Uptake and metabolism of circulating chylomicron triglyceride by rabbit aorta

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Abstract To determine if chylomicron triglycerides are taken up and metabolized by the arterial wall, rabbit abdominal aortas were perfused in situ for various times up to 2 hr with blood–buffer containing isotopically labeled substrates. Labeled chylomicrons were obtained by feeding [^{3}H]palmitic acid or [^{3}H]glyceryl trioleate to rats and rabbits with cannulated thoracic ducts. After aortic perfusion with these chylomicrons, more than 85% of aortic lipid ester radioactivity was in triglyceride; when labeled glycerol or palmitic acid was perfused, most aortic ester lipid radioactivity was in diglycerides and phospholipids. This indicated that, during perfusion with chylomicrons, intact triglyceride molecules were taken up by aorta. The rate of triglyceride fatty acid uptake by the inner avascular segment approached maximal values at low concentrations of perfusate triglyceride fatty acids (2 mM), whereas uptake in the outer capillary perfused segment increased with increasing triglyceride fatty acid concentration (0.4–25 mM). By double-radioisotope techniques it was shown that aortic free fatty acid was derived from both perfusate free fatty acids and from hydrolysis of lipoprotein glycerides within the aortic wall. Uptake of chylomicron triglyceride by perfused aorta was independent of triglyceride hydrolysis, which was quantitatively small.

Supplementary key words perfusion · triglyceride · lipolysis

Considerable information is now available about pathways of lipid synthesis in the mammalian aortic wall from precursors such as acetate, glucose, and long-chain free fatty acids (1–3). The role of circulating lipoprotein triglyceride in the metabolism of the arterial wall has not been studied previously despite much interest in the frequent association of severe atherosclerosis with abnormally high plasma triglyceride concentrations in man (4).

Uptake and metabolism of lipoprotein (chylomicron) triglyceride have been investigated most intensively in white adipose tissue in which there is a high correlation between rates of uptake of triglyceride fatty acid and the content of lipoprotein lipase (5). It has been suggested that in adipose tissue lipoprotein lipase acts at the capillary endothelium to control triglyceride fatty acid uptake (6). However, in experiments with perfused adipose tissue, labeled triglyceride derived from chylomicrons appears to be hydrolyzed after it is taken up by the tissue (7). Whether lipoprotein triglyceride is hydrolyzed at the endothelial surface or within the tissue, there is no doubt that in adipose tissue there is a correlation between the rate of uptake and hydrolysis of triglyceride. In aorta, as the present study will show, chylomicron triglyceride uptake rates are much greater than hydrolysis rates, thus permitting some resolution of mechanisms of tissue triglyceride uptake.

The aortic intima and inner media in man and the rabbit are avascular (8), and it is probable that lipoprotein lipids traverse the intimal endothelium to enter these aortic regions. For this reason, experiments were performed either in vivo or with the in situ perfusion technique in which intimal endothelial integrity is maintained (3), since we have had repeated difficulty in maintaining the total integrity of the aortic intimal endothelium in many varieties of in vitro preparations.

The perfusion technique employs a small mass of tissue and a large volume of perfusate so that constant specific radioactivities and concentrations of perfusate substrates are maintained and rates of aortic uptake and metabolism of these substrates are easily calculated.

The purpose of this study was to examine the uptake of circulating chylomicron triglyceride by perfused intact aortic wall and to assess the role of triglyceride hydrolysis in this uptake.

Abbreviations: FFA, free fatty acids; TGFA, triglyceride fatty acids; TLC, thin-layer chromatography.

MATERIALS AND METHODS

Animals

Male New Zealand rabbits (Canadian Breeding Laboratories, St. Constant, Que.) weighing 2.5–3.5 kg and fed Purina rabbit chow were used for perfusions and as a source of perfusate blood. Rabbits weighing 1 kg were used for the in vivo experiments.

Isotopic materials

[2-^{3}H]Glyceryl trioleate (specific activity 192 mCi/mmole), glyceryl [9,10-^{3}H]tripalmitate (1200 mCi/mole), [1-^{14}C]palmitic acid (36.6 mCi/mmole), [9,10-^{3}H]palmitic acid (266 mCi/mmole), and [2-^{14}C]glycerol (8.0 mCi/mmole) were obtained from the Radiochemical Centre, Amersham, England. The radiochemical purity of lipids was confirmed by TLC on silica gel with appropriate standard lipids as described below. With each lipid > 98% of the radioactivity was recovered with the appropriate standard. [2-^{14}C]Glycerol was chromatographed with unlabeled glycerol on a paper chromatogram developed with butanol–acetic acid–water (3), and its radiochemical purity was > 99%. The potassium salts of labeled palmitic acid were prepared as described previously (3).

Chemicals

Reagent grade chemicals were used throughout, and ethyl ether and light petroleum ether were redistilled before use. Standard preparations of glyceryl tripalmitate, glyceryl trioleate, and glyceryl trilinoleate with a stated purity of > 99% were obtained from Applied Science Laboratories, State College, Pa.

Crystalline bovine albumin, fraction V, was obtained from Miles Laboratories, Kankakee, Ill. A 10% solution in Krebs-Ringer–bicarbonate–phosphate buffer, pH 7.4, was dialyzed in boiled cellulose dialysis tubing against 10 vol of buffer for 24 hr with one change of buffer. After centrifugation the supernatant solution was sterilized by filtration through autoclaved Metricel GA membrane filters (Gelman Instrument Co., Ann Arbor, Mich.).

