The Impact of Intravenous Fat Emulsion Administration in Acute Lung Injury

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The aim of this study was to evaluate the effect of parenteral nutrition containing medium- and long-chain triglycerides on the function of the respiratory system and to investigate mechanisms involved in this process. We studied 13 patients with acute respiratory distress syndrome (ARDS), 8 receiving lipid and 5 placebo, and 6 without ARDS, receiving lipid. Bronchoalveolar lavage (BAL) was performed before and 1 hour after administration of lipid or placebo. In patients with ARDS, lipid administration resulted in deterioration of oxygenation (Pa_{O_2}/FI_{O_2}: from 129 ± 37 to 95 ± 42), compliance of respiratory system (from 39.2 ± 12 to 33.1 ± 9.2 ml/cm H_2O), and pulmonary vascular resistance (from 258 ± 47 to 321 ± 58 dyne·s·cm^{-5}). In the BAL fluid of the same group, an increase in total protein and phospholipid concentrations, phospholipase activities, platelet-activating factor and neutrophils, as well as alterations in BAL lipid profile were observed. No significant changes were observed in the control or in the ARDS-Placebo groups. In conclusion, this study indicates that administration of medium- and long-chain triglycerides in patients with ARDS causes alterations in lung function and hemodynamics. Inflammatory cells, possibly activated by lipids, release phospholipase A_2 and platelet-activating factor, enhancing edema formation, inflammation, and surfactant alterations.

Keywords: parenteral nutrition; medium-chain triglycerides/long-chain triglycerides; phospholipase A_2; platelet-activating factor–acetylhydrolase; platelet-activating factor

Total parenteral nutrition is the intravenous route of nutrient administration, and it includes proteins, carbohydrates and lipids. Lipids are required to prevent essential fatty acids deficiency. In addition, they provide a rich source of calories in a small volume that, compared with other fuel molecules, yields the lowest carbon dioxide burden.

Lipid emulsions consist of a continuous aqueous phase (usually containing glycerol), medium-chain triglycerides (MCT), and long-chain triglycerides (LCT) and emulsifiers (usually phosphatidylcholine). These emulsions have been designed to resemble the natural chylomicrons. They contain two different particle populations, the triglyceride-rich and phospholipid-rich particles. The triglyceride-rich particles have the metabolic fate of chylomicrons, whereas the phospholipid-rich liposomes play an important role in the stability of the emulsion (1).

When enteral nutritional support is not possible, total parenteral nutrition is required in a number of patients with multiple organs dysfunction, including cases with acute lung injury or acute respiratory distress syndrome (ARDS). Acute lung injury/ARDS is characterized by inflammation and pulmonary edema as a result of increased permeability of the alveolar–capillary barrier. The acute phase is characterized by rapid onset of respiratory failure and arterial hypoxemia (2). The pathologic findings include injury in both capillary endothelium and alveolar epithelium, resulting in an influx of a protein-rich edema fluid into the airspaces. Also, a massive accumulation and activation of polymorphonuclear leukocytes mediates the evolution of inflammation through the release of several inflammatory mediators, such as eicosanoids, platelet-activating factor (PAF), and oxidants. (3). Qualitative and quantitative alterations in lung surfactant composition may contribute to respiratory failure (4, 5).

Surfactant phospholipids are sensitive targets for phospholipases and especially for type II secretory phospholipase A_2 (PLA_2), a marker of inflammation, giving rise to lyso-phospholipids (6). Lyso-phosphatidylcholine (lyso-PC) plays a crucial role in the evolution of ARDS because it damages the alveolar epithelium (7), increases capillary permeability (8), and inactivates surfactant tensioactivity (9). PLA_2 activity also is responsible for the release of arachidonic acid and its biologically active metabolites, such as prostaglandins (PGs) and leukotrienes (10). Moreover, secretory PLA_2 could lead to the generation of PAF, a potent phospholipid mediator in inflammatory reactions, which also is possibly involved in the pathogenesis of acute lung injury (11, 12). PAF-acetylhydrolase (PAF-AcH) displays specificity for acetyl groups or oxidatively modified acyl chains at the sn-2 position of phospholipids and inactivates PAF.

