Lipid Metabolism in Perfused Human Coronary Arteries[1] (36516)

TAKASHI MORITA AND RICHARD J. BING

Huntington Memorial Hospital, Pasadena, California 91105; and University of Southern California, Los Angeles, California 90033

The turnover rate and exchange of cholesterol in human plasma and tissues have been studied in autopsy material from patients injected with ^{14}C-cholesterol (1–3). In most investigations carried out *in vitro*, human arteries derived from autopsy or during surgery can synthesize lipids, such as phospholipids from ^{32}P, ^{14}C-acetate and ^{14}C-fatty acids (4–6), glycerides from ^{14}C-oleate, and cholesterol esters from ^{14}C-oleate (6) and from lecithin labeled with ^{14}C-linoleic acid (7).

The results on cholesterol synthesis have been conflicting. Chobanian demonstrated cholesterol synthesis in the vascular wall using ^{14}C-acetate and ^{14}C-mevalonate (8), while others were unable to confirm this (9, 10). Ott, Lohss and Gergely (11) found low-density lipoproteins in the human aortic wall. This has led to the suggestion that the influx of cholesterol may be a passive process.

Cigarette smoking has been implicated in the mechanism of atherogenesis (12). Nicotine, in chronic experiments, leads to the accumulation of nonesterified fatty acids in the aorta of rabbits (13). In acute experiments with nicotine, the pattern of lipid synthesis in dog arteries may be altered (14).

The present report deals with experiments to study lipid synthesis, cholesterol uptake, and the effect of nicotine on lipid metabolism in human perfused coronary arteries. *In vitro* perfusion, with pulsatile pressure, was carried out in order to simulate physiological conditions existing *in vivo*. All arteries had moderate to severe atherosclerosis, according to the criteria of the World Health Organization (15).

Materials and Methods. Human coronary arteries were obtained from autopsy material up to 5 hr after death. Sterile techniques were used during the preparation and perfusion. Arteries were dissected free of fat and connective tissue. Perfusion was carried out for a period of 4 hr at 37° with a perfusion pressure of 130-100 mm Hg and a pulsatile rate of 80. A modified Carrel–Lindbergh pump was used in all experiments (16). The basic perfusion fluid consisted of sterile human plasma collected within 2 weeks prior to the experiment. A mixture of 75% nitrogen, 20% oxygen, and 5% carbon dioxide[2] was employed to drive the fluid through the artery.

The purity of sodium acetate-2-^{14}C (^{14}C-acetate, 2 Ci/mole) and of cholesterol-1,2-^{3}H (N) (^{3}H-cholesterol, 55 Ci/mmole)[3] was tested in each instance. ^{3}H-Cholesterol was purified by means of thin-layer chromatography 1 week prior to use. ^{3}H-Cholesterol (100–200 μCi) was added to about 0.05 ml of concentrated lipid extract from human serum which contained 118.4 μmoles/ml of total cholesterol, 15.2 μmoles/ml of free cholesterol, and 32.6 μmoles of phosphorus/ml of phospholipids. The mixture was then evaporated *in vacuo* to a small volume. Final evaporation was carried out under a stream of nitrogen. After addition of about 5 ml of human plasma, the mixture was sonicated in an ice water bath 3 times for 1 min each, at intervals of about 1 min, using a Biosonik III (20 kHz) with a microtip.[4] The sonicated mixture was made to a volume of about 40 ml with plasma and shaken for 2 hr at 37°. Cellulose acetate membrane electrophoresis of the incubated plasma mixture, carried out according to the method of Chin and Blankenhorn (17), showed that the tritium radioactivity was mainly located

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[2] Liquid Carbonic Corporation, San Carlos, CA.

[3] New England Nuclear, Boston, MA.

[4] Bronwill Scientific, Rochester, NY.

in the alpha-2 and beta lipoproteins. The incubated mixture was combined with the basic perfusion fluid to which 150 μCi of ^{14}C-acetate had been previously added. The total perfusion mixture was made up to 250 ml.

In the experiments in which the effect of nicotine was investigated, 6 mg of nicotine (99.9% pure)[5] dissolved in 5 ml of saline solution were added prior to adjusting the final volume of 250 ml with plasma. This compares approximately with the amount of nicotine administered to rabbits by Schievelbein and associates (13). In control experiments, saline was used in place of nicotine.

