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Identification of factors regulating lipoprotein lipase catalyzed hydrolysis in rats with the aid of monoacid-rich lipoprotein preparations

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Abstract

To identify the substrate specificity and regulatory factors in lipoprotein lipase (LPL) catalyzed hydrolysis of triacylglycerol-rich lipoprotein, monoacid-rich lipoproteins were used to study the kinetic parameters of LPL. Feeding growing rats with diets rich in palmitic acid (16:0), oleic acid (18:1) or linoleic acid (18:2) for 10 days increased the corresponding acid content in the triacylglycerols of the lipoproteins. Force-feeding the monoacid-rich triacylglycerols, particularly 16:0 or 18:1, increased the respective fatty acid content in both chylomicrons and VLDLs. Major apolipoproteins and lipid compositions were essentially similar among all lipoproteins differing in monoacid species, except for apo A-IV. The Vmax of LPL for 16:0-rich chylomicrons and VLDLs were higher than for 18:1- or 18:2-rich lipoproteins. Order parameter (S), an indicator of the surface fluidity of lipoproteins, decreased with the chain length and unsaturation of monoacid in similar manner as the Vmax. The Vmax of LPL increased linearly ($P < 0.05$) with an increase in either the palmitic acid content of the lipoprotein triacylglycerols or order parameter (S) of the lipoproteins. The order parameter (S) and Vmax of LPL were higher in 16:0 triacylglycerol emulsions with apo B than with 18:1 or 18:2 triacylglycerols. The apo A-IV in triacylglycerol emulsions stimulated Vmax of LPLs in the presence of apo B and apo C-II. The binding of apo A-IV to 16:0 triacylglycerol emulsions was higher than to other triacylglycerol emulsions. These findings suggest that lipoprotein catalysis by LPL is modulated by the 16:0 level in the lipoprotein triacylglycerol, which affects the surface fluidity and apo A-IV content of lipoproteins.

Keywords: Lipoprotein lipase; VLDL; Chylomicron; Surface fluidity; Apo A-IV; Fatty acid; Rat

1. Introduction

The obligatory step in the transport of triacylglycerol fatty acids from circulating chylomicrons and VLDL into tissues, is the hydrolysis of triacylglycerol cores in the lipoprotein particles by lipoprotein lipase (LPL, EC 3.1.1.3) present in adipose tissues, heart, diaphragm and skeletal muscles [1]. LPL is located both at capillary endothelial surfaces and in parenchymal cells of tissues [2]. The efficiency of LPL-catalyzed triacylglycerol hydrolysis of artificial substrates depends upon apolipoprotein composition, micellar size, phospholipid species, fatty acid composition of triacylglycerols and surface pressure [3].

Chylomicrons and VLDLs, as natural substrates for LPL, are considered to have micellar structures with an inner core consisting of neutral lipids, triacylglycerol and cholesteryl ester, and an outer monolayer of phospholipid, unesterified cholesterol, and apolipoproteins [4]. Botham *et al.* [5] reported that the triacylglycerol and total cholesterol concentrations in chylomicrons affected the apparent Km of LPL. Connelly *et al.* [6] demonstrated that the triacylglycerol/apo B ratio of VLDL particles was a factor determining the apparent Km of LPL. Jackson *et al.* [7] reported that the Apo C-II in lipoproteins decreased the apparent Km with a minor affect on the Vmax in LPL catalyzed hydrolysis.

We previously reported that the rate of rat LPL catalyzed hydrolysis of the monoacid triacylglycerol emulsion increased with chain length e.g., 16:0 > 14:0 > 12:0 and decreased with increasing acyl chain unsaturation e.g., 18:

Abbreviations used: LPL, lipoprotein lipase; VLDL, very low density lipoprotein; ESR, electron spin resonance.

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1 > 18:2 > 18:3 [8]. These findings suggest that the fatty acid composition in triacylglycerol-rich lipoprotein affects LPL catalyzed hydrolysis. There is, however, less information available on the relative catalytic activity of LPL for triacylglycerol-rich lipoproteins, chylomicrons and VLDL, with varying fatty acyl-chain lengths and degrees of saturation in the triacylglycerol fraction. Hence it would seem essential to provide monoacid-rich lipoproteins for the investigation of lipoprotein metabolism.

Bouziane *et al.* [9] observed that feeding of a salmon oil diet raised the n-3 fatty acid content in VLDL triacylglycerol fractions in growing rats. Suarez *et al.* [10] reported that oleic acid comprised about 30% of total fatty acids in VLDL in weanling rats fed a diet rich in oleic fat. These findings suggested that the dietary fat sources potentially modulated the fatty acid composition in both chylomicrons and VLDL triacylglycerols. Nir *et al.* [11] reported that fast re-feeding of diet led to a rise in plasma triacylglycerols in geese. Furthermore, we showed that triacylglycerols in chylomicrons and VLDL of chickens force-fed tripalmitin, triolein or trilinolein after starvation contained the corresponding unsaturated acid as more than 70% of the total acids [12]. Thus we have predicted that it should be possible to prepare chylomicrons and VLDLs rich in monoacids, by re-feeding monoacid triacylglycerols after starvation in mammals. Structural analysis of the monoacid–rich lipoproteins may lead to a more comprehensive understanding of regulatory factors for chylomicron and VLDL hydrolysis by LPL.

In the present work with growing rats, we report the preparation of triacylglycerol-rich lipoproteins rich in a variety of mono fatty acids, and the kinetic parameters of LPL for these lipoproteins. In addition, in order to identify a regulatory factor in LPL-catalyzed lipoprotein hydrolysis, we examined the importance of apolipoprotein profile, lipid composition and surface fluidity of monoacid-rich lipoproteins in lipoprotein hydrolysis.

2. Materials and methods

2.1. Animals

Male rats of the Wistar strain with body weights ranging from 150 to 160 g were provided. At the beginning of experiments, rats were allowed free access to a commercial standard diet for 7 days. Before feeding the experimental diets, five rats (Time 0 group) were decapitated and the blood collected with sodium citrate as anticoagulant. Plasma was prepared by centrifugation for 15 min at 1,500×g. Rats for experiments were randomly divided into three groups of 15 rats each, housed in sets of 5 rats per cage, in a room with controlled temperature (21 ± 3°C) and light intensity (0800–2000 h).