There is some evidence that intravenous administration of lipid-rich emulsions or their metabolites may induce or aggravate gas exchange abnormalities (13, 14). The aim of this study was to evaluate the effect of MCT/LCT parenteral nutrition on the function of the respiratory system and to investigate mechanisms involved in this process. Therefore, respiratory system mechanics, hemodynamics, blood gases, qualitative and quantitative surfactant alterations, total protein as a marker of capillary–alveolar permeability, and phospholipases A_2 and PAF as inflammatory mediators were assessed in bronchoalveolar lavage (BAL) after intravenous lipid administration.

METHODS

Nineteen mechanically ventilated patients were included in this study, eight with ARDS to whom lipid emulsion was administered (ARDS-Lipid group), five with ARDS to whom saline was given (ARDS-Placebo group), and six without acute lung injury/ARDS to whom lipid emulsion was administered (normal control group). Standard criteria for ARDS diagnosis were (*1*) acute hypoxemic respiratory failure requiring mechanical ventilation, (*2*) diffuse bilateral alveolar infiltrates on the chest roentgenogram, (*3*) refractory hypoxemia ($Pa_{O_2}/FI_{O_2} < 200$ regardless of positive end-expiratory pressure level), (*4*) pulmonary artery wedge pressure less than 18 mm H_2O or no clinical evidence for left atrial hypertension, and (*5*) recognized appropriate clinical setting or risk factor for the development of ARDS (15). All patients with ARDS had a direct type of ARDS at the early phase (within the first 76 hours from its onset). ARDS due to primary pulmonary injury was characterized as direct, whereas ARDS due to secondary pulmonary injury was characterized as indirect. The inclusion criteria for control

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patients were: absence of cardiopulmonary disease, normal chest radiograph, and Pa_{O_2}/FI_{O_2} greater than 300 mm Hg. Patients with dyslipidemia and with hepatic and renal failure were excluded. The patient's demographic data and clinical characteristics are shown in Table 1. The protocol was approved by the Ethics Committee of the University Hospital of Ioannina, and the patients or the next of kin gave an informed consent to the study.

Study Protocol

Patients were sedated, paralyzed, and ventilated through a cuffed endotracheal tube with a 300 Siemens Ventilator (Siemens-Elema, Solna, Sweden), using volume control or pressure support mode. The ventilatory settings in patients with ARDS of both groups were: frequency of 10 to 18 breaths/minute, VT of 6 to 7 ml/kg body weight, and positive end-expiratory pressure between 8 and 15 cm H_2O. FI_{O_2} was set at the minimal level, at which an Sa_{O_2} of 90% could be achieved. The Pa_{CO_2} level was not a target in the ventilatory settings. The ventilatory settings in control patients were: a frequency of 10 to 18 breaths/minute, VT of 9 to 12 ml/kg body weight, and positive end-expiratory pressure between 3 and 5 cm H_2O, to minimize the risk of atelectasis. FI_{O_2} was set at the minimal level, at which an Sa_{O_2} of 95% could be achieved. The Pa_{CO_2} level was kept between 35 and 45 mm Hg. We used different ventilatory settings in ARDS and normal control groups because patients with normal lungs needed higher VT to avoid basal atelectasis.

A pulmonary artery catheter (Opticath; Abbott, Chicago, IL) was introduced in all patients via an internal jugular or subclavian vein and an arterial line. When the patients were hemodynamically stable, the first BAL was performed. Soon after the blood gases, hemodynamics, and lung mechanics returned to the initial levels, lipid emulsion, consisting of a mixture of MCT and LCT (MCT/LCT 20%, Lipofundin; B. Braun Melsungen AG, Melsungen, Germany), was infused via a central venous catheter. The rate of infusion was 3.5 mg/kg/minute for 1 hour. Soon afterward, a second BAL was performed. In the placebo group, saline was given with the same infusion rate as lipid emulsion.

For each patient, the following parameters were recorded: Pa_{O_2}/FI_{O_2}, respiratory compliance, Pa_{CO_2}, pH, VT, plateau pressure, positive end-expiratory pressure, mean arterial blood pressure, mean pulmonary arterial pressure($\overline{Ppa}$), $\dot{Q}$, pulmonary vascular resistance (PVR), and systemic vascular resistance.

All measurements were obtained at the following time points: (*1*) before the first BAL, (*2*) before lipid infusion or placebo administration (baseline), (*3*) soon after the end of lipid infusion or placebo (after I), and (*4*) 2 hours after the second BAL (after II).