Labeled FFA and chylomicrons

To prepare ^{3}H-labeled FFA for intravenous injection, 200 μCi (0.75 μmole) of the potassium salt of [^{3}H]palmitic acid was bound to serum albumin by a 15-min incubation at 37°C with 2 ml of serum from a fed rabbit.

Chylomicrons (labeled or unlabeled) were obtained by cannulating rat thoracic ducts below the diaphragm with Silastic catheters (9). Heparin, at a concentration of 10 U/ml of saline, was used only to moisten the Silastic catheter and the operative site; no more than 50 units were used. To avoid any possibility of contamination of chylomicrons with heparin, lymph was always allowed to flow freely for an hour before any collection of unlabeled chylomicrons and for several hours before collection of labeled chylomicrons. Cannulated animals were kept in restraining cages permitting longitudinal movement. For preparation of labeled chylomicrons, rats were tube-fed the appropriate labeled lipid (0.5–1 mCi) in 0.1–0.2 ml of safflower oil emulsified in saline with 30–60 mg of sodium taurocholate. Rabbit chyle was obtained by cannulation of the left jugular vein (10), and the labeled feed was introduced through a gastrostomy tube.

Chyle was collected at room temperature and it was stored as whole lymph in stoppered, sterile 30-ml flasks at 10°C in order to prevent lipid peroxidation. Chylomicrons were isolated from whole lymph, 24–48 hr before perfusion, by layering the chyle below 0.9% saline in 30-ml polycarbonate screw-cap ultracentrifuge tubes and centrifuging at 65,000 *g* (30,000 rpm in an A-211 rotor in a B-60 International centrifuge) for 60 min at 14°C; the upper 2 cm of the 7-cm column was relayered below saline and the centrifugation was repeated. Chylomicrons were stored in flasks with 10,000 U of crystalline penicillin. The maximum time between the onset of lymph collection and perfusion was 4 and 5 days for labeled and unlabeled chylomicrons, respectively. Since degradation of lipids can occur during storage of chylomicrons, in each experiment labeled chylomicron lipids were examined for evidence of triglyceride hydrolysis or the appearance of polar peroxidized lipids as described by Ontko (11). There was no evidence of chylomicron deterioration (e.g., Table 5).

Perfusions

The technique of perfusion of the rabbit abdominal aorta was identical with that previously described (3). In summary, animals were fed until 2 hr before operation. They were anesthetized lightly with intravenous Nembutal, and anesthesia was maintained with Nembutal and ether. Under sterile conditions the abdominal aorta was isolated by ligating all branches of the abdominal aorta between the renal arteries and the aortic bifurcation. The arterial branches were ligated at least 5 mm from the aortic wall, and the aorta was perfused by the rabbit's own circulation for approximately 30 min before catheterization. Silastic catheters were inserted at the level of the renal arteries and the aortic bifurcation, and the aorta was then perfused with medium containing labeled substrates. The medium was a 1:1 mixture of defibrinated rabbit blood and Ringer–phosphate–bicarbonate buffer with added crystalline insulin (25 μU/ml), crystalline penicillin (200 U/ml), and D-glucose to give final glucose concentrations of 4.5–5 mM. In some experiments a 5%

bovine albumin–bicarbonate buffer containing 50 μU/ml of insulin, 5 mM D-glucose, and penicillin was used. The perfusion medium was maintained at 37°C, equilibrated continuously with humidified 5% CO_2, 18% O_2, and 77% N_2, and pumped through the isolated aorta at 100 mm Hg and 150 cycles/min with a pulsatile flow pump. After various times of perfusion with labeled substrates, the perfusate was flushed from the aorta with 6–10 ml of bicarbonate buffer and, using a second perfusion apparatus, blood– or albumin–buffer containing unlabeled substrate was perfused for a final 15 min. The purpose of the perfusion with unlabeled perfusate was to remove any adsorbed or residual intravascular labeled substrate or any rapidly exchangeable aortic labeled lipid.

During perfusions, samples of perfusate were removed for determination of the concentration and specific radioactivity of triglyceride, FFA, and glycerol and for analysis of distribution of radioactivity in lipid classes. Samples of the final unlabeled perfusate contained no significant radioactivity. The specific radioactivities of glycerol, triglyceride, and FFA were measured at the beginning, middle, and end of each perfusion, and they remained constant; the coefficients of variation were 5% for the specific radioactivities of glycerol and triglyceride and 13% for the specific radioactivity of FFA.

Treatment of aorta

After perfusion, the aorta was placed in ice-cold saline; it was stripped of periaortic fat, and adventitia and all arterial branches were removed. The aorta was then washed repeatedly in saline and split into an inner segment of tunica intima and inner media (intima) and an outer segment of media. The aortic segments and periaortic fat were then treated as previously described: homogenization, extraction of lipids with chloroform–methanol 2:1, and measurement of DNA in the defatted tissues (3).

The possibility of measuring labeled chylomicrons trapped in occluded arterial tributaries was minimized by ligating all these vessels at a distance from the aortic wall and perfusing them with the rabbit's own blood and subsequently with only defibrinated blood to avoid thrombosis in vessels during the labeled perfusion. Finally, after the unlabeled perfusion, all arterial branches were carefully removed before analysis of the aorta. The potential venous drainage of the aorta was left intact to prevent formation of venous trapping areas.