All groups received semipurified diets containing 10 g fat/100 g differing only in the source, *viz* palm oil (tripalmitin-rich), olive oil (triolein-rich) and safflower oil (trilinolein-rich), as shown in Table 1. Water and diets were consumed *ad libitum.* After 10 days, five rats in each group were subjected to blood collection and plasma preparation (Fed groups). Other rats were food-deprived for 24 h, then force-fed (5 g/kg body weight) three times during the subsequent 12 h, with emulsions containing respectively the monoacid triacylglycerols, tripalmitin, triolein or trilinolein. After 12 or 24 h of force-feeding, five rats from each treatment regime were also bled for plasma preparation (Re-fed 12 or 24 h groups).

Table 1
Composition of experimental diets

Ingredient (%)	Diet		
	16:0-rich	18:1-rich	18:2-rich
Palm oil	10.0	—	—
Olive oil	—	10.0	—
Safflower oil	—	—	10.0
Casein		18.9	
Starch		48.1	
Saccharose		15.0	
Cellulose		5.0	
DL-methionine		0.3	
Choline chloride		0.1	
Mineral mix[1]		2.4	
Vitamin mix[2]		0.1	
DL-α- tocoperol[3]		0.1	
Fatty acid profile (%)[4]			
12:0	0.2	0.1	0.1
14:0	0.3	0.2	0.3
16:0	63.4	12.1	6.2
18:0	3.2	1.3	1.3
18:1	24.8	71.4	13.7
18:2(n-6)	5.2	7.1	75.8
18:3(n-3)	0.2	0.8	0.6
18:3(n-6)	0.1	0.2	1.2

[1]American Institute of Nutrition (43).
[2]Composition of vitamin mix was as follows (g/100g): thiamin hydrochloride 0.51, riboflavin 0.51, pyridoxine HCl 0.59, para-aminobenzoic acid 2.53, calcium pantothenate 1.35, folic acid 0.17, vitamin B-12 0.008, retinal acetate 0.12, cholecalciferol 0.002, phyloquinone 0.004.
[3]The dl-α -tocopherol was added to avoid fatty acid oxidation.
[4]The fatty acid profiles were expressed as the mean proportion of total fatty acids in diets.

2.2. Preparation of chylomicrons and VLDL

Plasma lipoproteins, chylomicrons (d < 0.96) and VLDL (d = 0.96–1.006), were prepared by the method of Lindgren [13] using ultracentrifugation (Kontron Instrument K.K., Zurich, Switzerland) in a TFT65.13 rotor.

2.3. Emulsion preparation

Artificial substrates with various monoacid triacylglycerol emulsions were prepared as described by Tajima et al. [14] with slight modifications. Triacylglycerol (70 mg),

cholesterol oleate (1 mg) and phosphatidylcholine (10 mg) were evaporated to dryness with a stream of nitrogen gas, suspended in 2 ml 10 mM phosphate buffer containing 0.15 M NaCl, pH 7.4 and sonicated at 50 watts (5 × 1 min with 30 s intervals for cooling). Particles were isolated by ultacentrifugation at 17,000 rpm for 20 min at 4°C. For preparation of triacylglycerol-rich particles with apolipoproteins, apo B100, apo B48, apo A-I, apo A-II, apo A-IV, apo E, apo C-II or apo C-III were purified from rat plasma by chromatography on Sephadex G200 (1.5 × 60 cm) and Mono Q^R (Pharmacia Fine Chemicals, Uppsala, Sweden) columns equilibrated with 6 M urea, pH 8.0 at 4°C as described by Krul et al. [15]. Each purified apolipoprotein (0.1 mg protein) was mixed with triacylglycerol emulsion (1.0mg triacylglycerol) and incubated at 37°C for 30 min. The resulting triacylglycerol particles with apolipoproteins were isolated by centrifugation at 17,000 rpm for 20 min at 4°C. The apolipoprotein composition of the particles were verified by SDS-PAGE (12.5% gel).

2.4. Preparation of LPL

Adipose LPL from rats was purified by a procedure described previously [16]. The specific activity of purified LPL was 56.76 U/mg protein. The LPL activity was determined by quantifying released free fatty acids (FFA) following *in vitro* incubation at 37°C with substrates and LPL as described previously [17]. One unit of enzyme activity was defined as 1 mmol of FFA released per hour.

2.5. Fatty acid analysis of triacylglycerol fraction in lipoproteins

Lipoprotein lipids were extracted according to the method of Folch *et al.* [18]. Triacylglycerol fractions were isolated by chromatography on silicic acid (100 mesh, Mallinckrodt, USA) according to the method of Fleischer *et al.* [19]. Fatty acids present in the triacylglycerols were analyzed after acylation with phenacyl bromide at 50 °C for 2 h. HPLC analysis was performed [20], using a Shimadzu 6A system, Zorbax ODS (4.6 × 250 mm) (Shimadzu Co., Kyoto, Japan). The flow rate was set at 1.0 ml/min, with a gradient in acetonitrile concentration from 85 to 90%. Absorbance was measured at 254 nm. Fatty acids were identified by means of relative retention time. Areas were calculated with Shimadzu C-R6A integrator. All lipid samples were stored under N_2 gas in the dark at −20°C to prevent peroxidation of the unsaturated fatty acids.

2.6. Determination of different classes of lipids and phospholipids in lipoproteins with monoacid-rich triacylglycerol

The different classes of lipids in lipoproteins were quantified according to Sipos and Ackman [21] using TLC-FID equipment (IATROSCAN TH-10, Iatron Laboratories, Tokyo, Japan) connected to an electronic integrator. Known standards of tripalmitin, dipalmitin, cholesterol, cholesterol palmitate, palmitic acid and phospatidyl choline were used as standards. Phospholipid species were determined by the method of Nakamura and Hanada [22] using thin-layer chromatography (TLC). The phospholipids were applied to Silica gel 60 (Merck, Darmstadt, Germany) and developed with chloroform/methanol/NH_4OH (60:35:8, by volume). The TLC plate was then air-dried and immersed in a staining solution consisting of 0.03% Brilliant blue R (Sigma, St. Louis, USA) in 20% methanol. After 2 h, the plate was removed from the staining solution, immersed in 20% methanol and scanned with a densitometer (CS-9300PC, Shimadzu Co., Kyoto, Japan) under the reflectance mode at 580 nm. Authentic standards of phosphatidylcholine, phosphatidylethanolamine, phospatidylserine, phosphatidylinositol and sphingomyelin were included.

