UPTAKE OF CHYLE CHOLESTEROL ESTERS AND INTACT TRIGLYCERIDES BY SUSPENDED HEPATOCYTES

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1. Introduction

Most of the chyle triglycerides are hydrolyzed by lipoprotein lipase in extrahepatic tissues [1]. Particles enriched in cholesterol esters are formed [2] which are rapidly cleared from blood mainly by the hepatocytes [3–6]. The mechanism by which this occurs is poorly understood. In the present study the uptake of chyle cholesterol esters was studied in hepatocyte suspension. Addition of postheparin plasma that hydrolyzed most of the lymph triglycerides markedly increased the uptake of cholesterol ester. The uptake was limited during short term incubations but was improved if the cells were preincubated for 2 hr. This improvement was prevented by cycloheximide and colchicin. Significant uptake of triglyceride that had not been hydrolyzed by the postheparin plasma also occurred. This uptake was inhibited to about the same extent as the uptake of cholesterol ester if the cells had been preincubated with colchicin or cycloheximide. The data suggest that cholesterol ester was taken up by the cells in 'remnant particles' containing on average 10% of their original triglyceride. The inhibition of the uptake with cycloheximide and colchicin indicates that the affinity of these particles for the hepatocytes may depend on certain cell surface proteins, that are damaged during the cell isolation and in part reconstructed during incubation of the cells.

2. Methods

Male white Sprague-Dawley rats kept on a controlled feeding schedule [7] were used. Suspended hepatocytes were prepared by a collagenase procedure [8,9]. The details are given elsewhere [10]. After thoracic duct cannulations [11] animals had a constant infusion of 2.5% glucose, 0.5% NaCl and 0.05% KCl (3 ml/hr) through a stomach fistula. About 24 hr after operation the rats were given 1.2 ml cream (40% fat) mixed with 1,2 [$^3H_2$] cholesterol (200 μCi) and 1 [^{14}C] palmitic acid (25 μCi) (Radiochemical Centre, Amersham, England) that had been emulsified with 5 mg egg lecithin. The fat was given through the stomach fistula in three doses over 6 hr. Lymph was collected for 10 hr after the first dose. In one experiment [^3H] cholesterol was given in 0.5 ml triolein. After defibrination 50 units of benzyl penicillin and 50 μg streptomycin sulfate per ml were added and the lymph was stored at 4°C. The distribution of radioactivity in particles with $S_f > 400$ and with $S_f < 400$ was determined by gradient centrifugation [12].

The cells were incubated in Hanks' solution containing 25 mM $NaHCO_3$, 19.4 mM HEPES, 1.3% defatted and dialyzed bovine serum albumin [10] and amino acids in similar concentrations as used by East et al. [13].

After interrupting the incubations by cooling, the cells and media were separated by centrifugation [14]. Lipids were extracted from supernatants and cell pellets [15]. They were separated by thin-layer chromatography and radioactivity was determined as described earlier [10,15]. The triglycerides were eluted with diethyl ether, taken to dryness, and separated by argentation chromatography according to the number of double bonds [16]. The total lipid content of the lymph was determined by weighing a dried lipid extract. Cholesterol was determined [17] after saponification of the lipid extract [18]. The triglycer-

ide content and the fatty acid composition of lymph was determined by gas liquid chromatography of the fatty acid methyl esters [19]. Protein was determined according to Lowry et al. [20].

3. Results and discussion

3.1. *Hydrolysis of lymph triglycerides with postheparin plasma.*

Most of the triglycerides were rapidly hydrolyzed when chyle was incubated with postheparin plasma under the conditions used in the incubations with cells. For instance after 30 min only 12.8% of the triglycerides remained unhydrolyzed (table 1). The further hydrolysis was slow, and after 120 min 9.8% was still left intact. There was no net hydrolysis cholesterol ester.

Doubly labelled lymph obtained after feeding cream mixed with [^{14}C] palmitic acid and [$^{3}H_2$] cholesterol contained a large proportion of saturated fatty acids (32% palmitic, 14% stearic and 11% myristic acid). 47% of the [^{14}C] triglyceride was in completely saturated molecular species (table 2). The distribution of palmitate mass was similar to that of ^{14}C. After lipolysis with postheparin plasma the remaining triglycerides were enriched in fully saturated species, which contained 65% of the radioactivity after 30 min (table 2). Thus the remaining triglycerides consisted mainly of molecular species that were not synthesized to any significant degree by the cells, as shown by the distribution of [^{3}H] palmitic acid in the presence of linoleic acid (fig.1) [21].