BAL Procedure

BAL was performed by fiberoptic bronchoscopy, as described previously (16). Patients were ventilated with a control mechanical ventilation mode; they were sedated (midazolam) and paralyzed (atracurium) during the BAL procedure and measurements. Propofol was not used.

Six aliquots of 20 ml sterile normal saline at 37°C were infused through the working channel of the bronchoscope. The first aspirated fluid, reflecting a bronchial sample, was discharged, whereas the others were collected in ice-cold tubes. BAL was then filtered through sterile gauze and centrifuged at 500 × *g* for 15 minutes at 4°C to obtain BAL cells in the pellet. The supernatant of 500 × *g* BAL fluid was used to measure the biochemical parameters.

Biochemical Parameters

In BAL fluid, the following parameters were measured: total protein, total phospholipids and individual classes, and PAF, PLA_2, and PAF-AcH activities.

Total protein concentrations were measured according to the method of Lowry and coworkers (17). Total lipids were extracted according to Bligh and Dyer (18) and separated into classes with thin-layer chromatography, by using chloroform–methanol–water (65:35:7, vol/vol) as the solvent system. Phospholipids were visualized under ultraviolet lamp, after spraying with 2-(*p*-toluidinyl)-naphthylene-6-sulfonic acid. Then, they

TABLE 1. DEMOGRAPHIC DATA OF PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME

No.	Age (*yr*)	Sex	Disease	MV	ARDS	PMR	Outcome
Lipid administration group							
1	60	F	Lung contusion	2	2	48	S
2	49	M	Aspiration	3	3	70	S
3	67	F	Pneumonia	3	3	40	S
4	52	F	Aspiration	2	2	69	D
5	42	M	Viral pneumonia	2	2	30	D
6	70	M	Lung contusion	3	2	82	S
7	39	M	Aspiration	7	2	88	D
8	48	F	Pneumonia	5	3	66	S
Mean	53			3.4	2.4	61	
SD	11			1.8	0.5	20	
Placebo group							
1	32		Pneumonia	3	2	48	S
2	74		Aspiration	6	2	84	D
3	59		Pneumonia	7	3	55	S
4	67		Pneumonia	6	3	72	D
5	18		Lung contusion	2	2	30	S
Mean	50			4.8	2.4	58	
SD	24			2.2	0.5	21	
Normal control group							
1	52	M	CVA	5	–	42	S
2	46	F	CVA	2	–	36	S
3	19	M	H/S	5	–	61	D
4	75	M	CVA	6	–	77	D
5	75	M	NM	5	–	65	D
6	54	M	H/S	5	–	54	S
Mean	53.5			4.6		55	
SD	21			1.3		15	

Definition of abbreviations: ARDS = days from the onset of acute respiratory distress syndrome; CVA = cerebrovascular accident; D = died; F = female; H/S = head and/or spine trauma; M = male; MV = days of mechanical ventilation before starting the protocol; NM = neuromuscular disease; PMR = predicted mortality rate; S = survived.

were scraped off the thin-layer chromatography plate and measured from their lipid–phosphorus content after perchloric acid digestion, according to Bartlett (19).

PAF was purified from the lipid extract of BAL fluid with thin-layer chromatography, as described previously. The area between authentic sphingomyelin and lyso-PC, where PAF migrates, was scrapped off the plate, extracted, and quantified. PAF determination was based on the aggregation of washed rabbit platelets pretreated with creatine phosphate/creatine phosphokinase for scavenging adenosine diphosphate and acetylsalicylate-lysine, a cyclooxygenase inhibitor, as described previously (12, 20). Low temperatures were maintained throughout the BAL treatment to avoid PAF degradation due to PAF-AcH.

PLA_2 and PAF-AcH were measured by a fluorimetric assay (21). Briefly, the incubation mixture for PLA_2 contained 10 mM Tris–HCl, pH 7.4, 2 mM Ca^{2+}, and 5 nM 1-palmitoyl-2-[12-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino] dodecanoyl]-sn-glycero-3-phosphocholine (C_{12}-NBD-PC) as substrate. The incubation mixture for PAF-AcH contained 10 mM Tris–HCl, pH 7.4, 10 mM ethylenediaminetetraacetic acid, and 5 nM 1-palmitoyl-2-[6-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino] dodecanoyl]-sn-glycero-3-phosphocholine (C_6-NBD-PC). We have shown previously that secretory PLA_2 in the absence of Ca^{2+} does not hydrolyze C_6-NBD-PC. On the other hand, in chromatographic analysis (thin-layer chromatography and HPLC) of the reaction mixture without Ca^{2+} in the presence of both substrates, only 6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino] hexanoic acid (C_6-NBD-FA) was identified as a product. The fluorimetric assay correlated well with the radiometric assay (21).