The periaortic adipose tissue was analyzed to assess its role as a possible radioactive contaminant of aortic media, since in previous aortic perfusion studies with [^{14}C]acetate the content of labeled lipid per gram wet weight of adipose tissue had exceeded that in aortic intima and media by 75 and 20 times, respectively. However, in the present labeled chylomicron experiments the triglyceride radioactivity per gram wet weight of adipose tissue was only 12 and 2.2 times greater than that in intima and media, respectively. Contamination of aorta by trace amounts of labeled lipid was not, therefore, a major problem.

Separation of lipids and chemical analyses

The methods of lipid extraction, purification, and TLC (separation of phospholipids, triglycerides, cholesteryl esters, FFA, and diglycerides plus cholesterol on silica gel H in light petroleum ether–ethyl ether–acetic acid 45:5:1) have been previously described (3). Triglycerides were separated into classes, according to their degree of unsaturation, by TLC on silica gel H impregnated with 12.5% silver nitrate (12, 13). The triglyceride classes were identified by the simultaneous chromatography of standards of tripalmitate, trioleate, and trilinoleate and, after spraying the thin-layer plates with 0.2% dichlorofluorescein in methanol, they were visualized with ultraviolet light. Triglycerides were eluted from silica with ethyl ether, and the radioactivity was assayed. Recovery of radioactivity from the plates was > 90%.

Glycerol was determined by a modification of the enzymatic method of Wieland (14, 15) after perfusate proteins were precipitated with perchloric acid and the supernatant solution was adjusted to pH 9.0 with KOH. Aliquots were counted and assayed for glycerol with glycerokinase and the glycerol-1-phosphate dehydrogenase NAD–NADH linked reaction; the absorbance of the enzyme mixture was measured at 366 nm. Blanks and standards were prepared in 5% bovine serum albumin and assayed simultaneously. The change in absorbance was proportional to glycerol concentration between 0.02 and 0.15 μmole. Perfusate glycerol concentrations remained constant throughout all perfusions with [^{14}C]glycerol.

The triglyceride content of perfusate was measured by the technique of Van Handel and Zilversmit (16). The specific radioactivity of triglyceride was obtained by measuring radioactivity in aliquots of the chloroform extract after removal of the small quantities of labeled FFA and phospholipids. Triglyceride concentrations in the perfusate were constant throughout all perfusions.

The concentration and specific radioactivity of perfusate FFA were obtained after extraction of perfusate by the method of Dole (17) and purification of the heptane phase in alkaline ethanol as previously described (3). In the double-label experiments the radioactivity in ^{3}H-labeled triglyceride greatly exceeded that in ^{3}H-labeled FFA, and triglyceride was, therefore, initially removed from the perfusate samples by precipitating chylomicrons with heparin (18). FFA from the super-

natant solution was purified until a constant $^{3}H/^{14}C$ ratio was achieved. Both techniques of purifying FFA were used in the first double-isotope perfusion and gave identical results, but initial precipitation by heparin greatly reduced procedure time. Tissue FFA was also purified via alkaline ethanol in these experiments.

Radioactivity

The uptake or incorporation of labeled perfusate substrates by aorta was calculated from the mean specific radioactivity of the substrates and the total aortic lipid radioactivity. Radioactivity was counted in a Mark I Nuclear Chicago liquid scintillation system using techniques for simultaneous ^{3}H and ^{14}C counting previously described (3).

RESULTS

Uptake of ^{3}H-labeled FFA and ^{3}H-labeled TGFA by aorta in vivo

Initially the possibility was explored that hydrolysis of plasma triglyceride at the endothelial surface might be a prerequisite for aortic uptake of TGFA.

It was anticipated from a previous study of FFA esterification in perfused aorta (3) that aortic hydrolysis of chylomicron-labeled triglyceride with release and reesterification of labeled FFA would result in labeling aortic phospholipids and diglycerides predominantly, whereas uptake of intact triglyceride molecules would label only aortic triglyceride.

In preliminary experiments, rabbits received by rapid intravenous injection either ^{3}H-labeled palmitic acid (0.75 μmole) or [^{3}H]palmitate-labeled rat chylomicrons (42 and 55 mg of triglyceride) in which 90% of the radioactivity was in TGFA. Thoracic aortas were removed 10 min later. The results are shown in Table 1. After injection of the labeled fatty acid, aortic lipid ester radioactivity was found mainly in triglycerides and phospholipids. The only significant plasma radioactivity after 10 min was in plasma FFA; aortic ester lipid radioactivity was therefore derived only from FFA. In the experiments in which labeled triglycerides were injected, the distribution of aortic lipid ester radioactivity differed markedly from the latter result: 80% of the labeled ester lipid was recovered in triglyceride and only 13% in phospholipid. These results suggest that, in vivo, plasma TGFA enter aorta without hydrolysis of triglyceride and that the metabolism of plasma TGFA by the aorta is quite distinct from metabolism of plasma FFA. However, the possibility cannot be excluded that aortic lipid radioactivity in the experiments with labeled triglycerides represented surface adsorption or contamination with labeled lipid from plasma.

TABLE 1. Aortic lipid ester radioactivity after intravenous administration of [^{3}H]palmitic acid or [^{3}H]palmitate-labeled triglyceride

	Labeled Lipid Injected		
	Palmitic Acid	Triglyceride	
	Aorta	Aorta	Plasma
	% *ester lipid radioactivity*[a]		
Triglyceride	42 ± 5	79 ± 8	90 ± 5
Diglyceride	22 ± 5	6 ± 3	3.5 ± 0.5
Phospholipid	35 ± 5	13 ± 6	1.5 ± 0.5
Cholesteryl ester	0	1 ± 1	5.0 ± 4.0

[a] Mean values ± SEM of four aortic inner and outer segments obtained from two animals in each experimental group.

