REGULATION OF FATTY ACID SYNTHESIS IN ISOLATED HEPATOCYTES BY INTESTINAL CHYLOMICRONS AND THEIR REMNANTS

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1. Introduction

Dietary fatty acids are incorporated into chylomicrons and the chylomicron triglycerides are degraded by the action of lipoprotein lipase. The resultant particles, remnants, are selectively metabolized by the liver [1]. Sherrill and Dietschy demonstrated that unmetabolized chylomicrons were taken up at very low rates, whereas chylomicron remnants were taken up by the perfused liver at much higher velocities [2]. Recently, Carrella and Cooper demonstrated that there was a high affinity receptor for the chylomicron remnant on the surface of the hepatocytes [3]. This study was undertaken to examine the effects of chylomicrons and their remnants on the fatty acid synthesis in isolated rat hepatocytes.

Chylomicrons in the medium containing post-heparin rat plasma significantly inhibited fatty acid synthesis in hepatocytes. On the other hand, chylomicrons in the medium containing pre-heparin rat plasma caused inhibition to a much smaller extent. Remnants prepared from the chylomicrons injected into functionally hepatectomized rats inhibited fatty acid synthesis to a greater extent than unmetabolized chylomicrons. Thus the chylomicron remnants produced by the action of lipoprotein lipase may play an important role in the regulation of hepatic fatty acid synthesis.

2. Materials and methods

Normal fed male Wistar rats were used (250–350 g). Isolated hepatocytes were prepared by the method in [4] with the modification [5] at noon. These hepatocytes were 85–95% viable as tested by trypan blue dye exclusion.

The hepatocytes were suspended in Eagle's minimal essential medium (pH 7.4) containing 1.5% bovine serum albumin (fraction V, Wako Pure Chemicals Co.) and 5% lipoprotein poor serum (<3 mg cholesterol/ dl) prepared by ultracentrifugation [6]. For the studies on the incorporation of radioactivity into fatty acids, 1 μCi [1-^{14}C]acetate sodium salt (55 mCi/mmol, The Radiochemical Centre, Amersham) or 0.2 mCi $^{3}H_2O$ (1 mCi/g, New England Nuclear) was added to 1 ml incubation medium. All incubations were done in stoppered plastic vials at 90 osc./min. in a metabolic incubator at 37°C for 1 h in an atmosphere of 5% CO_2–95% O_2. Radioactive fatty acids were determined as in [7].

Chylomicrons were obtained from a thoracic duct fistula by a modification of the technique in [8]. Rats were given 2 ml of corn oil by stomach tube and 3 h–5 h later lymph samples were collected in plastic vessels. Chylomicrons were isolated by layering 1 ml chyle under 3 ml 0.15 M NaCl (pH 7.4) and floated at $4.5 \times 10^6 \times g$.min at 4°C. The chylomicrons were resuspended in 0.15 M NaCl (pH 7.4).

For chylomicron remnant formation by peripheral tissues functionally hepatectomized, eviscerated rats (300 g) were prepared under sodium pentobarbital anesthesia by the method in [9]. Chylomicrons containing ~60–100 mg triglycerides were injected intravenously and the animal was maintained under

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anesthesia for 1, 5, 10 or 30 min before a blood sample was taken from the abdominal aorta. Remnants (d =1.006) were isolated by floatation at 105 000 × g for 22 h and dialyzed at 4°C for 12–18 h against 0.15 M NaCl (pH 7.4). Post-heparin rat plasma was obtained at 10 min after rapid intravenous injection of 100 IU sodium heparin (Novo Industry A/S)/kg body wt.

Triglycerides were determined by the method in [10]. Free fatty acids were determined by the method in [11]. Cholesterol was extracted by petroleum ether after saponification of lipoprotein samples and was determined by the method in [12]. Protein was determined by the method in [13] using ethyl-ether to remove turbidity due to lipid [14].

Statistical significance of results was determined by Student's t-test.

3. Results

Incubation with intestinal chylomicrons in a medium containing post-heparin plasma caused significant inhibition of [1-^{14}C]acetate incorporation into fatty acids ($p < 0.001$) whereas incubation in a medium containing pre-heparin plasma decreased ^{14}C incorporation to a much smaller extent (n.s.) (table 1). The inhibition of fatty acid synthesis measured by ^{3}H incorporation from tritiated water into fatty acids in the medium containing chylomicrons and post-heparin plasma were dependent on the amounts of post-heparin plasma (fig.1). Remnants prepared in vitro by incubation of chylomicrons in rat post-heparin plasma also inhibited ^{14}C incorpora-