3.2. *Uptake of cholesterol ester and intact triglyceride by the hepatocytes*

In accordance with earlier observations [22], the uptake of chyle cholesterol ester and triglyceride by hepatocytes was small in the basic medium, and was further decreased by the addition of serum. The uptake of cholesterol ester was markedly increased by adding postheparin plasma, that hydrolyzed most of the triglyceride during the incubation (Å. Nilsson, unpublished data). During the initial period the uptake was limited and varied between different cell preparations. The uptake was improved significantly, if the cells had been preincubated for 2 hr before adding postheparin plasma and the chyle. This improvement was prevented by colchicin and cycloheximide (fig.2). In all experiments described below the cells were therefore preincubated for 120 min. The average uptake of [^{3}H] cholesterol ester by the cells in 30 min was

Table 1
Uptake of chyle cholesterol ester and intact triglyceride by suspended hepatocytes

Incubation time (min)	% Unhydrolyzed triglyceride in cell-free controls	% Decrease of radioactivity in the medium, compared to cellfree controls		% Radioactivity in cells	
		Cholesterol esters	Triglycerides	Cholesterol esters	Saturated triglycerides
30	12.8 ± 1.5	32.8 ± 9.1	26.7 ± 10.6	40.4 ± 7.8	36.1 ± 8.4
120	9.8 ± 0.3	72.2 ± 5.2	68.7 ± 2.9	68.7 ± 2.9	73.1 ± 7.8

Thoracic duct lymph (0.52 mg lipid, 2.3 g cholesterol, 1.7 g cholesterol ester in 25 μl), doubly labelled with 1,2[$^{3}H_2$]cholesterol (4500 cpm as cholesterol ester and 4456 cpm as nonesterified cholesterol) and 1-[^{14}C]palmitic acid (5663 cpm as triglyceride) was used. Particles with $S_f > 400$ contained 93.9% of the [^{14}C]triglyceride and 80.9% of the [^{3}H]cholesterol ester. Hepatocytes (13.2–28.0 mg protein) were preincubated for 2 hr before adding 400 μl chyle, 25 μl chyle and albumin bound linoleic acid (final concentration 1.0 mM). The total volume was 1.5 ml. The values for the decrease of radioactivity in the medium are means ± S.E.M. of five experiments. In three of the experiments the distribution of [^{14}C]triglyceride among different molecular species was determined. In the right part of the figure the uptake of fully saturated triglycerides by the cells in these experiments is compared to the uptake of cholesterol ester.

Table 2
Distribution of [^{14}C]palmitic acid among different trigluceride species of chyle before and after incubation with postheparin plasma

Chyle				Chyle incubated with postheparin plasma	
No of double bonds	Mass composition		% ^{14}C	% ^{14}C	
	% of total fatty acid	% of palmitic acid		30 min (3)*	120 min (3)
0	32.5	46.4	47.0	64.9 ± 4.9	78.7 ± 1.6
1	38.1	35.2	39.0	22.3 ± 3.6	12.4 ± 0.7
2	20.0	11.4	9.7	7.0 ± 1.5	3.7 ± 0.6
3	4.0	3.5	2.0	2.6 ± 0.5	2.2 ± 0.2
> 3	5.4	3.4	2.3	3.7 ± 0.7	3.0 ± 0.3

The left part of the table describes the distribution of fatty acid mass, palmitic acid mass and [^{14}C]palmitic acid into different triglycerides of the chyle that was used in the experiments described in talbes 1 and 2. The right part of the table shows % distribution of the remaining triglyceride into different molecular species after incubation with postheparin plasma. The values are from cellfree controls of the experiments described in table 1. *No of experiements.