Aortic perfusions with triglyceride-[2-^{3}H]glycerol and [2-^{14}C]glycerol

To examine in detail the kinetics and mechanisms of aortic uptake of circulating lipoprotein triglyceride, the in situ perfusion system for rabbit abdominal aorta (3) was used for all subsequent experiments. To measure rates of uptake of lipoprotein triglyceride, chylomicrons were labeled biosynthetically in either the glycerol or the fatty acid moiety of triglyceride.

The uptake of lipoprotein triglyceride-glycerol by aorta was examined in three perfusions. Chylomicrons obtained from rats fed triolein labeled with [2-^{3}H]-glycerol were perfused in blood–buffer for 80, 120, and 180 min; at the end of perfusions, labeled perfusate was flushed from the aorta with 10 ml of warm albumin–buffer. The results are shown in Table 2. In the perfusate, triglyceride accounted for > 93% of lipid radioactivity, and only 1.5% was recovered in phospholipid. The distribution of lipid radioactivity in aortic segments was almost identical with that in plasma. Aortic intima took up 21 ± 1 nmoles and media took up 170 ± 72 nmoles of triglyceride/mg of DNA/hr at a mean perfusate triglyceride concentration of 3.24 ± 0.86 mM. These values are similar to rates given below for aortic uptake of chylomicrons containing triglyceride labeled with [^{3}H]-palmitic acid.

To explore the possibility that these results might be

TABLE 2. Percentage distribution of aortic and perfusate lipid radioactivity in perfusions with chylomicron triglyceride labeled in the glycerol moiety[a]

	Perfusate	Aorta (Intimal and Medial Segments)
Triglyceride	93.3 ± 1.1[b]	93 ± 2.6
Diglyceride	5.0 ± 0.8	4.7 ± 1.3
Phospholipid	1.5 ± 0.3	2.8 ± 1.2

[a] n = 3.
[b] Means ± SEM.

explained by aortic hydrolysis of labeled chylomicron triglyceride and subsequent esterification of labeled free glycerol, the rates of incorporation of perfusate free [^{14}C]glycerol into lipids of perfused aorta and the distribution of aortic labeled lipids were measured. Two aortas were perfused for 2 hr with [2-^{14}C]glycerol in blood–buffer containing physiological concentrations of glycerol (0.25 mM and 0.28 mM) (19). The results are shown in Table 3. Perfusate glycerol carbon was incorporated into aortic lipids at rates which were quantitatively insignificant compared with rates of uptake of glycerol-labeled triglycerides by aorta described above. Aortic ^{14}C-labeled lipid was recovered predominantly in phospholipid, and the distribution of aortic lipid radioactivity after perfusions with [2-^{14}C]glycerol was similar to that previously described after perfusions of the same duration with ^{14}C-labeled glucose (3). It is evident from the low rate of incorporation of perfused [2-^{14}C]glycerol into aortic lipids and its distribution among aortic lipid classes that the aortic uptake of chylomicron triglyceride-glycerol, described above, cannot be attributed to triglyceride hydrolysis with reesterification of free labeled glycerol in aorta.

TABLE 3. Incorporation of [2-^{14}C]glycerol and [U-^{14}C]glucose into lipids of perfused aorta and periaortic fat

Aortic Lipid Glycerol Formation			Distribution of Aortic Ester Lipid Radioactivity			
	[^{14}C]Glycerol			[2-^{14}C]-Glycerol		[U-^{14}C]-Glucose
	Expt. 1	Expt. 2	Lipid Class[a]	Expt. 1	Expt. 2	(6 expts.)[b]
	nmoles/mg DNA/hr			*% of total*		*radioactivity*
Intima	0.39	0.55	TG	6	7	10 ± 2[c]
			DG	15	21	25 ± 3
			PH	77	72	65 ± 4
Media	0.85	0.63	TG	18	21	19 ± 1
			DG	18	26	25 ± 2
			PH	62	52	56 ± 3
Fat	1.80	1.28	TG	71	68	59 ± 4
			DG	29	32	38 ± 3
			PH	tr	tr	3 ± 1

[a] TG, triglycerides; DG, diglycerides; PH, phospholipids.
[b] Results of 2-hr perfusions with [U-^{14}C]glucose previously published; 97% of lipid radioactivity was in glycerol moiety of lipids (3).
[c] Means ± SEM.

As partial glycerides may be formed from lipoprotein triglycerides during hydrolysis by lipoprotein lipase (20), it was also possible that the aortic triglyceride radioactivity observed after perfusions with labeled chylomicron triglycerides was a result of reesterification of partial glycerides. As the labeled perfusate triglycerides had been obtained by feeding a tracer quantity of [^{3}H]-glyceryl trioleate with safflower oil, in which 70% of the fatty acids are linoleic acid (21), the labeled chylomicron triglycerides were highly unsaturated. In contrast, 70% of the TGFA in rabbit aorta and serum are saturated or monounsaturated (22); consequently, partial hydrolysis of these labeled triglycerides with reesterification by endogenous aortic fatty acids and FFA derived from the rabbit serum in perfusate would produce aortic labeled triglycerides less saturated than labeled chylomicron triglycerides. This possibility was explored in two perfusions with glycerol-labeled triglycerides by separating aortic and perfusate triglycerides by argentation TLC. The results are shown in Table 4. There was no difference in composition between perfusate and aortic triglycerides. There was, therefore, no evidence of partial hydrolysis and reesterification of partial glycerides during uptake of lipoprotein triglyceride by aorta.