BAL Cells

BAL cell differential counts were performed by counting at least 300 cells in cytocentrifuge preparations stained with hematoxylin and eosin. The lipid content of the cells was examined using the Sudan Black B staining procedure.

Statistical Analysis

Data are reported as mean ± SD. Statistical comparisons between groups were performed using two-way analysis of variance for repeated measurements. The significance of differences between values was determined with Bonferroni correction for multiple comparisons. Correlation between values was analyzed by linear regression. The level of significance was defined as a p value less than 0.05.

RESULTS

Patients' Data

Both groups of patients with ARDS exhibited lower respiratory compliance and Pa_{O_2}/FI_{O_2} compared with patients without ARDS (normal control group). After the lipid infusion, both respiratory compliance and Pa_{O_2}/FI_{O_2} were further decreased in the ARDS-Lipid group, whereas in the ARDS-Placebo and control groups, these parameters remained at the same levels. Furthermore, after the lipid infusion in the ARDS-Lipid group, PVR significantly increased ($p < 0.05$) from baseline values. A nonsignificant increase in $\overline{Ppa}$ ($p = 0.07$) and $\dot{Q}$ ($p = 0.2$) and decrease in systemic vascular resistance ($p = 0.08$) were also observed after lipid administration. Two hours after fat administration, all the parameters mentioned previously had the tendency for improving, but the differences were not significant. Nonsignificant changes were observed in ARDS-Placebo and control groups. There were no significant differences between ARDS groups in $\overline{Ppa}$, PVR, systemic vascular resistance and $\dot{Q}$, whereas baseline PVR and $\overline{Ppa}$ levels were significantly higher in ARDS groups compared with the normal control (Table 2).

BAL Protein Content

Total BAL protein was higher in the ARDS groups compared with the control group. After the lipid infusion, the protein content was further elevated in patients with ARDS, whereas in ARDS-Placebo and control groups, there was no significant change (Table 3).

Surfactant Phospholipids

A quantitative decrease in total BAL phospholipids was observed in the ARDS groups compared with the control group. After lipid administration, the amount of total phospholipids in ARDS-Lipid group increased, whereas in ARDS-Placebo and control groups, it remained at the same levels (Table 3). In addition, several alterations related to the quality of BAL fluid were observed: PC had the same fluctuation to total phospholipids. In particular, both its concentration and percentage were lower in the ARDS groups compared with the control group.

TABLE 2. BLOOD GASES, LUNG MECHANICS, AND HEMODYNAMICS OF PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME AND CONTROL PATIENTS