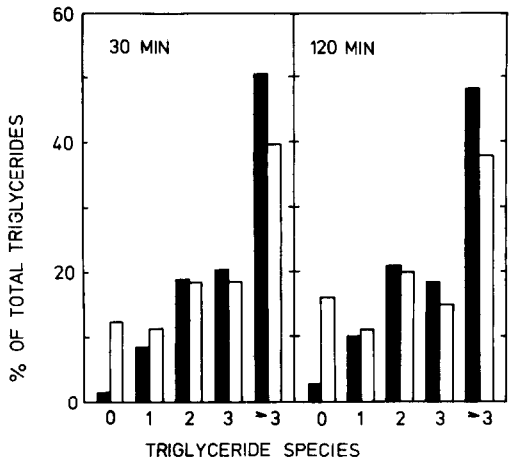

Fig.1. Distribution of lymph triglyceride [^{14}C] palmitic acid and non-esterified [^{3}H] palmitic acid into different cellular triglyceride species. Hepatocytes (21.6 mg cell protein) were incubated with 25 μl lymph labelled with [^{14}C] palmitic acid and [^{3}H] cholesterol and 400 μl postheparin plasma as described in table 1. The cells were preincubated for 120 min before adding lymph, postheparin plasma, albuminbound [^{3}H] palmitic acid (< 1 μM 62 000 cpm) and albuminbound linoleic acid (final concentration 1 mM). Data are from one of two similar experiments.
Open bars – distribution of [^{14}C] palmitic acid. Filled bars – distribution of [^{3}H] palmitic acid.

32.8%. In the same time 26.7% of the radioactivity remaining in triglyceride in the cellfree controls disappeared from the medium (table 1). If the uptake of cholesterol ester and triglyceride occurred in the same particles, these would contain on average 11.2% of their original amount of triglyceride (table 4). The

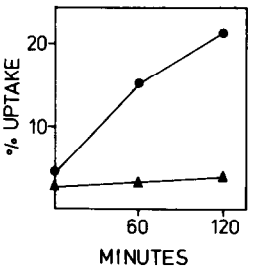

Fig.2. Improved uptake of lymph cholesterol ester after preincubation of the cells. Hepatocytes (8.8 mg protein) were incubated in Hanks' solution containing 2% bovine serum albumin for the times indicated. Then lymph labelled with [^{3}H] cholesterol (25 μl, 7560 cpm cholesterol ester, 4350 cpm unesterified cholesterol, 276 μg total lipid, 5.5 μg total cholesterol) and 400 μl post heparin plasma were added, and the incubations were continued for another 60 min.
Symbols: (▲) cells preincubated in the presence of 0.035 mM cycloheximide. (●) cells preincubated without inhibitor.

Table 3
Effect of colchicin and cycloheximide on the uptake of lymph cholesterol ester and triglyceride by hepatocytes

Inhibitor	Disappearance of [^{3}H]CE from medium %	Disappearance of [^{14}C]TG from medium[a] %	[^{14}C]TG in cells (cpm)	Saturated [^{14}C]TG in cells (cpm)
None	32.8 ± 9.1	26.7 ± 10.6	1547 ± 128	236 ± 67
Colchicin (0.033 mM)	11.3 ± 7.0 (70.3 ± 6.8***)	5.9 ± 2.9 (82.1 ± 4.7***)	1596 ± 145	111 ± 40 (55.9 ± 6.6**)
Cycloheximide (0.035 mM)	12.6 ± 8.4 (71.5 ± 8.5**)	8.7 ± 4.2 (74.2 ± 9.4**)	1495 ± 116	84 ± 24 (64.0 ± 3.4***)

Cells were preincubated for 2 hr, with and without inhibitors as described in table 1. Then 25 μl doubly labelled lymph, 400 μl postheparin plasma and albuminbound linoleic acid (final conc. 1 mM) were added and the incubations were continued for another 30 min. Data are means ± S.E.M. (n=5). The degree of inhibition (%) is shown within brackets. Abbreviations: CE, cholesterol ester; TG, triglyceride. The probability that the inhibition was significant was calculated by t-test.
***$P < 0.001$; **$0.001 < P < 0.001$
[a] Relative to cellfree controls.