After the perfusion, the plasma was tested for growth of microorganisms. Prior to and following perfusion, several pieces of arteries were cut for pathological gross and microscopic examination. No pathological changes were noticed following perfusion.

Lipid analysis on the perfusion fluid was carried out prior to and following perfusion. Extraction was carried out according to the method of Folch, Lees and Sloan Stanley (18).

After perfusion, the coronary arteries were opened longitudinally. The proximal and the distal 10 mm of vessels were discarded. The opened tissue was then washed three times with 1.26% sodium acetate, four times with 0.9% sodium chloride, and finally once with 0.02% calcium chloride. The final washing solution showed almost no trace of radioactivity.

Analyses were carried out on intima and media.

The tissues were weighed after removing moisture by blotting on filter paper and were crushed in a mortar after freezing with liquid nitrogen. The crushed tissue was then collected in an Erlenmeyer joint flask with the Folch mixture of chloroform and methanol (2:1 v/v) and kept under nitrogen gas for 20 hr at 5°. The mixture was then refluxed for 30 min and filtered using analytical filter paper.[6] The residue on the filter paper and in the Erlenmeyer flask was refluxed again with the Folch mixture for 30 min.

[5] Eastman Kodak Co., Rochester, NY.

[6] Scientific Products, Evanston, IL.

Lipids were separated on a thin-layer plate of silica gel (silica gel F-254)[7] according to the method of Freeman and West (19). Authentic samples were developed simultaneously. Location of spots on the plates was detected by ultraviolet light or by exposure to iodine vapors. The separated fractions were eluted from the thin-layer plate with elution solvents appropriate for the corresponding fraction (20). Recovery of the fraction was tested by counting the radioactivity of the eluate and comparing it with the radioactivity of the residue gel.

The samples for determination of the radioactivity of the lipid extracts or of the eluate were evaporated in a scintillation vial and counted in a Tri-Carb liquid scintillation spectrometer[8] in the presence of scintillator of toluene–dioxane solvent system (20). Aquasol[3] was used for direct determination of total radioactivity in the perfusion plasma. To determine the distribution of radioactivity in fractionated lipids, the individual spots on the thin-layer plate were scraped off and placed into scintillation vials, followed by addition of methanol prior to addition of scintillator in the ratio of 3 to 10 (v/v). ^{3}H-Toluene and ^{14}C-toluene[3] were used as internal standards.

Cholesterol was determined in plasma extract and in the eluate by the method of Zlatkis, Zak and Boyle (21) and by the method of Zak *et al.* (22), and phosphorus of phospholipids was analyzed according to Lowry *et al.* (23) modified by Wagner *et al.* (24). When the amount of cholesterol esters taken up was calculated, the mean value of the specific activity of ^{3}H-cholesterol esters in the plasma before and after perfusion was used.

Results. Distribution of incorporation of ^{14}C-acetate into lipids is presented in Table I. No synthesis of cholesterol from ^{14}C-acetate was demonstrated. Similar negative results were obtained by others in human and animal arteries (8, 9, 25).

In all experiments, with the exception of experiment Nos. 32-A, 33-A, and 40-A, little

[7] E. Merk, Darmstadt, West Germany.

[8] Model 3320, Packard Instrument Co., Downers Grove, IL.

TABLE I. Distribution of ^{14}C Radioactivity Incorporated into Human Coronary Artery Perfused with Acetate-2-^{14}C in the Absence of and the Presence of Nicotine.[a]

Expt. no.	Age and sex	^{14}C radioactivity in total lipids (dpm/g tissue)	% of ^{14}C radioactivity of TL[c]						Statistical[d] difference PL vs TG
			FC	CE	PL	FFA	DG	TG	
Control									
32-A	51 M	10,862	1.2	7.2	26.4	7.0	0.2	57.4	
34-A	51 M	3998	0	0	33.1	11.6	0	58.7	
36-A	49 M	5371	0	0	36.4	19.2	0	41.6	
38-A	70 M	25,085	1.0	0	34.7	7.6	0	51.2	
40-A	60 M	11,352	0	7.0	31.3	4.7	2.6	49.7	
38-B	70 M	18,138	0	2.6	37.8	7.2	0	49.0	
40-B	60 M	4480	0	0	37.6	4.3	10.9	39.1	
42-B	77 M	1855	0	0	42.9	7.7	2.3	46.1	
Mean ± SE		ND	ND	ND	35.02 ± 1.74	8.66 ± 1.69	ND	49.10 ± 2.43	$2p < .001$
Nicotine addition									
33-A	51 M	26,461	1.8	21.4	22.5	7.0	0.8	40.7	
35-A	51 M	7036	0	1.6	29.4	10.3	4.9	49.6	
41-A	60 M	13,642	0	2.0	33.0	8.1	9.7	40.6	
43-A	77 M	6999	0	2.6	41.4	6.0	1.5	48.0	
37-B	49 M	9670	1.4	1.2	53.5	14.4	0	12.0	
39-B	70 M	7623	0	3.9	42.5	10.4	2.8	36.8	
41-B	60 M	3870	0	0	39.6	16.3	0	42.4	
Mean ± SE		ND	ND	ND	37.41 ± 3.81	10.35 ± 1.43	ND	38.58 ± 4.73	NS
Statistical difference control vs nicotine					NS	NS		$2p < .05$	

