Metabolism of lipid in the human fatty streak lesion

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Abstract The composition and synthesis of lipid have been examined in normal intima and adjacent fatty streak lesions of human arterial segments which were incubated with [2-^{14}C]acetate. The incorporation of acetate into lipid was greater in fatty streaks than in normal intima. There was increased acetate incorporation into all major lipid groups in the fatty streak, with the greatest relative increase in the cholesteryl ester fraction. The major radiolabeled arterial fatty acid was a long-chain polyenoic fatty acid with chromatographic properties of a C22:4 acid. The content of fatty acid in the fatty streaks differed from that in normal intima, with substantial increases in the C18:1 acid and decreases in C16:0, C18:0, and C18:2 acids of the fatty streak. It is concluded that lipid synthesis is increased in the human fatty streak lesion and that the local metabolism of lipid contributes to its accumulation within the fatty streak.

Supplementary key words arterial lipid metabolism · human arterial metabolism · atherosclerosis

PREVIOUS STUDIES have suggested an important role of local arterial metabolism in the accumulation of lipid in human fatty streak lesions. In the fatty streak, most of the lipid appears to be intracellular in location, whereas in normal intima, whatever lipid is present is predominantly extracellular in nature (1). Immunochemical analyses of human arteries for plasma low density lipoprotein content have indicated that proportionately less cholesterol is bound as low density lipoprotein in the fatty streak than in either normal intima or in advanced atheromata (2). Furthermore, the composition of cholesteryl esters in fatty streaks differs considerably from that in adjacent normal intima or in plasma (3). These findings suggest that much of the fatty streak lipid is synthesized locally rather than being derived from the plasma.

Our recent investigations in which we have utilized isolated human arterial segments incubated with labeled acetate have suggested that lipid synthesis in the fatty streak lesion is much greater than that observed in either normal intima or advanced atheromata (4). These studies have now been expanded to include a characterization of the newly synthesized lipid and a comparison of the pattern of lipid synthesis with that of its chemical composition in normal intima and adjacent fatty streaks.

MATERIALS AND METHODS

Human aortas removed at postmortem examination within 5 hr of sudden death were prepared for incubation as previously described (4, 5). Fatty streaks, which were identified by gross inspection as slightly raised, nonulcerated, yellow tissue segments, were carefully dissected free from underlying tissue and from adjacent normal intima, and the individual lesions were pooled. Because of difficulty in dissecting the intima completely free from media, the intimal segments utilized generally contained small amounts of media as well. Comparable quantities of pooled normal tissue and fatty streaks (0.16–0.34 g dry wt) were incubated for 3 hr in 3 ml of Krebs bicarbonate buffer (pH 7.35) containing sodium [2-^{14}C]acetate (1.0 μCi/ml, specific activity 34.0 mCi/mmole). A representative segment of tissue was also fixed in formalin, imbedded in paraffin, stained with hematoxylin and eosin and oil red O, and examined by light microscopy.

After incubation, the tissues were washed with 5 × 10 ml of physiological saline. The tissue lipids were extracted with chloroform–methanol 2:1 (v/v), and the

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; Cm:n, m = number of carbon atoms, n = number of double bonds.