These results and the results of the [2-^{14}C]glycerol perfusions indicate that perfusions with chylomicron triglycerides in the labeled triglycerides are taken up by aorta without hydrolysis.

Aortic perfusions with [^{3}H]palmitate-labeled triglycerides

To examine the aortic uptake of the fatty acids of circulating lipoprotein triglyceride, all subsequent perfusions were with labeled rat chylomicrons (labeled by

TABLE 4. Aortic perfusions with chylomicron triglyceride labeled in the glycerol moiety; distribution of labeled triglyceride classes in aorta and perfusate

Triglyceride Class[b]	Experiment 1[a]		Experiment 2		
	Plasma (n = 2)	Media	Plasma (n = 2)	Intima	Media
	% total triglyceride radioactivity				
0	1.2	2.3	0.6	1.4	0.6
1	10.3 ± 0.4[c]	9.4	5.2	7.2	5.2
2	14.9 ± 0.4	15.5	10.0 ± 0.2	10.3	10.1
3	26.0 ± 0.6	24.4	24.5 ± 0.6	24.9	24.7
4	20.5 ± 0.3	19.7	24.1 ± 0.5	25.3	24.1
5	16.7 ± 0.4	15.2	24.9	22.1	26.0
>5	10.4 ± 0.6	13.2	10.5	8.7	9.2

[a] Intima contained insufficient radioactivity for analysis.
[b] Triglycerides were classified according to number of double bonds.
[c] Ranges of values.

feeding rats glyceryl [9,10-^{3}H]tripalmitate or [9,10-^{3}H]-palmitic acid as described earlier); each labeled perfusion was followed by a 15-min perfusion with unlabeled chylomicrons. The results of a series of six perfusions are shown in Table 5. The distribution of perfusate lipid radioactivity was similar in all experiments, and no consistent change was noted in the aortic distribution of labeled lipid with either perfusion time or concentration of perfusate triglyceride. In aorta, triglyceride contained 85% of lipid ester radioactivity, and in the perfusate > 95% of lipid ester label was in triglyceride. However, a second possible precursor of labeled aortic triglyceride, perfusate FFA, accounted for 2.5 ± 0.5% of total perfusate lipid radioactivity. As shown in parentheses in Table 5, labeled triglyceride has previously been shown to be a minor product of aortic lipid synthesis from labeled perfusate FFA in aortas perfused for 2 hr (3). Thus, only a small fraction of the labeled aortic triglyceride in these experiments with ^{3}H-labeled TGFA could have been derived from esterification of perfusate FFA. These results suggest that perfusate TGFA remained esterified during uptake by the aorta.

In these experiments with labeled chylomicrons, the proportion of lipid label in aortic diglycerides and phospholipids of 13.4% (12 ± 3% in intima and 13 ± 3% in media) was higher than that in perfusate. In contrast, in the experiments with [^{3}H]glycerol-labeled triglyceride, the distribution of lipid label in perfusate and aorta was almost identical (Table 2). As discussed above, this difference was anticipated from aortic esterification of the ^{3}H-labeled FFA in the chylomicrons of [^{3}H]palmitate-labeled chylomicrons to form predominantly phospholipids and diglycerides; in these experiments, the calculated mean aortic esterification rates to produce this increase in phospholipid and diglyceride radioactivity were 27 and 106 nmoles of FFA/mg of DNA/hr in intima and media, respectively. These rates approximate aortic esterification rates of perfusate FFA previously reported (37 and 70 nmoles of FFA/mg of DNA/hr). There was no evidence from these calculations that hydrolysis of chylomicron triglyceride contributed to newly esterified ^{3}H-labeled fatty acid although the specific radioactivity of chylomicron TGFA was 3.0 ± 0.56 greater than that of perfusate FFA.

TABLE 5. Aortic perfusions with chylomicrons containing [^{3}H]palmitate-labeled triglycerides

	% Distribution of Ester Lipid Radioactivity		
	Perfusate	Aorta[a]	(Intima Media)[b]
Triglyceride	95.6 ± 0.7[c]	85 ± 2.9	(8 22)
Diglyceride	2.3 ± 0.3	5.3 ± 0.8	(17 18)
Phospholipid	1.2 ± 0.3	8.1 ± 2.2	(74 60)
Cholesteryl ester	0.9 ± 0.2	1.4 ± 0.3	(<1 <1)

The duration of labeled perfusion was 105 min in four experiments and 30 min and 10 min in two experiments.

[a] Results of intimal and medial analyses.

[b] Values in parentheses are mean values in five 2-hr perfusions with [1-^{14}C]palmitic acid (3).

[c] Means ± SEM (n = 6).

As only rat chylomicrons had been used, it was of interest to compare the results obtained with chylomicrons from a rabbit. Rabbit chylomicrons, labeled by feeding [^{3}H]palmitic acid, were perfused in a single 105-min experiment. The distribution of lipid radioactivity in the aorta and perfusate was identical with that obtained with rat chylomicron perfusions, and the rate of aortic triglyceride uptake from the perfusate was similar to that subsequently observed with rat chylomicrons. Since there were no obvious differences in metabolism of chylomicrons from the two animals, all other experiments were performed with rat chylomicrons, which were obtained more easily.