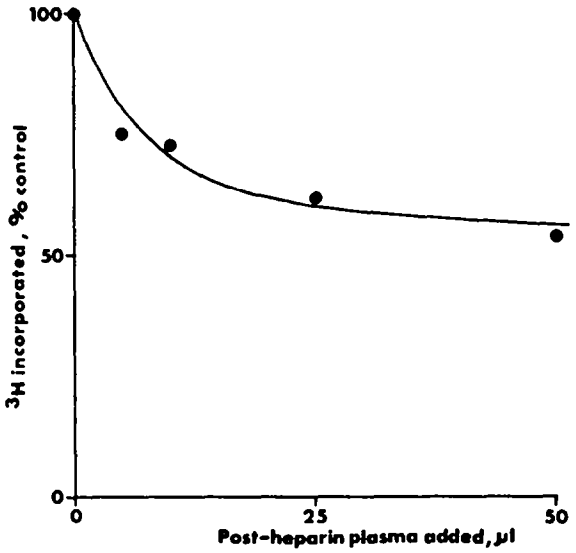

Fig.1. Effect of the amounts of post-heparin rat plasma in the incubation medium on fatty acid synthesis in hepatocytes. Chylomicrons (triglyceride 8248 µg, cholesterol 74 µg) were incubated with isolated hepatocytes (12.5 mg dry wt) in the medium containing various amounts of post-heparin plasma for 1 h at 37°C. Each point represents the average of duplicate determinations.

Table 1
Effects of chylomicrons incubated with pre-heparin or post-heparin rat plasma on fatty acid synthesis in hepatocytes

Chylomicron	Plasma added	^{14}C incorporation into fatty acids (×10^{5}dpm .g cells^{-1}.h^{-1})	
		Expt. 1	Expt. 2
–	pre-heparin	19.2 ± 0.9	–
–	post-heparin	18.2 ± 0.4	–
+	pre-heparin	18.1 ± 0.1	16.2 ± 1.3
+	post-heparin	4.4 ± 0.1[a]	5.0 ± 0.1[a]

[a] $p < 0.001$

Chylomicrons (triglyceride: 3760 µg in expt.1; 3688 µg in expt.2. cholesterol: 51 µg in expt.1; 74 µg in expt.2) were incubated with isolated hepatocytes (20 mg dry wt in expt.1 and expt.2) in the medium containing 50 µl pre-heparin or post-heparin plasma for 1 h at 37°C. Each value is mean ± SEM of triplicate determinations

Table 2
Effects of chylomicrons and their remnants produced in vitro on fatty acid synthesis in hepatocytes

	Triglyceride/ protein	^{14}C incorporation into fatty acids ($\times 10^5$ dpm .g cells^{-1} .h^{-1})
Chylomicron	49.2	6.9 ± 0.2
Remnant	20.3	3.4 ± 0.5[a]

[a] $p < 0.01$

Chylomicrons (protein 50 μg) or their remnants (protein 50 μg) were incubated with isolated hepatocytes (17.5 mg dry wt) for 1 h at 37°C. Remnants were prepared in vitro by incubating chylomicrons in rat post-heparin plasma for 1 h at 37°C and floated at 4.5 × 10^6 × *g* .min at 4°C under 0.15 M NaCl. Each value is mean ± SEM of triplicate determinations

tion from [1-^{14}C]acetate into fatty acids significantly ($p < 0.01$) (table 2). These results indicate that chylomicron remnants produced by the action of post-heparin plasma inhibited fatty acid synthesis in isolated rat hepatocytes.

Chylomicron remnants catabolized at the vascular surface of the peripheral tissues were prepared from the chylomicrons injected into functionally hepatectomized rats. The remnants obtained at 5 and 30 min after the injection of chylomicrons inhibited fatty acid synthesis at an equivalent protein concentration. The inhibition by the remnants obtained at 30 min was significant ($p < 0.05$) in comparison with unmetabolized chylomicrons. At an equivalent triglyceride concentration, cholesterol-rich particles

Table 3
Effects of unmetabolized chylomicrons and their remnants on fatty acid synthesis in hepatocytes

Expt. 1

Lipoprotein samples	Unmetabolized chylomicron	5 min remnant	30 min remnant
Protein (μg)	20	20	20
Triglyceride (μg)	1072	790	388
Cholesterol (μg)	12.7	14.1	20.2
Free fatty acid (μmol)	0.22	0.22	0.24
^{14}C incorporation into fatty acids ($\times 10^5$ dpm .g cells^{-1} .h^{-1})	10.7 ± 0.6	9.9 ± 0.1	8.9 ± 0.2[a]

Expt. 2

Lipoprotein samples	Unmetabolized chylomicron	1 min remnant	10 min remnant	30 min remnant
Triglyceride (μg)	618	618	618	618
Cholesterol (μg)	7.0	9.3	14.3	24.7
Free fatty acid (μmol)	0.09	0.23	0.20	0.23
^{3}H incorporation into fatty acids ($\times 10^4$ dpm .g cells^{-1} .h^{-1})	9.6 ± 0.6	9.3 ± 0.03	7.1 ± 0.2[b]	6.2 ± 0.3[c]

[a] $p < 0.05$,
[b] $p < 0.02$,
[c] $p < 0.01$, comparison with unmetabolized chylomicrons