sterol and sterol ester contents were determined in duplicate aliquots of the extract (6). Individual lipid groups were separated by TLC, and their radioactivity was assayed as previously described (7). The remaining lipid extracts were pooled to insure sufficient lipid material for the following analyses. The lipids in the pooled extracts were fractionated into lipid classes by TLC, and the lipids were extracted from the silica gel with 5 × 5 ml of chloroform–methanol 2:1. The phospholipid, triglyceride, and sterol ester fractions were saponified with KOH, and the methyl esters of the fatty acids were prepared (8). The samples were analyzed in duplicate by GLC on a Packard 7400 series gas chromatograph, using a 6-ft glass column packed with 20% diethylene glycol succinate coated on Chromosorb W (90–100 mesh) (Analabs, Inc., Hamden, Conn.). Nitrogen was the carrier gas at a flow rate of 40 ml/min. Column temperature was 160°C, injection temperature was 200°C, and heater temperature of the fraction collector was 200°C. The column effluent was split so that 91% of the sample was diverted from the flame detector to a Packard model 852 fraction collector. Each fatty acid peak and the intervening areas were collected separately in anthracene-containing cartridges. After the C18:3 peak, the collections were made at 10-min intervals for a total of 2 hr. The radioactivity of the cartridges was assayed directly in counting vials using a liquid scintillation spectrometer. The bulk of the fatty acid radioactivity was isolated in a GLC fraction with retention time equal to C22:4, C24:0, and C24:1 fatty acids. This fraction from the cholesteryl ester and phospholipid assays was analyzed further by argentation TLC. The anthracene cartridges containing this GLC fraction were treated with 5 × 10 ml of diethyl ether. The extracts were combined, concentrated under nitrogen, and analyzed on silica gel G impregnated with silver nitrate (20%, w/w) (9). The developing solvent was petroleum ether–diethyl ether 6:1 (v/v). Known fatty acid standards (National Heart Institute Fatty Acid Standards) were utilized to identify the saturated, monoene, diene, triene, and polyene fractions. These moieties were isolated and their radioactivities were determined as previously described (7).

The total radioactivity of the samples assayed by GLC ranged from 675 to 2100 cpm. The recovery of standards of methyl [1-^{14}C]palmitate and methyl [1-^{14}C]oleate (New England Nuclear Corp., Boston, Mass.) utilizing this technique was 86–93%. The peaks on the GLC tracing were identified by comparison of the retention times with those of known standards. For quantitation, the relative areas were calculated by multiplication of peak height by width at 50% height. The duplicate analyses of the tissue extracts for the major fatty acids (C16:0, C16:1, C18:0, C18:1, and C18:2) varied less than 11%, and the assays of standard mixtures of these compounds agreed within 6% of their stated compositions. Duplicate assays of the chemical compositions of the minor components (fractions with R_F values less than C16:0 or greater than C18:2) varied from one another by 4–29%. Duplicate assays of radioactivity of the major fatty acid components agreed within 12%, and of the minor components, within 28%. Between analyses, all samples were stored under nitrogen at −20°C.

Control experiments were carried out with segments of arterial tissue boiled in water for 2 min prior to incubation. As we have previously reported (7), no significant incorporation of labeled acetate into arterial lipids was apparent.

RESULTS

The total cholesterol content of the fatty streak was 2.0–6.4 times greater than that of adjacent, normal-appearing intima (Table 1). In all instances, the percentage of esterified cholesterol in the fatty streak exceeded that in normal intima. The DNA content of normal intima (mean 1.00, range 0.75 to 1.28 mg/g dry

TABLE 1. Incorporation of [2-^{14}C]acetate into total lipid by human aortic intima

Patient	Age and Sex	Degree of Atherosclerosis	Cholesterol Content			DNA Content	Incorporation of [2-^{14}C]Acetate into Total Lipid	
			Total	Free	Ester			
			mg/g dry wt	%	%	*mg/g dry wt*	*dpm/g dry wt*	*dpm/mg DNA*
1	18, M	0	9.9	58	42	1.17	24,300	20,800
		Fatty streak	20.2	43	57	1.36	37,600	27,500
2	27, M	0	11.6	49	51	0.75	12,300	16,500
		Fatty streak	45.5	44	56	0.67	15,500	23,000
3	51, F	0	16.0	56	44	1.28	13,100	10,200
		Fatty streak	53.4	32	68	1.61	33,700	20,900
4	57, F	0	8.9	53	47	1.00	19,100	19,100
		Fatty streak	57.0	31	69	0.83	29,300	35,100
5	58, M	0	30.5	54	46	0.80	17,700	21,600
		Fatty streak	156.0	38	62	0.64	57,300	88,900