presence of cells might, however, increase the hydrolysis of triglyceride in the medium. It was therefore necessary to prove directly that uptake of intact [^{14}C] triglyceride occurred. Since a large proportion of the free fatty acids formed during the lipolysis of lymph triglycerides with postheparin plasma are used for triglyceride biosynthesis by the hepatocytes [21] (table 3), this could not be done by measuring the triglyceride radioactivity of cells and medium. In the presence of 1 mM linoleic acid in the medium, however, the cells synthesized only small amounts of fully saturated triglycerides (fig.1). The presence of saturated [^{14}C] triglycerides in the cells must then indicate uptake of intact triglycerides. After 30 min of incubation 36.1% of the recovered saturated triglycerides and 40.4% of the cholesterol ester had actually been taken up by the cells (table 1). A parallel uptake of cholesterol ester and intact triglyceride continued between 30 and 120 min of incubation. The total radioactivity recovered in saturated triglycerides was not significantly lower than in the cellfree controls, indicating that no extensive hydrolysis of saturated triglycerides occurred in the cells. The average net hydrolysis of [^{3}H] cholesterol ester was 16.8% in

Table 4
Relative amounts of cholesterol ester and intact triglyceride taken up by the hepatocytes

Inhibitor	TG disappearance × 100 / CE disappearance	Inhibited part of TG disappearance × 100 / Inhibited part of CE disappearance
None	11.2 ± 1.6	
Colchicin (0.033 mM)	8.0 ± 2.3	10.2 ± 1.5
Cycloheximide (0.035 mM)	6.5 ± 1.4	11.9 ± 2.3

The disappearance from the medium of cholesterol ester (CE) and unhydrolyzed triglyceride (TG), expressed as percent of original labelled CE or TG was calculated. TG-disappearance = % of added TG remaining unhydrolyzed in cellfree controls minus % of added TG in medium after incubation with cells. Since there was no significant net hydrolysis of cholesterol esters in the cellfree controls CE-disappearance = % of added CE that had been taken up by the cells. If it is assumed that the intact triglyceride and the cholesterol ester were taken up in the same particles the calculated ratio gives the triglyceride content of these particles expressed as % of that in the original chyle lipoproteins. The given values are all based on radioactivity data. From the data in table 2 the proportion of triglyceride mass taken up intact can be calculated, and was found to be only 5% less than the proportion of radioactivity taken up as intact triglyceride. Data are means ± S.E.M. from 5 experiments. Incubation time after lymph addition: 30 min.

120 min, and was below 5% in 30 min. Thus the rate of cholesterol ester hydrolysis was slower than in the rat liver in vivo, where the hydrolysis is essentially completed in an hour [3,4]. Yet the observation that it can be inhibited by metabolic inhibitors and with colchicin (Å. Nilsson, unpublished data) indicates that it is due to a physiologic process and does not occur in the small proportion of damaged cells.

3.3. *Effects of colchicin and cycloheximide*

The presence of colchicin or cycloheximide during the preincubation period inhibited the disappearance from the medium of intact triglyceride and cholesterol ester to about the same degree (table 3). The data suggested (table 4) that colchicin inhibited the uptake of particles containing on average 10.2% of their original triglyceride. Calculations based on the data obtained with cycloheximide gave similar results. This suggested that the uptake of cholesterol ester and of intact triglyceride from the medium was due to a common mechanism. Redgrave [2] found that after intravenous injection of chylomicrons into functionally hepatectomized rats, particles were formed that contained on average 10% of their original amount of triglyceride. These particles were rapidly cleared by the liver, when injected into a normal animal. Although it is recognized that the lymph lipoproteins acting as substrates for the lipases in postheparin plasma, as well as those formed during the lipolysis are likely to be heterogenous in size and in their relative content of triglyceride and cholesterol ester, our data indicate that particles with a similar triglyceride/cholesterol ester ratio may be substrates for the suspended hepatocytes.

The results are compatible with the hypothesis that the action of lipoprotein lipase in postheparin plasma yields cholersterol ester rich particles with affinity for the hepatocytes. The data also suggest that certain cell surface proteins are necessary for this affinity. Such proteins may be damaged during the enzymatic cell isolation and reconstructed during the preincubation of the cells. This hypothesis would explain why the uptake of cholesterol ester was not improved during preincubation with cycloheximide. By analogy with the known effects of colchicin on a number of secretory processes [22–24] the same inhibitory effect of colchicin might be due to inhibited incorporation of newly synthesized protein into the plasma membranes.

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