Kinetics of triglyceride uptake by perfused aortas

The effects of perfusion time and triglyceride concentration on aortic uptake of perfusate lipoprotein triglyceride were explored by perfusing rat chylomicrons (^{3}H-labeled TGFA) for various times (10–105 min) and at TGFA concentrations varying from 0.4 to 25 mM. Very low TGFA concentrations (< 1.3 mM) were achieved by perfusing chylomicrons in albumin–buffer instead of blood–buffer. Chylomicron triglyceride uptakes in both types of media were compared in experiments at perfusate TGFA concentrations of 2–4 mM.

The effects of concentration on rates of TGFA uptake by aortic intima and media are shown in Figs. 1 and 2. In intima the TGFA uptake was dependent on perfusate TGFA concentration only below 1.95 mM. In the three perfusions at concentrations of < 1.95 mM, rates of triglyceride uptake by intima were significantly less than in the other perfusions ($t = 7.2$, $P < 0.01$). Analysis of uptake by intima of TGFA at concentrations of 1.95 mM and above showed that there was no correlation between uptake and concentration; the regression line of these values is shown in Fig. 1. It is of interest that the rate of uptake was not increased despite increasing concentrations of TGFA in perfusate to as high as 25 mM. The variations in data do not permit a detailed kinetic analysis, but they suggest that there may be a maximal intimal uptake of TGFA of approximately 90 nmoles of TGFA/mg of DNA/hr. This rate is approached at a TGFA concentration of 2.0 mM, a value in the lower range of physiological levels described for the rabbit (23).

The kinetics of TGFA uptake by media are shown in Fig. 2; the rate of medial uptake of TGFA for all perfusions was significantly correlated with TGFA concentration ($r = 0.712$, $t = 3.93$; $P < 0.005$). There was no evidence of the saturation phenomenon observed in

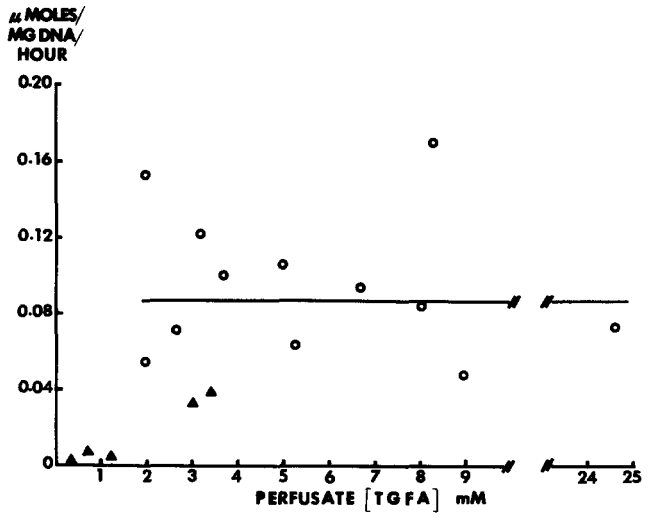

FIG. 1. Uptake of perfusate TGFA by aortic intima relative to perfusate TGFA concentrations. 12 perfusions were defibrinated blood (○) and 5 perfusions were with albumin–buffer (▲). Durations of labeled perfusions were from 10 to 105 min and, as shown in Fig. 3, uptake increased linearly with time. The perfusate contained chylomicrons labeled with [^{3}H]palmitate triglyceride. The regression line is for values at perfusate TGFA concentrations of 1.95–25 mM.

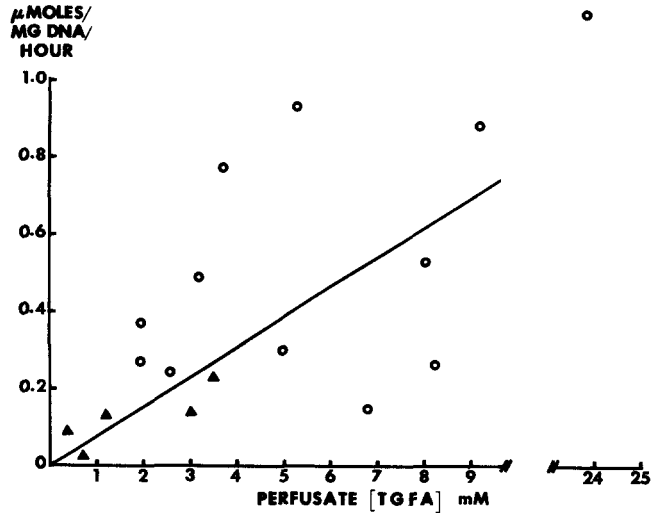

FIG. 2. Uptake of perfusate TGFA by aortic media relative to perfusate TGFA concentrations. Experiments and symbols are explained in Fig. 1. Uptake and concentration of TGFA were significantly correlated ($P < 0.005$), and the regression line for all values is shown but interrupted at 10 mM TGFA.

intima, and very high rates of uptake were observed with high triglyceride concentrations.

In both intima and media the calculated TGFA uptake from albumin–buffer was less than that from blood–buffer at approximately the same TGFA concentrations, but the differences were not significant. It is evident from the albumin experiments that chylomicron triglyceride can be taken up by aorta in the absence of other lipoproteins.