TABLE 2. Incorporation of [2-^{14}C]acetate into lipid classes in human aortic initima

Patient	Degree of Atherosclerosis	Incorporation of [2-^{14}C]Acetate: Phospholipid	Triglyceride	Free Fatty Acid	Free Sterol	Sterol Ester
		dpm/g dry wt				
1	Normal	9,990	8,470	4,520	535	753
	Fatty Streak	13,500	13,400	7,630	714	2,260
2	Normal	5,450	3,830	2,560	338	159
	Fatty Streak	6,220	5,610	2,670	372	651
3	Normal	6,940	3,580	2,030	131	328
	Fatty Streak	13,500	10,300	4,990	1,010	3,500
4	Normal	6,040	6,360	4,050	936	860
	Fatty Streak	10,800	7,470	7,970	1,440	1,850
5	Normal	10,200	2,730	3,950	550	266
	Fatty Streak	29,700	12,600	11,100	1,430	2,460

wt) was comparable to that in the fatty streak (mean 1.02, range 0.64 to 1.61 mg/g dry wt).

Acetate incorporation into lipid groups

The incorporation of acetate into total lipid in fatty streak was 26–224% greater than that into normal intima when compared on the basis of the dry weight of tissue, and 32–312% greater when compared according to arterial DNA content (Table 1). The greatest incorporation generally occurred into the phospholipid fraction, with decreasing amounts into triglyceride, free fatty acid, sterol ester, and free sterol moieties (Table 2). In the fatty streak, there was increased acetate incorporation into all the major lipids. The greatest relative increase occurred in the sterol esters, which exhibited 2–11-fold greater rates of acetate incorporation in fatty streak than in normal intima.

Fatty acid composition

The saturated acids (C16:0 and C18:0) represented the major long-chain fatty acids in all major lipid fractions of normal intima and in the phospholipids and free fatty acids of the fatty streak (Table 3). Oleic acid (C18:1) was the major constituent of the cholesteryl ester and triglyceride moieties of the fatty streak. The greatest differences in composition between normal intima and fatty streak occurred in the cholesteryl esters, where there were relative increases in the C18:1, C16:1, and > C20:0 fractions and decreases in the C16:0, C18:0, and C18:2 fatty acids.

Acetate incorporation into fatty acids

In all fractions examined, the major incorporation of acetate occurred into a fraction with retention time by GLC equal to the C22:4, C24:0, and C24:1 fatty acids (Table 4). The relative retention times of these and certain other fatty acids are presented in Table 5. On further analysis of the highly radiolabeled fraction by argentation TLC (Table 6), this radioactivity was recovered predominantly with the polyenoic fatty acids. The data therefore suggest that the major acetate incorporation occurred into a C22:4 acid. Much of the remaining lipid radioactivity (Table 4) was recovered with C16:0 and C18:0 acids and to a lesser degree with C18:1 and C18:2 acids.

Microscopic examination

The intima of the fatty streak lesions contained numerous foam cells which stained heavily for lipid. In many of the sections, stainable lipid and fibrous tissue were present in the extracellular space. The adjacent, grossly normal intima also appeared normal by light microscopy. It was essentially free of foam cells and fibrous tissue, although small amounts of stainable lipid were occasionally seen in the extracellular space.

DISCUSSION

These data suggest that lipid synthesis is increased in the human fatty streak lesion. The results are in agreement with previous observations in rabbits and pigeons demonstrating accelerated lipid synthesis in atheromata (10–12). They contrast, however, with our results in advanced human atheromata where lipid synthesis appears to be depressed (4).

