Plasma activities of lipoprotein lipase, hepatic lipase and lecithin: cholesterol acyltransferase in patients considered for parenteral nutrition with fat emulsion[1,2]

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ABSTRACT Intralipid® is a fat emulsion which is widely used for intravenous nutrition in very ill patients. In order to know more about the capacity of these patients to metabolize exogenous triglycerides, the plasma activities of lipoprotein lipase (LPL), hepatic lipase (HL) and lecithin: cholesterol acyltransferase (LCAT), the key enzymes in the metabolism of serum lipoproteins were measured by a radioisotope technique in 23 critically ill patients and 20 patients with recent major surgery. Compared with normal volunteers, the activities were significantly decreased. On the other hand, the capacity to clear intravenously given Intralipid® (0.1 g/kg), expressed as fractional removal rate (K_2), was retained in patients. It is suggested that the measurement of K_2 could not be useful to evaluate the capacity of Intralipid® administration to satisfy the metabolic needs and also that its utilization must be reevaluated in terms of potential harmful effects. *Am J Clin Nutr* 1985;41:748–752.

KEY WORDS Plasma clearance of Intralipid®, lecithin: cholesterol acyltransferase, lipoprotein lipase, hepatic lipase, critically ill patients

Introduction

Intralipid® (Kabivitrum, Stockholm, Sweden) is a fat emulsion which is widely used in humans for intravenous nutrition. The triglyceride particles share many properties with chylomicrons, in terms of physical behavior, chemical composition and kinetics. However, to be suitable for metabolic needs, these exogenous triglycerides must be handled in the same way as the endogenous ones.

The activities of the enzymes which are mainly involved in the catabolism of triglyceride-rich lipoproteins, are lipoprotein lipase (LPL) and hepatic lipase (HL); moreover, lecithin: cholesterol acyltransferase (LCAT) contributes to the mobilization of cholesterol from peripheral cells by esterifying free cholesterol contained in the HDL fraction. Therefore, patients considered for parenteral nutrition with fat emulsion and particularly critically ill patients must display a capacity not only to clear the exogenous triglycerides from plasma but also to metabolize them in such a way that they are used to meet the metabolic requirements of the organism.

The aim of this work is to appraise, in such patients, the plasma activities of LPL, HL and LCAT, particularly in relation with their capacity to clear intravenously given Intralipid®.

Material and methods

Forty-three patients (25 men, 18 women) admitted to the Intensive Care Department were studied. The mean age was 57 ± 2 (mean ± SEM) years (range: 31–82 years). Twenty-three patients (mean age ± SEM: 57 ± 3 years) (13 men, 10 women) were critically ill (CI patients) with

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multiple organ failure and nine (6 men, 3 women) of them had cirrhosis of the liver; twenty other patients (mean age ± SEM: 57 ± 3 years) (12 men, 8 women) were evaluated after major elective surgery such as total gastrectomy, colectomy, etc in the immediate postoperative period (PO patients). All subjects were infused with 5% dextrose (100–150 g/24 h) at the start of the study. Within twenty-four hours, blood was taken for measurement of serum lipid and lipoprotein concentrations and plasma LCAT activity; immediately after, heparin (100 U/kg) was injected intravenously and another blood sample was taken 10 min later for the assay of plasma LPL and HL activities. Twenty-four hours later, an intravenous fat tolerance test was performed. Normal volunteers were used as controls: 8 normolipemic, non-obese male volunteers aged between 25 to 40 years; they underwent the same experiment protocol as the patients. Approval for the study was obtained from the Ethical Committee of the School of Medicine of the Catholic University of Louvain.

The plasma LPL and HL activities were measured following the technique described by Nilsson-Ehle and Ekman (1). The substrate contains glycerol trioleate (9.15 mg), glycerol tri[1-14C]oleate (10.10^6 cpm) (The Radiochemical Center, Amersham), lysolecithin (0.04 mg), Tris-HCl buffer 0.2 M containing fatty acid free albumin in a total volume of 3.3 ml; this emulsion is stabilized by sonication (Sonifier® B-12, Branson Sonic Power Company, Danbury, CT). LPL but not HL, rapidly hydrolyses the substrate emulsion to which albumin is added after sonication; for HL, the same emulsion is used but albumin is added before sonication. When LPL is measured, normal human serum (0.35 ml) is added as a source of ApoC-II to the substrate emulsion.