2.7. Electrophoretic determination of chylomicrons and VLDL apolipoproteins

After partial delipidation, chylomicrons and VLDL apolipoproteins were estimated using SDS-PAGE (12.5 and 17.5%) by the method of Bouziane *et al.* [9]. Electrophoresis was performed in a Mini-PROTEAN II electrophoresis cell (Bio-Rad Laboratories, CA, USA) at 4°C, for 3 h with 25 mA/gel slab. Apo C-II or apo C-III was determined using the PhastGel IEF 3–9 on PhastSystem (Pharmacia Fine Chemicals, Uppsala, Sweden). Gels were then stained with Coomassie brilliant blue G250. Destained gels were scanned at 595 nm with a densitometer (CS-9300PC, Shimadzu Co., Kyoto, Japan). Apoliprotein concentrations were determined semiquantitatively from the densitometer tracing. To estimate each apolipoprotein, the percentage of area relative to each apolipoprotein was multiplied by the total apolipoprotein content of each serum sample. Results were expressed as arbitrary units (AU).

2.8. Electron spin resonance (ESR) analysis of lipoproteins with mono fatty acid-rich triacylglycerol

The measurements of order parameter (S) as the index of fluidity were performed using the 5-doxyl-stearic acid (5-DSA; Sigma Chemical Co., St. Louis, USA) [12,23,24]. To 1 ml of each lipoprotein or artificial emulsion (10 mg triacylglycerols/ml) was added 5 μl (1 μg/μl) of 5-DSA solution in ethanol. The mixtures were incubated at 37°C for 15 min and analyzed by ESR. ESR spectra were recorded on a JES-RE1X (JEOL, Tokyo, Japan) operated at a microwave frequency of 9.4 GHz at 20 °C as described previously [12].

2.9. Enzyme kinetics

Kinetic constants (Apparent Km and Vmax) of LPL for the hydrolysis of natural or artificial substrates were deter-

mined using double reciprocal plots [25]. Incubations were performed at 37°C with increasing amounts of substrate (0.5, 0.75, 1.5, 2.5 and 4 mmol triacylglycerols/L) in a total volume of 0.6 ml 10 mM sodium barbital-HCl buffer, pH 8.6, containing 20 mg defatted bovine serum albumin (fraction V, Sigma, St. Louis, USA) [15]. All assays were performed in triplicate.

2.10. Apolipoprotein binding assay

The various monoacid triacylglycerol emulsions (1 mg triacylglycerols in 10 mM phosphate buffer containing 0.15 M NaCl, pH 7.4), were incubated with ^{125}I-labeled apolipoproteins (50 μg), apo B100, apo A-IV, apo E, apo C-II or apo C-III, for 20min at 37°C. Each emulsion was isolated by centrifugation at 17,000 rpm for 20 min at 4°C. Apolipoproteins were labeled with ^{125}I by the method of Stifani *et al.* [26] and ^{125}I radioactivity counted in a Cobra II auto gamma counter (Packerd Instrument Company, Meriden, CT, USA).

2.11. Other assays

The protein content of enzymes was determined by the method of Lowry *et al.* [27] using bovine serum albumin as standard. Triacylglycerol concentrations of plasma lipoprotein were quantified by the method of Fletcher [28].

2.12. Statistical analysis

A computer generated SAS applications package was used for statistical calculations (Statistical Analysis System Version 6.03, SAS Institute Inc., Cary, NC). Group data for multiple comparisons were analyzed by ANOVA using a general linear models procedure followed by Duncan's multiple range test to test for differences. The level of significance used in all studies was $p < 0.05$.

3. Results

3.1. Fatty acid composition of triacylglycerols in chylomicrons and VLDL in rats fed or re-fed with monoacid triacylglycerol

At the beginning of feeding experimental diets (Time 0 group), 18:1- and 18:2-acids accounted for more than 70% of total fatty acids in both chylomicrons and VLDL. Feeding palmitic acid (16:0) -rich, oleic acid (18:1) -rich or linoleic acid (18:2) -rich diets resulted in a higher proportion of the corresponding fatty acid in both chylomicrons and VLDL, irrespective of the feeding schedule (Tables 2 and 3). The fatty acid composition of chylomicrons and VLDL of fed rats was generally comparable to that of the diets, although the fatty acid composition of VLDL mirrored the dietary composition less than that of the chylomicrons.

Table 2
Effects of diets rich in mono fatty acid on the fatty acid composition of chylomicron triacylglycerol prepared from Time 0, Fed, Refed 12 h or 24 h groups

Fatty acid	Treatment	Pre-feeding	Feeding		
			16:0-rich	18:1-rich	18:2-rich
14:0	Time 0	1.3			
	Fed		0.1	0.2	0.3
	Refed 12 h		0.2	0.2	0.3
	Refed 24 h		0.9	0.3	0.3
16:0	Time 0	12.2			
	Fed		57.1^{a}	14.7^{b}	7.2^{c}
	Refed 12 h		74.3^{a}	8.2^{b}	4.3^{c}
	Refed 24 h		83.6^{a}	5.4^{b}	4.8^{b}
18:0	Time 0	4.6			
	Fed		3.2	1.3	1.3
	Refed 12 h		3.0	2.4	1.2
	Refed 24 h		2.9^{b}	2.3^{a}	1.6^{b}
18:1	Time 0	36.2			
	Fed		30.9^{b}	69.3^{a}	18.9^{c}
	Refed 12 h		12.8^{b}	73.9^{a}	14.6^{b}
	Refed 24 h		6.2^{c}	86.1^{a}	16.2^{b}
18:2(n-6)	Time 0	42.5			
	Fed		6.8^{b}	7.1^{b}	64.3^{a}
	Refed 12 h		4.8^{b}	6.5^{b}	71.4^{a}
	Refed 24 h		3.5^{b}	4.3^{b}	74.1^{a}
18:3(n-3)	Time 0	2.2			
	Fed		0.3	0.4	0.5
	Refed 12 h		0.2	0.4	0.4
	Refed 24 h		1.1	0.3	0.2
18:3(n-6)	Time 0	1.0			
	Fed		0.1	0.1	1.6
	Refed 12 h		0.3	0.6	1.4
	Refed 24 h		1.2	1.2	1.6

Results are expressed as mean proportions (%) of total fatty acids in each group with five animals. Values with different superscripts within a row are significantly different ($p < 0.05$).