The relationship of TGFA uptake to time of perfusion is shown in Fig. 3. Since intimal uptake had been shown to be independent of TGFA concentration at 1.95 mM or above, only perfusions at or above this concentration are shown. The linear increase of TGFA uptake with time in aortic intima is evident. This demonstrates that the apparent saturation of intimal TGFA uptake rate, observed at concentrations of 1.95 mM and above (Fig. 1), was not due to saturation of intimal capacity for lipoprotein triglyceride nor was it due to reaching a steady state in which intimal triglyceride influx from perfusate was balanced by efflux from intima into media. The uptake of TGFA in media had been shown to increase in an approximately linear fashion with increasing perfusate TGFA concentrations (Fig. 1). Medial uptakes in each experiment were therefore multiplied by the ratio of the mean TGFA concentration to the TGFA concentration of that experiment; these corrected values for uptake are shown in Fig. 3. TGFA uptake in media showed large variations but it was approximately linear with time.

The 15-min perfusion with unlabeled chylomicrons was designed to remove intravascular or adsorbed labeled chylomicrons. To determine if this time was adequate, two experiments were carried out in which a 1-hr perfusion with [^{3}H]palmitate-labeled chylomicrons was followed by a 1-hr perfusion with unlabeled chylomicrons. The distribution of aortic and plasma lipid

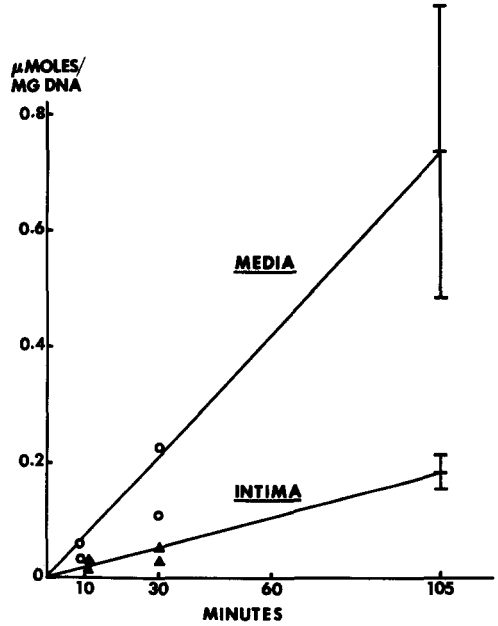

FIG. 3. Relationship of TGFA uptake to time in intima (▲) and media (○). All medial uptakes were corrected for TGFA concentrations as described in the text. Intimal uptake was independent of concentration with the exception of the three values at TGFA concentration < 1.95 mM, and these three were omitted from this figure.

radioactivity was similar to that of the other [^{3}H]palmitate-labeled chylomicron experiments, and the mean rates of aortic triglyceride uptake in intima and media (290 and 1080 nmoles/mg of DNA/hr, respectively) were greater than the predicted 1-hr uptakes of 110 and 600 nmoles obtained from Fig. 1. Prolonging the unlabeled perfusion did not produce a fall in aortic lipid radioactivity, and the standard 15-min perfusion was probably sufficient.

The intra- and extracellular distributions of endogenous aortic triglyceride and of chylomicron ^{3}H-labeled triglyceride taken up by aorta were not determined, but it was of interest to compare the quantity of endogenous aortic triglyceride with the rates of triglyceride uptake. The triglyceride content of abdominal aorta in two pools of four rabbits of the same weight and nutritional state as the perfused animals was 990 ± 140 μg of triglyceride/100 mg wet weight. The mean triglyceride uptake in these chylomicron perfusions (excluding the three perfusions with TGFA concentration < 1.95 mM) was 6.1 μg/100 mg wet weight/hr.

Simultaneous aortic perfusion with [^{3}H]- and [^{14}C]palmitate-labeled FFA and [^{3}H]palmitate-labeled triglyceride

Although the results of all perfusion experiments were consistent with the aortic uptake of intact triglyceride molecules, they did not exclude the possibility of triglyceride hydrolysis in aorta. To investigate triglyceride hydrolysis in aorta by release of ^{3}H-labeled fatty acids it was necessary to distinguish the labeled perfusate TGFA from the labeled FFA in perfusate. This was done by preparing chylomicrons with glyceryl [^{3}H]tripalmitate in the usual manner and equilibrating them, before perfusion, with defibrinated blood containing [^{14}C]-palmitic acid. The ^{3}H/^{14}C ratios in FFA in all double-isotope perfusions were constant throughout the perfusions. Each labeled perfusion lasted 30 min and was followed by a prolonged unlabeled perfusion period (30 or 60 min) so that measurement of any continuing hydrolysis of labeled ^{3}H-labeled triglyceride within the aortic wall would not be obscured by further influx of labeled FFA from perfusate. The results are shown in Table 6. Perfusate FFA and aortic phospholipid had a ^{3}H/^{14}C ratio of 1.0. Aortic labeled phospholipid was, therefore, derived by esterification of aortic FFA with a ^{3}H/^{14}C ratio of 1.0, and perfusate ^{3}H-labeled phospholipid was not a major precursor of aortic labeled phospholipid. The ^{3}H/^{14}C ratio of aortic FFA measured at the end of perfusion was, however, significantly higher ($P <$ 0.025) than ratios in plasma FFA, aortic phospholipid, and, by inference, aortic FFA during the major time of aortic synthesis of labeled lipid. Evidently aortic FFA was partly derived from a precursor with a high ^{3}H/^{14}C ratio. The only lipid with a ^{3}H/^{14}C ratio exceeding FFA was triglyceride, and these results, therefore, indicate that hydrolysis of ^{3}H-labeled triglyceride occurred. The results are compatible only with hydrolysis within the aortic wall since hydrolysis at the endothelial surface, in association with uptake of ^{3}H-labeled triglyceride, would have increased the ^{3}H/^{14}C ratios of aortic FFA and its ester product, aortic phospholipid, above that of perfusate FFA. Hydrolysis of triglyceride at the endothelium would also have produced ^{3}H-labeled FFA only during the labeled perfusion and would not have increased the ^{3}H/^{14}C ratio of aortic FFA thereafter. These results suggest that triglyceride derived from the perfusate was hydrolyzed within the aortic wall.