Postheparin plasma (5–10 μl) is added to the substrate emulsion (100 μl) and the mixture is incubated (10 min at 37°C) at pH 8.0 in the presence of 0.15 M NaCl for LPL, and at pH 9.0 and in the presence of 0.5 M NaCl for HL. The reaction is stopped by addition of methanol, chloroform and heptane (1.45/1.25/1.0; v/v/v). The free fatty acids liberated into the medium by the hydrolytic reaction are extracted by addition of a 0.1 M potassium carbonate-borate buffer (pH 10.5). The counting of the radioactivity present in 1 ml of the extract permits estimation of the rate of lipolysis, expressed as mU/ml plasma. One milliunit of enzyme activity represents the release of 1 nmol fatty acid/minute. For plasma LPL and HL, a coefficient of variation of about 30% (28.2% and 31.3% respectively for LPL and HL) is observed when measuring the enzyme activities in different plasma samples taken from healthy normolipidemic subjects. The interassay variation is probably due partially to the difficulty of obtaining a highly reproducible emulsion. To reduce the variability of the results as much as possible, the plasma activities of LPL and HL were measured using the same triolein emulsion. Using this method, the coefficient of variation is 4.0% and 12.7%, respectively for LPL and HL when calculated from the enzyme activities measured in 6 plasma samples taken from one volunteer on different days during a 10 days period (2).

The plasma LCAT activity was assayed by the technique described by Stokke and Norum (3). The fresh plasma (100 μl) is preincubated at 37°C for four hours with 30 μl of an albumin-stabilized emulsion of cholesterol [1a, 2a, (n)3H] (around 50.000 cpm) (The Radiochemical Center, Amersham) and in the presence of 20 μl phosphate buffer 0.2 M pH 7.1 containing DTNB (1.4 mM); this preincubation permits the radiolabeled cholesterol to be distributed into the various plasma lipoproteins in the absence of active LCAT. Addition of 20 μl mercaptoethanol (0.1 M) reactivates the enzyme and, during a 20-min incubation, the LCAT reaction takes place. The reaction is stopped with methanol/chloroform; lipids are extracted and the free and esterified cholesterol are separated by thin layer chromatography. The spots containing free and esterified cholesterol are visualized by exposure to iodine vapor and scraped off into counting vials. The determination of the amount of radioactivity in each spot permits the calculation of the free cholesterol/total cholesterol ratio in each plasma sample before and after the LCAT reaction and thus estimation of the esterification rate. The fractional esterification rate ($\% \cdot h^{-1}$) expressed the percentage of free cholesterol esterified in the plasma sample per hour. The interassay variation estimated by the coefficient of variation of the LCAT activity measured in the plasma samples was 12.6% (2). Validation of the techniques for measurement of plasma LPL, HL and LCAT activities in humans, such as used in the study has been described previously (2) and normal values of these plasma enzyme activities have been established in normolipemic subjects (2).

The intravenous fat tolerance test was performed following the method described by Carlson and Rössner (4). A single intravenous injection of triglycerides emulsion was given (0.1 g/kg 10% Intralipid® emulsion) in two minutes time and blood was sampled at 0, 5, 10, 15, 20, 25, 30 and 45 min to measure the serum triglycerides concentrations.

The K_2 coefficient (percentage of the emulsion cleared from plasma per minute) expressing the fractional plasma removal rate of Intralipid® was calculated from the disappearance curve. The serum total cholesterol and triglycerides were measured by automated enzymatic techniques (WAKO). The serum HDL-cholesterol concentration was determined by the method described by Lopes-Virella et al (5). The statistical difference between mean values was estimated by the Student's *t* test.