The major incorporation of acetate into lipid occurred

TABLE 3. Fatty acid composition of human aortic intima

Fatty Acid	Phospholipid: Normal	Phospholipid: Fatty Streak	Triglyceride: Normal	Triglyceride: Fatty Streak	Cholesteryl Ester: Normal	Cholesteryl Ester: Fatty Streak	Free Fatty Acid: Normal	Free Fatty Acid: Fatty Streak
	% of total fatty acids							
C16:0	2.6	2.0	2.5	1.5	1.4	2.1	5.6	2.8
C16:0	41.2	39.0	38.2	30.3	32.5	24.4	36.0	34.9
C16:1	3.2	2.2	3.4	3.3	2.2	4.6	4.9	2.2
C18:0	30.8	29.3	27.0	18.9	25.8	5.6	32.1	31.4
C18:1	12.0	16.4	22.6	34.5	19.2	50.0	14.6	23.5
C18:2	6.9	7.0	3.5	5.9	16.7	8.2	3.6	2.7
C18:3 + C20:0	1.0	1.5	1.0	2.3	1.0	1.2	1.7	1.0
>C20:0	2.0	2.6	1.8	3.2	1.0	3.9	1.6	2.1

TABLE 4. Distribution of fatty acid radioactivity in human aortic intima following incubation with [2-^{14}C]acetate

Fatty Acid	Phospholipid		Triglyceride		Cholesteryl Ester		Free Fatty Acid	
	Normal	Fatty Streak	Normal	Fatty Streak	Normal	Fatty Streak	Normal	Fatty Streak
	% of total radioactivity in fatty acids							
C16:0	1.0	1.0	0.8	0.6	1.3	5.8	1.0	6.4
C16:0	7.3	17.5	14.4	9.0	13.9	15.1	37.8	15.6
C16:1	6.8	2.1	0.6	0	2.2	0	2.1	0
C18:0	13.6	6.5	25.6	13.9	9.3	12.1	13.4	22.1
C18:1	2.2	1.3	5.8	11.2	3.9	5.2	2.4	2.6
C18:2	0	2.0	2.9	6.9	1.9	2.4	0	0
C18:3 + C20:0	0	0	0.8	1.4	0	0.9	1.0	3.1
>C20:0								
R_T[a] 2.1–6.0	7.1	4.6	5.3	4.7	6.9	8.9	6.1	9.5
R_T 6.0–7.8	52.4	54.7	36.4	41.7	53.2	41.1	29.6	40.4
R_T >7.8	9.0	9.3	5.8	6.9	4.1	4.4	3.9	0

[a] R_T = relative retention time; C18:0 = 1.0.

TABLE 5. Relative retention times by GLC of some fatty acid methyl esters on diethylene glycol succinate (20%)

Fatty Acid	Relative Retention Time (180°C)
C18:0	1.0
C20:0	1.9
C20:2	2.4
C20:4	3.2
C22:0	3.5
C22:4	6.3
C22:5	8.0
C24:0	6.6
C24:1	7.5

TABLE 6. Argentation TLC analysis of distribution of radioactivity in a GLC fraction containing fatty acids >C20:0 from fatty streak

Fatty Acid Saturation	R_F	Cholesteryl Ester	Phospholipid
		% of total radioactivity	
Saturated	0.91	8	8
Monoene	0.62	4	2
Diene	0.30	2	0
Triene	0.19	9	6
Polyene	0.08	77	84

in a highly unsaturated long-chain fatty acid which, by GLC analysis, had a retention time equivalent to a C22:4 fatty acid. The exact identity of this material is uncertain. The synthesis of a similar substance has been observed with cell-free systems of rabbit and monkey aorta (13, 14) and with intact monkey aorta (15), although in these latter investigations, the proportion of fatty acid radioactivity represented by the very long-chain unsaturated fatty acids was less than that observed in the current study. Both de novo synthesis and chain elongation of fatty acids have been described in monkey and pigeon aorta (15, 16). Studies by Howard (14) with subcellular fractions of monkey aorta have demonstrated de novo fatty acid synthesis in the high-speed supernatant fraction and have indicated that malonyl CoA is the major substrate for this reaction. The incorporation of acetate into arterial fatty acid occurred in the microsomal and mitochondrial fractions and involved predominantly chain elongation. In the current investigation, the unusually high level of radioactivity in a long-chain polyenoic fraction and the marked discrepancy between the isotope incorporation patterns and the fatty acid composition suggest that chain elongation is the major mechanism by which acetate is incorporated into fatty acids of human aorta. Geer, Panganamala, and Cornwell (17) have suggested, on the basis of analyses of fatty acid isomers, that most of the long-chain polyene fatty acids present in the aorta are derived from chain elongation and desaturation of aortic linoleic and linolenic acids. The present results lend support to this hypothesis. Since the relative contributions of de novo synthesis and chain elongation have not been determined in the current investigation, we are unable to determine the relative amounts of each fatty acid synthesized. Those fatty acids arising from de novo synthesis would be expected to contain approximately eight times greater radioactivity than those fatty acids originating from chain elongation.