Re-feeding monoacid triacylglycerols, particularly 16:0 and 18:1, for 12 and 24 h, increased the respective fatty acid content in both chylomicrons and VLDL. The 16:0 and 18:1 contents in both chylomicrons and VLDL in rats re-fed for 12 and 24 h following starvation exceeded those in diets (Refed 12 h and Refed 24 h groups). Irrespective of the feeding schedule or nature of the dietary fatty acids, the VLDL triacylglycerols were characterized by higher 16:0 content than chylomicron triacylglycerols.

3.2. Kinetic parameters of rat LPL for monoacid-rich lipoproteins

Apparent Km and Vmax of LPL were determined with the Time 0 control group and the monoacid-rich lipoproteins from rats re-fed with monoacid triacylglycerols at 24 h following starvation (Table 4). Vmax values of rat-LPL for 16:0-rich chylomicrons and VLDL were higher than those for 18:1- and 18:2-rich lipoproteins. The Vmax of LPL for

Table 3
Effects of diets rich in mono fatty acid on the fatty acid composition of VLDL triacylglycerol prepared from Time 0, Fed, Refed 12 h or 24 h groups

Fatty acid	Treatment	Pre-feeding	Feeding		
			16:0-rich	18:1-rich	18:2-rich
14:0	Time 0	0.4			
	Fed		0.1	0.2	0.3
	Refed 12 h		0.4	0.2	0.3
	Refed 24 h		0.4	0.0	0.5
16:0	Time 0	19.4			
	Fed		41.3^{a}	24.7^{b}	17.2^{c}
	Refed 12 h		52.8^{a}	18.2^{b}	14.3^{b}
	Refed 24 h		78.2^{a}	14.7^{b}	14.2^{b}
18:0	Time 0	2.7			
	Fed		3.2	1.3	1.5
	Refed 12 h		3.0	2.4	1.3
	Refed 24 h		2.7	2.8	1.5
18:1	Time 0	38.9			
	Fed		43.5^{ab}	59.3^{a}	38.9^{b}
	Refed 12 h		30.8^{b}	63.2^{a}	34.6^{b}
	Refed 24 h		11.5^{c}	74.0^{a}	10.6^{b}
18:2(n-6)	Time 0	34.1			
	Fed		7.2^{b}	8.1^{b}	34.3^{a}
	Refed 12 h		6.8^{b}	6.5^{b}	41.2^{a}
	Refed 24 h		4.1^{b}	4.4^{b}	70.4^{a}
18:3(n-3)	Time 0	3.2			
	Fed		0.3	0.4	0.5
	Refed 12 h		0.2	0.4	0.4
	Refed 24 h		1.2	1.6	1.2
18:3(n-6)	Time 0	1.3			
	Fed		0.1^{b}	0.1^{b}	1.3^{a}
	Refed 12 h		0.3	0.6	1.4
	Refed 24 h		2.2	1.8	1.2

Results are expressed as mean proportions (%) of total fatty acids in each group with five animals. Values with different superscripts within a row are significantly different ($p < 0.05$).

18:1-rich chylomicrons was higher than for 18:2-rich chylomicrons, whereas those for 18:1- and 18:2-rich VLDLs were almostly identical. The LPL catalyzed hydrolysis of VLDL demonstrated a high Vmax with a low apparent Km when compared to that of chylomicrons, but the differences were not significant with 16:0-rich lipoproteins as substrates. The kinetic parameters in the Time 0 control group were similar to those of the 18:1- or 18:2-rich lipoproteins.

3.3. Different classes of lipids and phospholipids in chylomicrons and VLDL with monoacid-rich triacylglycerols

In all groups with Time 0 and Re-fed 24 h, triacylglycerols comprised approximately 85 and 65% of the chylomicrons and VLDL, respectively, followed by phospholipids with less than 20% (Table 5). However, the lipid compositions of plasma chylomicrons and VLDLs were not significantly affected by the monoacid present in the triacylglycerol. In the phospholipid species, every chylomicron and VLDL with monoacid-rich triacylglycerol resulted in a high proportion (more than 71%) of phosphatidylcholine but there were no significant differences among treatments.

Table 4
Kinetic parameters of rat-LPL for mono fatty acid-rich chylomicron and VLDL

Lipoprotein		Apparent Km (mM)	Vmax (U/mg protein)
Fraction	Triacylglycerol		
Chylomicron	Time 0	2.12 ± 0.09^{a}	90.3 ± 3.2^{bc}
	16:0-rich	1.89 ± 0.05^{b}	186.5 ± 11.3^{a}
	18:1-rich	2.18 ± 0.08^{a}	93.3 ± 2.8^{b}
	18:2-rich	2.11 ± 0.11^{a}	88.2 ± 1.3^{c}
VLDL	Time 0	1.71 ± 0.07	167.3 ± 9.3^{b}
	16:0-rich	1.63 ± 0.08	200.4 ± 9.8^{a}
	18:1-rich	1.73 ± 0.06	166.3 ± 8.2^{b}
	18:2-rich	1.72 ± 0.05	166.3 ± 7.2^{b}

Apparent Km and Vmax of LPL were determined on double reciprocal plots with plasma lipoproteins as the substrate at 37°C, pH 8.6. Lipoproteins were prepared from plasma of rats refed the 16:0-, 18:1- and 18:2-rich triacylglycerols for 24 h or rats in Time 0 group, respectively, as referred to Table 2 and 3. Results are expressed as mean ± SD ($n = 5$). Values with different superscripts within a column are significantly different ($p < 0.05$).

3.4. Apolipoproteins

Apolipoproteins, apo B100, apo B48, apo A-I, apo A-II, apo A-IV, apo E, apo C-II and apo C-III, were quantified in lipoproteins with monoacid-rich triacylglycerol groups, obtained in Time 0 or Re-fed 24 h samples (Fig. 1). Each apolipoprotein in the VLDLs was approximately twice as high as that in chylomicrons irrespective of differences in the monoacid species. No significant changes produced by monoacid-rich triacylglycerols were observed in apo B100, apo B48, apo A-I, apo A-II, apo E apo C-II and apo C-III of both chylomicrons and VLDLs. However, for apo A-IV in 16:0-rich lipoproteins, both chylomicrons and VLDLs, were highest among all lipoproteins.