Results

*Serum lipids and lipoprotein concentrations (**Table 1**)*

The patients had low serum triglycerides and, overall, low serum total cholesterol and HDL-cholesterol concentrations. In CI patients, the serum concentrations of total cholesterol and HDL-cholesterol were significantly lower and the serum concentration of triglycerides significantly higher than in PO patients.

*Plasma LPL, HL and LCAT activities (**Table 2**)*

When compared to normal volunteers studied in the present study, plasma activities

TABLE 1
Serum triglycerides (TG), total cholesterol (tot Chol) and HDL-cholesterol (HDL-C) concentrations (mean ± SEM)

	Number	TG	Tot Chol	HDL-C
		mg/dl	*mg/dl*	*mg/dl*
Patients	43	104.4 ± 8.6	102.9 ± 5.8*‖	26.0 ± 2.5*§
Critically ill	23	124.3 ± 12.7*‡	84.1 ± 7.2*‖†‖	16.4 ± 2.1*§†‖
With cirrhosis	9	106.7 ± 17.7	92.9 ± 13.2	17.0 ± 2.9
Without cirrhosis	14	135.6 ± 17.2	78.5 ± 8.2	16.1 ± 2.9
Postoperative	20	81.5 ± 9.3*‡	124.5 ± 6.8*‖	36.9 ± 3.5
Normals	8	126.3 ± 12.6	183.5 ± 9.5	45.4 ± 4.4

*† Mean values of the particular group are significantly different from respectively those of normals (*) and of postoperative patients (†).
‡§‖ Mean values of the particular group are significantly different of those of the other group considered at a p value superior respectively to 0.01, 0.0025 and 0.0005.

of LPL, HL and LCAT were found to be significantly low in patients ($p < 0.0005$), respectively 54.3, 21.5 and 49.8% of the values found in the volunteers. No significant difference was found between CI and PO patients and between cirrhotics and noncirrhotics.

*Plasma clearance of Intralipid® (**Table 3**)*

The fractional removal rate of Intralipid® was not significantly different in patients from that found in volunteers, whether the patients were considered as a whole or as critically ill, postoperative, cirrhotic or not cirrhotic.

Discussion

The present study clearly demonstrates that patients considered to be suitable for total parenteral nutrition because of a severe catabolic state due to serious illness or major surgical procedures, display a capacity to clear intravenously given fat emulsions (Intralipid®) similar to that found in normal volunteers. Accordingly, all previous studies have also shown that the plasma clearance rate of fat emulsion is not decreased in severely ill patients, being even higher than normal in patients suffering from burns (6), sepsis or trauma (7, 8). On the other hand, the plasma LPL, and HL, which are considered as key factors in the interconversion of triglyceride-rich lipoproteins and the plasma LCAT, were found to have significantly lower activities in patients compared to normal volunteers.

The low activity of plasma LPL and HL found in our patients is in accordance with the low LPL activity demonstrated in the

TABLE 2
Plasma activities (mean ± SEM) of lipoprotein lipase (LPL), hepatic lipase (HL) and lecithin: cholesterol acyltransferase (LCAT)

	Number	LPL	HL	LCAT
		mu/ml	*mu/ml*	*% · h⁻¹*
Patients	43	120 ± 11*‡	76 ± 7*‡	2.45 ± 0.20*‡
Critically ill	23	124 ± 19*‡	68 ± 11*‡	2.02 ± 0.30*‡
With cirrhosis	9	121 ± 31*†	66 ± 19*‡	2.54 ± 0.50*‡
Without cirrhosis	14	125 ± 25*‡	65 ± 14*‡	1.97 ± 0.41*‡
Postoperative	20	116 ± 13*‡	88 ± 9*‡	2.73 ± 0.25*‡
Normals	8	221 ± 30	354 ± 65	4.92 ± 0.51

* Mean values of the particular group are significantly different from those of normals.
†‡ Mean values of the particular group are significantly different from those of normal with a p value superior respectively to 0.0125 and 0.0005.

adipose tissue and the skeletal muscle of septic patients with and without trauma (9) and in the adipose tissue of endotoxin-sensitive mice killed 16 h after injection of endotoxin (10). Thus, it appears to be paradoxical that patients which clear very efficiently exogenous triglycerides from plasma are found to have low activities of the enzymes which normally catabolize endogenous triglycerides. Given the low plasma activity of LPL in our patients, hypertriglyceridemia would be expected. On the contrary, low levels of serum triglycerides were noted, which could be explained by a reduced synthesis of triglyceride-rich lipoproteins by the liver such as found in fasting state.