TABLE 6. Simultaneous perfusion of aorta with [^{3}H]palmitate-labeled chylomicron triglycerides and FFA ([^{3}H,^{14}C]palmitic acid)

	^{3}H/^{14}C
Serum triglyceride	∞
Serum FFA	1.00 ± 0.01[a]
Aortic phospholipid	1.05 ± 0.14
Aortic triglyceride	13.7 ± 3.2
Aortic FFA	2.5 ± 0.5[b]

[a] Means ± SEM (n = 3).
[b] Significantly greater than serum FFA ($P < 0.01$) and aortic phospholipid ($P < 0.025$).

DISCUSSION

In these perfusion experiments, extensive efforts were made to remove all labeled chylomicrons from surfaces and intravascular spaces of aorta. However, the possibility that labeled chylomicrons remained in these areas cannot be entirely excluded. The absence of detectable reduction in aortic triglyceride radioactivity after prolonged unlabeled perfusion and the linear increase in aortic triglyceride uptake for 2 hr suggest that rapid efflux of labeled triglyceride from aorta was not quantitatively important or was not measurable by these techniques.

In this investigation of aortic uptake of labeled chylomicron triglyceride, advantage was taken of the demonstrated preferential incorporation of labeled glycerol and fatty acids into phospholipids of perfused aorta. Complete hydrolysis of triglyceride labeled in either the glycerol or the fatty acid moiety would have produced, by reesterification, labeling predominantly in phospholipid with little radioactivity in triglyceride. After perfusion or intravenous injection of chylomicron triglyceride labeled in either the glycerol or the fatty acid moiety, aortic lipid ester radioactivity was recovered largely in triglyceride, indicating aortic uptake of intact triglyceride molecules both in vivo and in the perfused preparation.

Chylomicron triglyceride was taken up by aorta at similar rates in the presence or absence of other lipoproteins, but this does not exclude the possibility of triglyceride transfer between chylomicrons and other lipoproteins before aortic uptake in vivo. It may be inferred from these results that, in intact animals, exogenous TGFA may pass into the blood stream from the thoracic duct and enter arterial walls before sequestration of chylomicron triglyceride in other tissues can occur.

Chylomicron triglyceride transport into aortic intima approached saturation at triglyceride concentrations similar to those observed in plasma of fasting rabbits (24). The kinetics of triglyceride uptake by media did not show this effect nor were the large variations in medial uptake rates accompanied by large variations in intimal uptake. These observations suggest that intimal uptake of chylomicron triglyceride was directly from the perfusate and independent of medial uptake.

The nature of transport of chylomicron triglyceride across continuous endothelium such as aortic intimal endothelium is unknown, nor is it known whether triglycerides dissociate from other lipoprotein components during transport into tissues. The size of chylomicrons (approx. 5000 Å) would appear to exclude the passage of intact chylomicrons across the normal intercellular junctions of continuous endothelium (20–50 Å), although in newborn rats electron microscopic studies have indicated that chylomicrons may penetrate between unusually large intercellular junctions (25). The concept of triglyceride transport in the caveolae of the endothelial cells is unproven. The saturation of intimal triglyceride uptake may be due to saturation of endothelial lipoprotein binding sites, transport mechanisms, or transport sites or simply to mechanical hindrance of lipoprotein triglyceride by basement membrane or mucopolysaccharide matrix of the intima. Whatever is the nature of this process it is possible that the saturation phenomenon has biological importance in preventing excessive triglyceride influx into normal aortic intima during periods of high plasma triglyceride concentration.

In the perfused aorta, triglyceride is derived partly from intracellular synthesis from α-glycerophosphate and FFA and partly from uptake of chylomicron triglyceride which remains largely unhydrolyzed. It is now generally agreed that in adipose tissue little extracellular triglyceride is incorporated into intracellular storage triglyceride without lipolysis (26), and lipolysis similarly precedes uptake of extracellular TGFA by heart muscle (27). It is probable, therefore, that unhydrolyzed lipoprotein triglyceride in these perfused aortas was also confined to an extracellular compartment.

The present results suggest that chylomicron triglyceride was hydrolyzed within the aortic wall and not at the endothelial surface. Aortic hydrolysis of chylomicron triglyceride may be mediated by the aortic lipase a described by Korn for rabbit and rat aortas, using chylomicrons as a substrate (24). Aortic lipolytic enzymes with some characteristics of lipoprotein lipase have been extensively reviewed by Zemplenyi (28), who noted that rabbit aorta has the lowest activity of a number of animal species. This would suggest that lipoprotein triglyceride hydrolysis may be of greater quantitative importance in aortas of species other than the rabbit. Perfusion of rabbit aortas in vitro with activated Ediol (coconut oil triglyceride, monoglycerides, and polyoxyethylene sorbitan monostearate) has produced marked increase of FFA in the medium inhibited by protamine, but this substrate is not specific for triglyceride hydrolysis (29). In the present study there was no detectable hydrolysis of chylomicron-labeled triglyceride in the perfusate.

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