3.5. ESR analysis

The incorporation of 5-DSA into the phospholipid monolayer of lipoproteins was observed by the ESR spectra, and anisotropic movement was found. Parameter (S) of each VLDL was significantly higher than that of corresponding chylomicrons except for 16:0-rich lipoproteins, and increased in the order 16:0 > 18:1 > 18:2 in monoacid-rich lipoproteins (Table 6).

3.6. Identification of the regulatory factor of LPL catalyzed hydrolysis

In order to identify the relationship between the fatty acid content in lipoproteins and the LPL catalyzed hydrolysis, correlations between the 16:0, 18:1 or 18:2 content in

Table 5
Lipid composition and phospholipid species in mono fatty acid-rich cylomicron and VLDL

Lipoprotein		Lipid composition (%)				Phospholipid species (%)			
Fraction	Triacylglycerol	PL	TG	FC	CE	PC	SM	PS	PE
Chylomicron	Time 0	13.1	85.3	0.7	0.9	73.6	21.6	3.2	1.6
	16:0-rich	14.0	84.0	0.8	1.2	72.7	22.9	3.1	1.3
	18:1-rich	12.7	85.4	0.9	1.0	73.5	20.4	3.5	2.6
	18:2-rich	11.8	86.9	0.8	1.5	75.7	20.6	3.1	0.6
VLDL	Time 0	17.5	67.5	1.9	13.1	73.9	20.5	4.1	1.5
	16:0-rich	18.2	65.6	2.5	13.7	72.3	22.0	4.0	1.7
	18:1-rich	19.8	66.2	1.4	12.6	71.5	21.5	4.4	2.6
	18:2-rich	17.5	69.4	1.8	11.3	78.2	18.0	3.3	0.5

The lipoproteins were preapred from plasma of rats refed for 24 h with 16:0-rich, 18:1-rich, 18:2-rich diets or rats in Time 0 group. Results are expressed as mean proportion (%) of total lipids and of phospholipids, respectively, with five animals for each group.

Abbreviations: PL, phospholipid; TG, triacylglycerol; FC, free cholesterol; CE, cholesteryl ester; PC, phoshatidylcholine; SM, sphingomyelin; PS, phosphatidylserine; PE, phoshatidylethanolamine.

chylomicrons and VLDL triacylglycerols, and the Vmax for chylomicrons and VLDLs were determined. The Vmax of rat LPL for chylomicrons and VLDLs increased linearly with an increase in 16:0 content (Fig. 2), whereas no significant correlation between the 18:1 or 18:2 content and Vmax was detected (data not shown). In addition, statistically significant ($p < 0.01$) correlation existed between the order parameter (S) of chylomicrons or VLDLs and the Vmax of LPL for triacylglycerol-rich lipoproteins (Fig. 3).

3.7. Effects of apolipoproteins on the fluidity of lipid emulsions with mono fatty acid triacylglycerol

To identify the involvement of apolipoproteins in lipoprotein fluidity, the order parameter (S) of various triacylglycerol emulsions, with or without apolipoproteins, was determined (Table 7). No significant changes in the order parameter (S) by fatty acid species of triacylglycerol emulsions with apo A-I, apo A-II, apo E, apo C-II or apo C-III were observed. In contrast, the order parameter (S) for 16:0 emulsions with apo B100, apo B48 or apo A-IV was highest whereas for emulsions with apo B100, apo B48 or apo A-IV, it decreased in the order 16:0 > 18:1 > 18:2. The order parameter (S) of emulsions with apo B100 was unchanged by addition of either apo C-II or apo A-IV.

The binding of apo B100, apo E, apo C-II or apo C-III to the triacylglycerol emulsions was not significantly influenced by the species of monoacid contained in the triacylglycerols, but the binding of apo A-IV to 16:0 triacylglycerol emulsions was higher than to other triacylglycerol emulsions (Fig. 4).

3.8. Kinetic parameters of rat-LPL for monoacid triacylglycerol emulsions containing various apolipoproteins

The results of *in vitro* hydrolysis of various monoacid triacylglycerol emulsions, with or without apolipoproteins, by

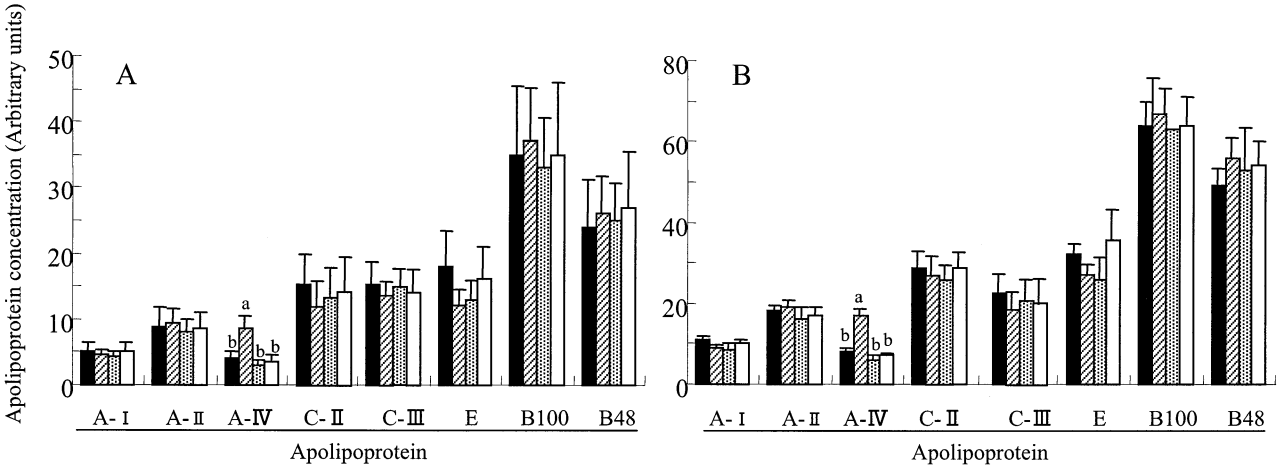

Fig. 1. Apolipoprotein species in mono fatty acid-rich chylomicron (A) and VLDL (B). Lipoproteins were prepared from plasma of rats refed for 24 h with C16-rich, C18:1-rich and C18:2-rich diets or rats in Time 0 group. Results are expressed as mean ± SD (n = 5) of arbitrary units. Different superscripts are significantly different ($p < 0.05$). Time 0 (■), 16:0-rich lipoprotein (▨), 18:1-rich lipoprotein (▩), 18:2-rich lipoprotein (□).