[a] The artery was perfused with plasma containing 150 μCi of ^{14}C-acetate in the absence of or the presence of nicotine (about 150 μM) for 4 hr at 37°. FC = free cholesterol; CE = cholesterol ester; PL = phospholipids; FFA = free fatty acids; DG = diglycerides; TG = triglycerides; ND = not determined; SE = standard error of the mean; NS = not significant or $2p > .05$.

[b] Each pair of experiments with the arabic numbers, 32 and 33, 34 and 35, etc., had the same origin of the tissue and were carried out using the plasma of the same lot. Alphabetic mark A indicates noncalcified, and B indicates calcified.

[c] Distribution percentage of ^{14}C radioactivity of less than 5% may be considered within the experimental error.

[d] Student's t test.

TABLE II. Uptake of Cholesterol by Human Coronary Artery in Absence or Presence of Nicotine.[a]

Expt.[b] No.	Sp act in perfusate[c] (dpm ^{3}H/mmole)		Uptake by tissue			
			FC		CE	
	FC	CE	dpm ^{3}H/ g tissue	mμmoles/ g tissue	dpm ^{3}H/ g tissue	mμmoles/ g tissue
Control						
32-A	1792	1.21	19,089	10.6	0	0
34-A	1851	7.80	147,964	79.9	0	0
36-A	1878	1.28	168,193	89.5	0	0
38-A	2882	7.30	15,353	5.3	121	16.5
40-A	2043	12.7	28,368	13.8	0	0
38-B	2882	7.30	37,231	12.9	796	109
40-B	2043	12.7	36,295	17.7	0	0
42-B	871	ND	30,114	34.5	0	0
Mean ± SE				33.02 ± 11.70		ND
Nicotine addition						
33-A	1669	0.92	65,859	39.4	65	70.6
35-A	1851	7.62	33,702	18.2	0	0
41-A	2451	14.3	85,848	35.0	0	0
43-A	884	1.77	35,308	39.9	0	0
37-B	1878	0.97	337,968	179.9	492	507
39-B	2972	5.70	171,068	57.5	0	0
41-B	2451	14.3	27,931	11.3	280	19.6
Mean ± SE				54.45 ± 21.67		ND
Statistical difference control vs nicotine addition[d]				NS		

[a] The artery was perfused with ^{3}H-FC under the same condition as in Table I. FC = free cholesterol; CE = cholesterol ester; ND = not determined; SE = standard error of the mean; NS = not significant or $2p > .05$.

[b] See footnote [b] in Table I.

[c] See text.

[d] Student's t test.

incorporation of ^{14}C radioactivity into cholesterol esters could be demonstrated. Whether in these experiments incorporation of acetate occurred into cholesterol or fatty acid moiety was not determined.

In control experiments in which nicotine was not added to the perfusion fluid, distribution of ^{14}C-radioactivity was greater in triglycerides (TG) than in phospholipids (PL) ($2p < .001$). However, no statistical difference was observed between PL and TG in the presence of nicotine.

In our series, human coronary arteries showed an uptake of free cholesterol (FC) from the perfusion fluid (Table II). Although this occurred in every artery examined, more than 50% of the experiments failed to demonstrate an uptake of cholesterol esters (CE). Statistically, nicotine did not affect the uptake of FC.