Unmetabolized chylomicrons and their remnants prepared in vivo with 20 μg protein (expt.1) or with 618 μg triglyceride (expt.2) were incubated with isolated hepatocytes (11.1 mg in expt.1 and 8.5 mg in expt.2). Unmetabolized chylomicrons were prepared by the mixture of 1.5 ml of intestinal chylomicrons (7118 mg triglyceride/dl) and 4 ml functionally hepatectomized rat plasma floated at d = 1.006. Each value is mean ± SEM of triplicate determinations

Table 4
Effects of the amounts of chylomicron remnants in fatty acid synthesis in hepatocytes

Expt. 1		Expt. 2	
Triglyceride (μg)	^{14}C incorporation into fatty acids ($\times 10^5$ dpm .g cells^{-1}.h^{-1})	triglyceride (μg)	^{3}H incorporation into fatty acids ($\times 10^4$ dpm .g cells^{-1}.h^{-1})
0	12.6	0	17.6 ± 0.3
53	11.3	1229	12.1 ± 0.3
106	10.4	2457	10.5 ± 0.3

The isolated hepatocytes were incubated in the presence of increasing amounts of the remnants (triglyceride/cholesterol: 6.2 in expt.1; 69.2 in expt.2) for 1 h at 37°C. The remnants were obtained at 30 min after the injection of chylomicrons into functionally hepatectomized rats. Each value is mean of duplicate determinations in expt.1 and mean ± SEM of triplicate determinations in expt.2

caused more inhibition of fatty acid synthesis than triglyceride-rich particles (table 3). When the isolated hepatocytes were incubated in the presence of increasing concentration of the remnants obtained at 30 min, fatty acid synthesis was progressively decreased (table 4).

4. Discussion

Dietary fatty acids are carried in the circulation as triglyceride constituents of chylomicrons. The chylomicrons may function as physiological mediators effecting the feedback inhibition of hepatic fatty acid synthesis. Inhibition of fatty acid synthesis in isolated hepatocytes by chylomicrons which were prepared from the plasma of rats given corn oil was demonstrated [15]. Here, rat intestinal chylomicrons have caused a much smaller inhibition on fatty acid synthesis compared with their remnants. Fatty acid synthesis was estimated from the incorporation of [1-^{14}C]-acetate or tritiated water in the present study. The incorporation of tritiated water is not likely to be affected by fluctuations in the specific radioactivity of different precursor pools but proportional to the number of incorporated acetyl units [16]. Chylomicron remnants produced in vitro or in vivo caused significant inhibition of ^{14}C and ^{3}H incorporation into fatty acids. The added remnant samples produced in vivo contained no significant different amounts of free fatty acids (table 2, 3). These data indicate that the inhibition of fatty acid synthesis in isolated hepatocytes by chylomicron remnants is not due to a contamination by free fatty acids. The cholesterol-rich particles produced by the action of lipoprotein lipase upon longer incubation caused a greater inhibition of fatty acid synthesis than the triglyceride-rich particles at the same concentration of triglycerides. In addition the increasing of the amounts of remnants decreased fatty acid synthesis. Thus, the degree of catabolism and the amounts of chylomicron remnants may be important for the regulation of hepatic fatty acid synthesis.

It is apparent that the hepatocytes recognize the remnant rather than the unmetabolized chylomicron. Some changes of lipoprotein composition due to the action of lipoprotein lipase may be important for the recognition of the remnants. Thereafter, the remnant taken up by the hepatocytes regulates fatty acid synthesis in the cells.

References

[1] Felts, J. M., Itakura, H. and Crane, R. T. (1975) Biochem. Biophys. Res. Commun. 66, 1467–1475.
[2] Sherrill, B. C. and Dietschy, J. M. (1978) J. Biol. Chem. 253, 1859–1867.
[3] Carrella, M. and Cooper, A. D. (1979) Proc. Natl. Acad. Sci. USA 76, 338–342.
[4] Berry, M. N. and Friend, D. S. (1969) J. Cell Biol. 43, 506–520.
[5] Seglen, P. O. (1973) Exp. Cell Res. 82, 391–398.
[6] Brown, M. S., Dana, S. E. and Goldstein, J. L. (1974) J. Biol. Chem. 249, 789–796.

[7] Endo, A., Tsujita, Y., Kuroda, M. and Tanzawa, K. (1977) Eur. J. Biochem. 77, 31–36.
[8] Bollman, J. L., Cain, J. C. and Grindlay, J. H. (1948) J. Lab. Clin. Med. 33, 1349–1352.
[9] Redgrave, T. G. (1970) J. Clin. Invest. 49, 465–471.
[10] Fletcher, M. J. (1968) Clin. Chim. Acta. 22, 393–397.
[11] Itaya, K. and Ui, M. (1965) J. Lipid Res. 6, 16–20.
[12] Zak, B. (1956) Am. J. Clin. Pathol. 27, 583–588.
[13] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[14] Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) J. Biol. Chem. 177, 751–766.
[15] Lakshmanan, M. R., Muesing, R. A., Cook, G. A. and Veech, R. L. (1977) J. Biol. Chem. 252, 6581–6584.
[16] Jungas, R. L. (1968) Biochemistry 7, 3708–3717.