Table 6
ESR analysis of mono fatty acid-rich chylomicron and VLDL

Lipoprotein		Order parameter (S)
Fraction	Triacylglycerol	
Chylomicron	Time 0	0.426[b]
	16:0-rich	0.543[a]
	18:1-rich	0.417[bc]
	18:2-rich	0.398[c]
VLDL	Time 0	0.482[bc]
	16:0-rich	0.569[a]
	18:1-rich	0.497[b]
	18:2-rich	0.477[c]

The lipoproteins were prepared from plasma of rats refed for 24 h with 16:0-rich, 18:1-rich, 18:2-rich diets or rats in Time 0 group. Results are expressed as mean (n = 3) of order parameter (S). Values with different superscripts within a column are significantly different ($p < 0.05$).

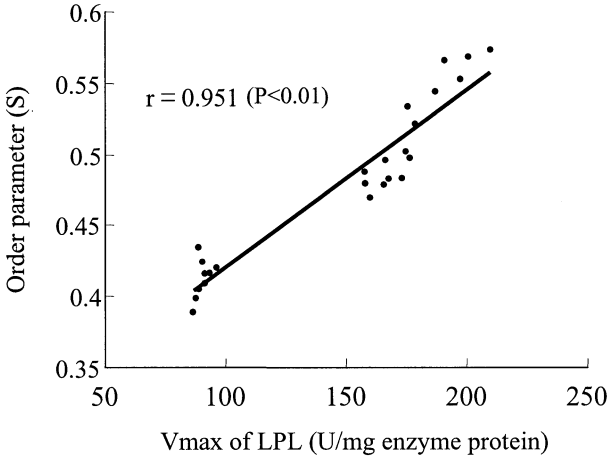

Fig. 3. The relationship between order parameter (S) of lipoproteins and Vmax of LPL.

rat LPL is shown in Table 8. When apolipoproteins were not included (lipid only), the Vmax of LPL for 16:0 triacylglycerols was slightly higher than for 18:1 or 18:2 triacylglycerols. Addition of apo C-II enhanced the hydrolysis of triacylglycerol emulsions, but the responses of the various monoacid triacylglycerols did not differ. In the presence of apo B100, the Vmax of LPL for 16:0 triacylglycerols containing apo C-II was higher than for 18:1or 18:2 triacylglycerols, and further addition of apo A-IV extensively stimulated the Vmax of LPL. Apparent Km values for all the triacylglycerol emulsions assayed were not significantly different.

4. Discussion

The present data show that lipoproteins rich in monoacid could be prepared by re-feeding monoacid triacylglycerols for 24h following starvation. These findings are in accord with an earlier observation by Bouziane *et al.* [9] that the fatty acid composition in diets reflected those in rat VLDL triacylglycerols. Suarez *et al.* [10] reported that the fatty acid compositions in diets influenced the composition in body tissues and plasma of weanling rats. The present study demonstrated, in addition, that the fatty acid composition of VLDLs was less influenced by dietary treatments than that of chylomicrons.

Re-feeding the monoacid triacylglycerols for 12 or 24 h after 24 h starvation, increased the corresponding fatty acid content in the lipoproteins as compared to feeding monoacid rich diets for 10 days. Nir *et al.* [11] demonstrated that fast-refeeding of diets was followed by a rise in plasma triacylglycerol concentrations in geese. The 16:0, 18:1 or 18:2 content in VLDL fractions at 24 h after re-feeding was roughly comparable with the corresponding fatty acid content of the diets and reflected the fatty acid profiles in the chylomicrons at 12 h after re-feeding. It is, therefore, suggested that efficient VLDL secretion occurs about 12 h after chylomicron secretion, and that receptor-mediated endocytosis of the lipoprotein occurs in the liver. The present study demonstrated that the monoacid-rich lipoproteins, having more than 70% of total acids incorporated even into VLDLs, are prepared, and used for kinetic studies of LPL specificity on lipoproteins differing in fatty acid composition.

Wang *et al.* [29] showed that the LPL-catalyzed hydrolysis of artificial substrates with monoacid triacylglycerols increased in the order, C8 > C10 > C4 > C12 > 18:1 > C6. McLean *et al.* [30] demonstrated that the fatty acyl-

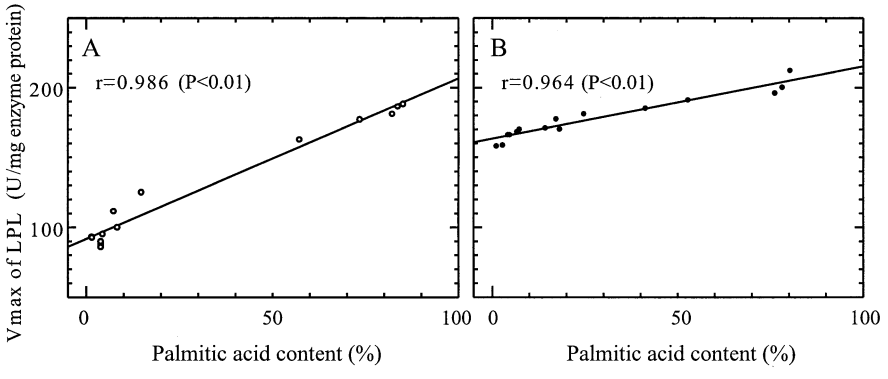

Fig. 2. The relationship between Vmax of LPL for chylomicron (A) or VLDL (B) and palmitic acid content in the lipoproteins.