Day and associates have demonstrated that foam cells may be an important site of lipid synthesis (18–20). The consistent presence of foam cells in the lesions studied by us suggests that the observed increase in lipid synthesis may be a secondary manifestation of the cellular accumulation in the fatty plaque. However, the source of the newly synthesized lipid in normal intima cannot be explained on this basis, since no foam cells were apparent in the normal tissue. While the fatty streak represents the earliest atherosclerotic lesion which could be utilized, we recognize that the atherosclerotic process may have been quite advanced in the fatty plaques examined. The current findings, therefore, do not clarify the mechanisms involved in the early phases of arterial

lipid deposition. Recent studies by Smith and Slater (2) suggest that the lipid in normal intima is derived primarily from plasma lipoproteins whereas much of the lipid in fatty plaques may have a local origin.

The major changes in fatty acid composition occurred in the cholesteryl ester fraction, where there was a striking increase in oleic acid and decrease in palmitic, stearic, and linoleic acids of fatty streak as compared with normal artery. Similar changes have been noted in other studies of human arteries (2, 21) and of arteries from cholesterol-fed monkeys and pigeons (15, 16). The observed increases in free and esterified cholesterol content of the fatty streak are likewise comparable to those previously described (22). A greater relative rise in esterified than in free cholesterol accompanies the development of atherosclerosis in other species (15, 16, 23, 24) and may even precede the appearance of grossly visible atherosclerotic lesions (15). At least some of the cholesteryl ester in the human fatty streak appears to be in the form of liquid crystals (25). As such, the cholesteryl ester may be in a static state and difficult to mobilize from the intima. In addition, cholesteryl esters, particularly of the monounsaturated type, may be sclerogenic to connective tissue and could conceivably contribute to the inflammatory reaction within the artery (26, 27).

The relative increases in the rates of acetate incorporation into the cholesteryl ester fraction of the fatty streaks generally paralleled the increases in total cholesteryl ester content. However, the pattern of fatty acid radioactivity differed markedly from that of fatty acid content. Both the synthesis of fatty acids and their incorporation into cholesteryl esters are reflected in the acetate incorporation rates, and no estimation can be made of the contribution of local cholesterol esterification to the arterial accumulation of these esters. Recent studies in rabbits have demonstrated marked increase in cholesterol esterification rates in atheromata (18) and have estimated that local synthesis accounts for 50% of the cholesteryl esters accumulating there (28). Other factors which may also contribute to the accumulation of cholesteryl esters in atherosclerotic tissue include a marked binding of these esters to arterial elastin (29) and a lesser capacity for hydrolysis of cholesteryl esters in atherosclerotic as compared with normal intima (30, 31).

The process by which arterial cholesterol esterification occurs remains unresolved. Abdulla, Orton, and Adams (32) have reported that the activity of the lecithin–cholesterol acyltransferase enzyme is increased in the human fatty streak and have suggested that cholesterol transacylation from phospholipid may be an important factor in arterial cholesteryl ester accumulation. However, studies in pigeon aorta by St. Clair (33) have indicated that arterial cholesterol esterification proceeds primarily by transfer of fatty acyl CoA to cholesterol rather than by transacylation from lecithin.

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