Discussion. The experiments reported here were carried out on 12 human perfused coronary arteries obtained at autopsy. Perfusion was maintained for 4 hr with human plasma containing ^{14}C-acetate and ^{3}H-cholesterol. The experiments were divided into groups according to whether or not nicotine was added to the perfusion fluid. All arteries showed various degrees of atherosclerosis. The number of experiments was too small to permit a differentiation of the incorporation of ^{14}C-acetate and uptake of ^{3}H-cholesterol between moderate and severe vascular lesions.

Our results demonstrate that (1) atherosclerotic human coronary arteries cannot synthesize cholesterol from acetate, (2) only very limited synthesis of cholesterol esters from acetate occurs, (3) free cholesterol is taken up by the artery, and (4) nicotine has some effect on lipid synthesis from acetate, but does not influence free cholesterol uptake.

Synthesis of cholesterol and cholesterol esters from acetate. Different results have been published concerning cholesterol synthesis in human arteries (8–10). Such a discrepancy is probably the result of differences in experimental condition. In most of our experiments, synthesis of CE from acetate is very low. There is no esterification of FC taken up. Wahlquist, Day and Tume (6) and Stein and Stein (26), however, demonstrated esterification of cholesterol from fatty acids in various arteries from both humans and animals. This divergence of opinion is explained by Silversmith (27), who pointed out that serum cholesterol and newly synthesized free fatty acids are located in different subcellular pools: in addition, plasma free cholesterol penetrates slowly to the esterifying sites.

Uptake of free cholesterol and cholesterol esters. There was uptake of FC in the coronary arteries (Table II). No conclusions on the mechanism of this uptake are possible from these experiments. Several investigators have suggested physicochemical processes or imbibition of cholesterol to explain the mechanism of uptake of cholesterol by the arterial wall (28–30). However, the intima may also act as a metabolic barrier to cholesterol influx (27, 31, 32). This is in agreement with the concept of Zemplényi, who considers the aortic wall metabolically as a defense barrier (33).

More than 50% of our experiments failed to demonstrate an uptake of cholesterol esters (Table II). The finding could be the result of insufficient radioactivity of ^{3}H in the perfusion fluid.

Effect of nicotine. The results in Table I demonstrate that nicotine influences lipid synthesis from acetate. In the absence of nicotine, the distribution of ^{14}C radioactivity in TG was greater than in PL. In the presence of nicotine, this difference was absent. In the three paired experiments (32-A 33-A, 34-A 35-A, 40-A 41-A), incorporation of ^{14}C-acetate into lipids (particularly TL, FFA, and PL) was increased by nicotine. This was almost exclusively due to ^{14}C radioactivity in the fatty acid moiety after alkaline hydrolysis and subsequent separation (more than 90% of the total radioactivity of ^{14}C in total lipids). This suggests that the increase in incorporation of ^{14}C-acetate into lipids results from stimulation of fatty acid and phospholipid synthesis by nicotine.

The possibility also exists that the increase in lipid synthesis is the result of an increased uptake of acetate by the tissues. This, however, cannot be confirmed, since there was insufficient tissue for analysis of total uptake of ^{14}C-acetate.

The results reported here must be considered in the light of the limitation of the experimental conditons. All perfused coronary arteries had some degree of atherosclerosis [from *b* to *d*, according to the World Health Organization classification (15)]. It is possible that the degree of severity of the pathological process may have influenced the results (34). Furthermore, the possibility exists that postmortem changes may have altered the permeability of the vascular endothelium and interfered with the synthetic processes within the vascular wall. This must be considered, even in the absence of obvious histological changes. Finally, the fact that these experiments were carried out *in vitro* may have introduced factors which do not operate in the intact organism. However, *in vitro* perfusion made possible stricter controls and permitted the elimination of a series of variables, which, in *in vivo* experiments, would have made the interpretation of the results difficult.

Summary. The experiment dealt with lipid synthesis and cholesterol uptake in atherosclerotic human coronary arteries perfused *in vitro* with pulsatile pressure. ^{14}C-acetate and ^{3}H-cholesterol were added to the perfusion fluid.

The results showed that under the experimental conditions, the arterial wall failed to synthesize cholesterol from acetate. Only

very limited synthesis of cholesterol esters from acetate was observed.

In contrast, there was uptake of free cholesterol by the artery from perfusion plasma.

Nicotine added to the perfusion fluid did not influence arterial cholesterol uptake, but influenced lipid synthesis from acetate by changing the distribution of ^{14}C-acetate in phospholipids and triglycerides.

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