Table 7
ESR analysis of mono fatty acid-rich triacylglycerol emulsions with or without apolipoproteins

Apolipoprotein	Order parameter (S)		
	Fatty acid species of triacylglycerols		
	16:0	18:1	18:2
No (Lipid only)	0.079 ± 0.009	0.084 ± 0.017	0.084 ± 0.008
Apo B100	0.408 ± 0.020[a]	0.370 ± 0.010[b]	0.302 ± 0.005[c]
Apo B48	0.385 ± 0.004[a]	0.310 ± 0.009[b]	0.244 ± 0.033[c]
Apo A-I	0.081 ± 0.010	0.082 ± 0.015	0.079 ± 0.011
Apo A-II	0.077 ± 0.020	0.079 ± 0.019	0.081 ± 0.016
Apo A-IV	0.146 ± 0.010[a]	0.123 ± 0.010[b]	0.101 ± 0.002[c]
Apo E	0.097 ± 0.030	0.081 ± 0.015	0.086 ± 0.016
Apo C-II	0.079 ± 0.009	0.080 ± 0.011	0.080 ± 0.011
Apo C-III	0.078 ± 0.014	0.082 ± 0.015	0.084 ± 0.008
Apo B100+ apo C-II	0.410 ± 0.014[a]	0.368 ± 0.011[b]	0.314 ± 0.008[c]
Apo B100+ apo A-IV	0.418 ± 0.023[a]	0.374 ± 0.008[b]	0.328 ± 0.007[c]

Each apolipoprotein (0.1 mg protein) was mixed with triacylglycerol emulsion (1.0 mg triacylglycerol) and incubated with at 37°C for 30 min, and then the emulsion particles were isolated by centrifugation for analyzing order parameter.

Results are expressed as mean ± SD (n = 3) of order parameter (S). Values with different superscripts within a row are significantly different ($p < 0.05$).

chain specificity of phospholipase A1 activity of bovine LPL followed the sn-1 acyl-chain length of the artificial substrates, with an order of 14:0 > 16:0 > 18:0. In the present experiments with chylomicrons and VLDLs, we demonstrated that the Vmax of LPL for 16:0-rich lipoproteins exceeded those of 18:1- and 18:2-rich lipoproteins. Since Vmax for 18:1 was higher than that for 18:2 in the chylomicrons but not in the VLDLs, it was suggested that fatty acid saturation in the chylomicrons was also involved in the substrate specificity of LPL.

The LPL-lipoprotein interaction involves many factors such as apolipoproteins, phospholipids and particle size, as reported by Carrero *et al.* [31]. In order to clearly characterize the involvement of fatty acid composition of lipoprotein triacylglycerols in the kinetic parameters of LPL for chylomicrons and VLDLs, it would be most relevant to provide monoacid-rich lipoproteins with no changes in these factors other than the fatty acid profile. In this context, the present study determined the lipid composition and phospholipid species of plasma chylomicrons and VLDLs prepared from rats fed monoacid-rich triacylglycerols. Feeding of 16:0, 18:1 or 18:2 triacylglycerols modified neither the lipid composition nor the phospholipid species. It is, hence, unlikely that the substrate specificity of LPL for lipoprotein hydrolysis is associated with the lipid class or phospholipid specificity.

Choi *et al.* [32] have described that an amino-terminal fragment of apo B100 binds to LPL. Baum *et al.* [33] observed that the re-feeding for 48 h with high carbohydrate diet after 48 h starvation increased the expression of apo B48 mRNA in rat liver. These data suggested that changes in apolipoprotein composition affected the LPL-catalyzed hydrolysis. In the present study, apolipoproteins in plasma chylomicrons and VLDLs were not significantly changed by re-feeding with 16:0, 18:1 or 18:2 triacylglycerols, except that apo A-IV was quantitatively changed by the monoacid species of the lipoprotein. Hence our data showed, that the difference of LPL-catalyzed hydrolysis for lipoproteins with monoacid was mainly due to differences in fatty acid composition, and not to the apolipoprotein species in the lipoproteins, except for apo A-IV.

The present data show that apo A-IV content in 16:0-rich lipoproteins, both chylomicrons and VLDLs, were highest among all lipoproteins. Sun *et al.* [34] reported that triacylglycerol-rich lipoprotein apo A-IV concentrations were correlated with the secretion rate. Intestinal apo A-IV synthesis is markedly stimulated by fat absorption [35], whereas fatty acid chain length and degree of unsaturation have little effect altering apo A-IV transcript and biogenesis [36]. Therefore, in our study, the absorption of chickens refed with 16:0 triacylglycerol might be higher than that with 18:1 or 18:2 triacylglycerols.

The surface structure has been regarded as one of factors determining the fluidity of phospholipid monolayer in lipoprotein particles and thereby the biochemical properties of lipoproteins [23]. Order parameter (S) decreased in the order, 16:0 > 18:1 > 18:2 for lipoproteins. We previously demonstrated that order parameter (S) also decreased with increasing monoacid chain length and degree of unsaturation for chicken chylomicrons and VLDLs [12]. These results may imply that the fluidity of lipoprotein is partly dependent upon the fatty acid composition of the triacylglycerol; decreasing with carbon number and degree of unsaturation. To be emphasized in the present study are findings that Vmax of LPL or 16:0 contents of lipoprotein triacylglycerol increased with the increase of order parameter (S) for lipoproteins. It is, therefore, likely that the 16:0 content in chylomicron and VLDL triacylglycerols is one of the major factors in the fluidity of lipoproteins.

In analysis of artificial substrate specificity, the order parameter (S) of 16:0 triacylglycerol emulsion with apo B100 and 48 was higher than that of 18:1 or 18:2 triacylglycerol emulsions, whereas no significant changes were observed in order parameters (S) for various triacylglycerol emulsions in the absence of apolipoproteins. These results suggest that triacylglycerol acyl chains in the presence of apo B, affect the fluidity of lipid emulsions with phospholipid monolayers and therefore that apo B plays a crucial role in LPL catalyzed hydrolysis of triacylglycerol-rich lipoproteins. In addition, the Vmax of LPL was higher for 16:0 triacylglycerol emulsions with apo B, than for 18:1 or 18:2 triacylglycerols, and these differences were exaggerated by the inclusion of apo A-IV. Goldberg *et al.* [32] have reported that apo A-IV is required for the efficient release of apo C-II from VLDLs to nascent chylomicrons, thereby increasing LPL-mediated hydrolysis of triacylglycerols. Hockey *et al.* [38] demonstrated that apoA-IV is displaced from the chylomicron surface by high density lipoprotein-