A relevant question to the problem of Intralipid® utilization is whether the activities of plasma LPL and HL could be increased by the intravenous fat emulsion. It has previously been shown that the LPL activity of adipose tissue but not of the skeletal muscle is increased by glucose and Intralipid® plus glucose infusions (11); no study has been performed so far, to analyze the effects of Intralipid® on LPL activity and work is in progress in our laboratory to answer this important question.

Although many data suggest that fat emulsion behaves like chylomicrons, few studies have evaluated the relationship between the K_2 value and the activity of plasma LPL and HL (12, 13). Nordenström et al (8) did not observe in their patients, any significant correlation between the plasma fractional clearance rate of triglyceride and the oxidation rate of lipids, as measured by the cumulative radioactive CO_2 production, during administration of intravenous fat emulsion containing radioactive Intralipid®. Moreover, in septic rats, the cumulative amounts of ^{14}CO in the expired breath were lower than in control rats, after intravenous infusion of ^{14}C-Intralipid® (14). Thus, serious doubt must be thrown upon the validity of the plasma fractional clearance rate of triglycerides to evaluate the metabolic utilization of Intralipid®. One can ask whether that fat given in patients such as studied in the present work is deposited in particular sites through mechanisms not necessitating prior hydrolytic reactions. Old artificial fat emulsions were found to be taken up by the reticuloendothelial system;

TABLE 3
Values (mean ± SEM) of plasma clearance of fat emulsion (K_2)

	Number	K_2 (%/min^{-1})
Patients	43	5.64 ± 0.58
Critically ill	23	6.23 ± 0.93
With cirrhosis	9	6.06 ± 1.63
Without cirrhosis	14	6.53 ± 0.61
Postoperative	20	4.95 ± 0.61
Normals	8	5.18 ± 0.90

even Intralipid® was incriminated to induce a distinctive splenopathy (lipidosis) (15) and lipid deposition in various organs (16), attributed to the property of acute-phase sera (C-reactive protein) to cream the fat emulsion (17).

It is known that Intralipid® infusion produces alterations in serum lipoproteins; some lipoproteins enriched with core lipids (triglycerides and cholesteryl esters) may be produced in excess of the normal hepatic clearance capacity, and bound and internalized by the β-VLDL receptors on macrophages (18).

Moreover, cultured macrophages secrete LPL into the culture medium (19), therefore accumulation of triglycerides in macrophages can occur by uptake of intact VLDL particles, by uptake of a triglyceride-depleted particle produced by the action of LPL and by the direct uptake of free fatty acids generated by the activity of LPL (20).

If confirmed, these findings would suggest that Intralipid® infusion given to very ill patients, could not only be without metabolic interest but also harmful to some patients.

The role of LCAT in lipoprotein metabolism is thought to favor cholesterol mobilization from peripheral tissue to the liver. The mechanism possibly involves esterification of the free cholesterol in the HDL, allowing this latter to accept more free cholesterol from the cells and transport it towards the hepatocytes. The present work demonstrates a reduction, not only in LCAT activity but also in the concentration of cholesterol contained in the HDL fraction, the substrate for LCAT.

In conclusion, the finding that the activities of LPL, HL and LCAT are lower than normal

in severely ill patients, suggests that lipoprotein metabolism may be severely disturbed in these patients. Despite these findings, the K_2 expressing the plasma clearance rate of Intralipid® was not found to be significantly altered in the same patients; this suggests that measurement of K_2 in these cases could be of no value to evaluate the usefulness of Intralipid® administration to satisfy the metabolic needs. Moreover, its utilization must also be reappraised in terms of potential harmful effects (blockade of the reticuloendothelial system).

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