1	37.8 ± 1.6	26.9 ± 2.9	37.3 ± 2.4	25.8 ± 3.5
18	2	25.1 ± 6.4	39.4 ± 8.1	16.3 ± 9.1	43.3 ± 4.7
18	3	.4 ± .2	.5 ± .4	.2 ± .2	.7 ± .4
20	0				.7 ± .4
20	3				.3 ± .2
20	4	1.8 ± 1.9	5.1 ± 2.2	3.0 ± 1.2	7.5 ± 2.2

* Values are avg for 6 subjects.

by diffusion, in contrast to the plaque material.

In the liver, a larger portion of the CEFA were monenoics and saturated fatty acids and a smaller portion were polyunsaturated acids than were found in the serum. These findings agree with those of a study of turnover and synthesis of cholesterol, using single tracer doses of cholesterol-4-C^{14}, which indicated that synthesis of cholesterol esters in the liver was much too slow to account for all of the esters of the plasma(8). The differences between CEFA of liver and of plasma might be due to a selectivity of synthesis in, or of release from the liver, of certain cholesterol esters. One or both could explain the very high content of arachidonic acid in CEFA of serum of rats.‡

This study does not support the concept of indiscriminate deposition of blood cholesterol esters in the intima of the aorta(4). Data of the present study indicate that a specificity of deposition or synthesis in different tissues exist, since the tissues studied differed in CEFA composition, except serum and media.

These results offer some support for Sinclair's hypothesis(2) insofar as there was found a significantly larger amount of saturated and monoenoic acids in the CEFA of isolated plaque material than in those of serum. They do not provide information on the mechanism thereof. Degree of atherosclerosis, as evaluated by extent of calcification of the abdominal aorta demonstrable in lateral roentgenograms of the abdomen, had no correlation with the arachidonic or linoleic acid content in CEFA of the serum. That negative finding is of doubtful significance. Composition of CEFA of the serum now does not necessarily indicate what it was during the approximately 50 years in which atherosclerosis was progressing.

Summary. The fatty acids esterified with cholesterol have been determined by gas-liquid chromatography in the media, free-lying lipid containing material of far-advanced plaques, and livers obtained at autopsy from 6 atherosclerotic subjects. For comparison, serum CEFA in 6 well-nourished subjects with occlusive atherosclerosis was also determined. Of tissues studied, composition of CEFA of the aortic media was very similar to that of serum. Plaque material contained a greater proportion of saturated and monoenoic fatty acids than either media or serum. Liver CEFA contained considerably less polyunsaturated fatty acids and more saturated and monoenoic acids than did serum or media. The major CEFA of media and serum was linoleic acid and major fatty acid of plaque material and liver was oleic acid. The significance of these findings in relation to the role of unsaturated fatty acids in atherogenesis is discussed.

‡ Unpublished data.

1. Alfin-Slater, R. B., Aftergood, L. Wells, A. F., Deuel, H. J., Jr., *Arch. Biochem. and Biophys.,* 1954, v52, 180.

2. Sinclair, H. M., *Lancet,* 1956, v1, 381.

3. Lewis, B., *ibid.,* 1958, v2, 71.

4. Tuna, N., Reckers, L., Frantz, I. D., Jr., *J. Clin. Invest.,* 1958, v37, 1153.

5. Luddy, F. E., Barford, R. A., Riemenschneider, R. W., Evans, J. D., *J. Biol. Chem.,* 1958, v232, 843.

6. Wright, A. S., Pitt, G. A. J., Morton, R. A., *Lancet,* 1959, v2, 594.

7. Bottcher, C. F. J., Keppler, J. G., Romeny-Wachter, C. C. H., Van Houte, E. B., Van Gent, C. M., *ibid.,* 1958, v2, 1207.

8. Field, H., Jr., Swell, L., Schools, P. E., Jr., Treadwell, C. R., *Circulation,* in press.

9. Swell, L., Trout, E. C., Jr., Hopper, J. R., Field, H., Jr., Treadwell, C. R., *J. Biol. Chem.,* 1958, v232, 1.

10. Fillerup, D. L., Mead, J. F., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 574.

11. Stoffel, W., Chu, F., Ahrens, E. H., Jr., *Anal. Chem.,* 1959, v31, 307.

12. Lipsky, S. R., Landowne, R. A., Godet, M. R., *Biochim. Biophys. Acta,* 1959, v31, 336.

13. James, A. T., Wheatley, V. R., *Biochem. J.,* 1956, v63, 269.

Received January 25, 1960. P.S.E.B.M., 1960, v103.