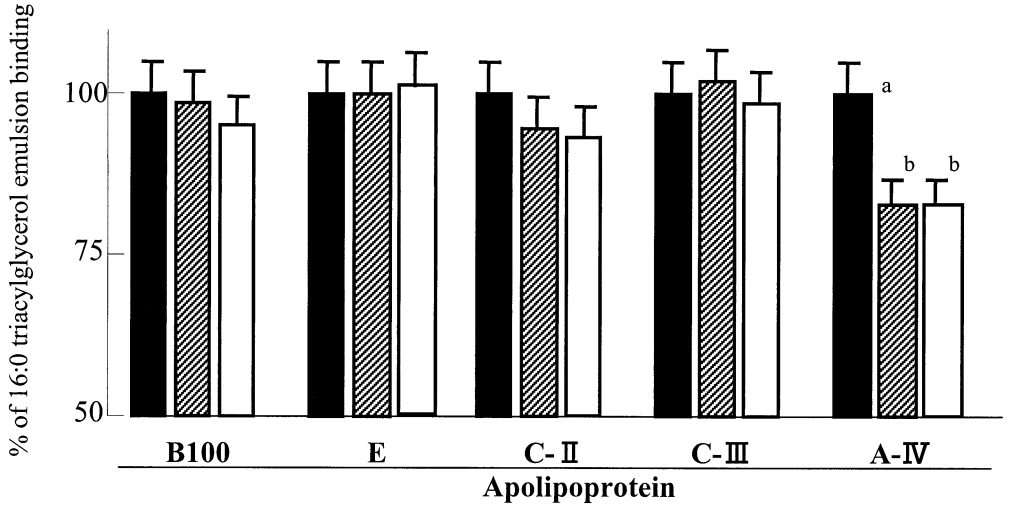

Fig. 4. Binding of rat apolipoproteins for monoacid triacylglycerol emulsion. Results are expressed as mean ± SD (% of 16:0 triacylglycerol emulsion binding, n = 4). Different superscripts are significantly different ($p < 0.05$). 16:0 triacylglycerols (■), 18:1 triacylglycerols (▨), 18:2 triacylglycerols (□).

associated C and E apolipoproteins, which are critical for activation of lipoprotein lipase and chylomicron remnant clearance. Our findings showed that the apo C-II and apo E levels in various monoacid-rich chylomicrons and VLDLs were not modulated by the fatty acid profile in lipoproteins, whereas binding of apo A-IV to 16:0 triacylglycerol emulsions was higher than to 18:1 and 18:2 triacylglycerol emulsions. It is, hence, probable that the 16:0 in lipoprotein triacylglycerols stimulates the binding of apo A-IV to lipoproteins, and that apo A-IV consequently modifies the lipoprotein fluidity which alters the rate of triacylglycerol hydrolysis by LPL.

The incidence of obesity is rapidly rising in a number of countries. Of the multiple etiologies involved in human obesity, a consistent association between obesity and abnormalities in lipoproteins has been reported [39]. Kern [40] reviewed that the metabolic changes resulting from LPL likely played a role in regulating body adipose tissue during much of human evolution and continue to affect human obesity today. Furthermore, Weinstock *et al.* [41] reported that obese mice rendered deficient in adipose tissue LPL demonstrated increased endogenous fatty acid synthesis but had diminished fat mass. These findings provide strong evidence for a role of adipose tissue LPL in one type of genetic obesity. The present data indicate that high palmitic acid content in lipoproteins modifies lipoprotein fluidity, thereby increasing the plasma lipoprotein hydrolysis. Increased intake of palmitic acid mainly from animal fats is partly concerned in obesity [42]. It is therefore suggested that diets high in palmitic acid increase the Vmax of adipose tissue LPL for lipoprotein, thus stimulating lipid accumulation in adipose tissues, and thereby leading to obesity.

In conclusion, the re-feeding of monoacid triacylglycerols to rats for 24 h following 24 h starvation following monoacid-rich diets for 10 days, is a possible procedure for generating lipoproteins having more than 70% of the corresponding monoacid in the triacylglycerol. The Vmax of rat LPL for chylomicrons and VLDLs increased linearly with the increased 16:0 content in the lipoprotein triacylglycerol, thereby modifying the fluidity of lipoproteins with

Table 8
Kinetic parameter of rat-LPL for mono fatty acid triacylglycerol emulsions with or without apolipoproteins

Apolipoprotein	Vmax (U/mg LPL)			Fold of Vmax		Apparent Km (mM)		
	16:0	18:1	18:2	16:0/18:1	16:0/18:2	16:0	18:1	18:2
Lipid only	36.8 ± 4.8[a]	30.2 ± 1.4[b]	30.2 ± 1.4[b]	1.22	1.22	1.57 ± 0.11	1.58 ± 0.12	1.58 ± 0.12
Apo B100	56.8 ± 4.8[a]	40.8 ± 3.2[b]	39.1 ± 1.0[b]	1.39	1.45	1.56 ± 0.15	1.56 ± 0.15	1.55 ± 0.17
Apo C-II	110.5 ± 3.1[a]	95.6 ± 3.7[b]	94.8 ± 5.2[b]	1.16	1.17	1.57 ± 0.12	1.59 ± 0.19	1.57 ± 0.08
Apo A-IV	46.5 ± 5.8[a]	33.3 ± 1.2[b]	32.3 ± 1.0[b]	1.40	1.44	1.57 ± 0.07	1.57 ± 0.08	1.58 ± 0.12
Apo B100+ apo C-II	110.5 ± 3.1[a]	92.6 ± 1.4[b]	93.4 ± 6.8[b]	1.19	1.18	1.61 ± 0.21	1.58 ± 0.14	1.58 ± 0.12
Apo B100+ apo A-IV	66.2 ± 1.7[a]	41.8 ± 3.5[b]	42.1 ± 2.8[b]	1.58	1.57	1.53 ± 0.15	1.57 ± 0.11	1.57 ± 0.15
Apo B100+ apo C-II + apo A-IV	168.4 ± 1.4[a]	115.6 ± 6.8[b]	112.2 ± 7.4[b]	1.47	1.50	1.53 ± 0.16	1.57 ± 0.11	1.56 ± 0.15

Apparent Km and Vmax of LPL were determined on double reciprocal plots with triacylglycerol emulsions as the substrate at 37°C, pH 8.6. Results are expressed as mean ± SD (n = 3). Values with different superscripts within a row are significantly different ($p < 0.05$).

apo B. The binding of apo A-IV to lipoproteins is stimulated by the 16:0 level in lipoprotein triacylglycerols, and the apo A-IV level in lipoproteins modifies the Vmax of LPL. These results may provide a clue to understanding not only the substrate specificity of LPL, but also, the regulatory factors for lipoprotein hydrolysis.

Acknowledgments

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