	ARDS-Lipids Group			ARDS-Placebo Group			Normal Control Group		
	Baseline	After (I)	After (II)	Baseline	After (I)	After (II)	Baseline	After (I)	After (II)
V_T	535 ± 102*	526 ± 102	529 ± 97	551 ± 78*	546 ± 83	550 ± 92	710 ± 10	710 ± 12	715 ± 13
Ppl	23.1 ± 6.1†	24.9 ± 6	25.1 ± 6.2	26.2 ± 6.3†	27.1 ± 7.1	27.5 ± 5.9	16.4 ± 4.7	17 ± 4.2	16.2 ± 4.3
PEEP	9.2 ± 2.7*	9.2 ± 2.7	9.2 ± 2.7	11.1 ± 3.3*	11.1 ± 3.3	11.1 ± 3.3	4 ± 1.5	4 ± 1.5	4 ± 1.5
pH	7.42 ± 0.04	7.39 ± 0.06	7.40 ± 0.06	7.37 ± 0.04	7.37 ± 0.06	7.38 ± 0.05	7.43 ± 0.08	7.43 ± 0.02	7.40 ± 0.02
Pa_{CO_2}	45 ± 9	48.7 ± 11	47.5 ± 10	50.1 ± 8	51.2 ± 9	49.0 ± 11	40 ± 5	37 ± 8	39 ± 8
HCO_3	26.2 ± 3	27.1 ± 3	27.5 ± 4.5	25.9 ± 3.2	27.2 ± 3.1	27.2 ± 3.6	24 ± 3	24 ± 2.5	24 ± 2.5
Crs, ml/cm H_2O	39.2 ± 12*	33.1 ± 9.2‡	35.2 ± 11.2	43.4 ± 12*	41.3 ± 10.3§	42.9 ± 12.9§	58.3 ± 10	55.2 ± 9	58.1 ± 9
Pa_{O_2}/FI_{O_2}	129 ± 37*	95 ± 42‡	108 ± 45	148 ± 43*	155 ± 36§	142 ± 55§	346 ± 37	342 ± 40	351 ± 53
mABP, mm Hg	86 ± 2.8	82 ± 3.1	85 ± 2.5	94 ± 12	93 ± 12	99 ± 11	89 ± 2.3	84 ± 4.5	82 ± 6.1
HR, per min	88 ± 11	93 ± 7	95 ± 12	99 ± 17	84 ± 20	81 ± 16	82 ± 8	85 ± 16	95 ± 13
$\dot{Q}$, L/min	4.9 ± 0.7	5.4 ± 0.9	5.2 ± 0.8	5.5 ± 0.8	5.6 ± 0.9	5.3 ± 1.1	5.7 ± 1.1	5.6 ± 1.3	5.9 ± 1.4
$\overline{Ppa}$, mm Hg	26.3 ± 4.1*	29.1 ± 3.8	27 ± 3.5	27.8 ± 6.0*	26.8 ± 5.6	27.1 ± 5.5	19.6 ± 2.3	19.9 ± 3.1	19.2 ± 3.6
SVR, dyne·s·cm^{-5}	1,140 ± 184	1,029 ± 108	1,088 ± 123	1,265 ± 2,453	1,208 ± 123	1,349 ± 228	960 ± 195	908 ± 108	858 ± 108
PVR, dyne·s·cm^{-5}	258 ± 47*	321 ± 58‡	279 ± 69	291 ± 72*	281 ± 63	307 ± 58	131 ± 32	135 ± 39	141 ± 29

Definition of abbreviations: ARDS = acute respiratory distress syndrome; Crs = respiratory compliance; HCO_3 = bicarbonate; HR = heart rate; mABP = mean arterial blood pressure; PEEP = positive end-expiratory pressure; $\overline{Ppa}$ = mean pulmonary arterial pressure; Ppl = plateau pressure; PVR = pulmonary vascular resistance; $\dot{Q}$ = cardiac output; SVR = systemic vascular resistance.

* Significant difference ($p < 0.01$) compared with control group.

† Significant difference ($p < 0.05$) compared with control group.

‡ Significant difference ($p < 0.05$) compared with baseline.

§ Significant difference ($p < 0.05$) between ARDS groups.

TABLE 3. BIOCHEMICAL CHARACTERISTICS OF BRONCHOALVEOLAR LAVAGE FLUID

	ARDS-Lipids Group		ARDS-Placebo Group		Control Group	
	Before	After	Before	After	Before	After
Total protein, mg/ml	0.51 ± 0.19*	0.72 ± 0.28†	0.67 ± 0.44*	0.58 ± 0.21	0.29 ± 0.13	0.24 ± 0.07
PLA_2, nmol FA/h/ml	1.61 ± 0.33*	2.51 ± 1.0†	1.22 ± 0.24*	1.19 ± 0.45‡	0.30 ± 0.10	0.35 ± 0.12
PAF-AcH, nmol FA/h/ml	7.29 ± 2.74*	10.76 ± 3.9†	5.8 ± 3.6*	5.1 ± 2.1‡	1.72 ± 0.4	1.32 ± 0.48
PAF, pg PAF/9 ml BAL	59 ± 42*	238 ± 49§	86 ± 37*	82 ± 43‡	0	0
Total PL, μg P/ml BAL	1.13 ± 0.82*	1.62 ± 0.89†	1.42 ± 0.78*	1.38 ± 0.73	2.45 ± 0.57	2.51 ± 0.66
PC, μg P/ml BAL	0.53 ± 0.37*	0.91 ± 0.57†	0.59 ± 0.33*	0.55 ± 0.41‡	1.52 ± 0.45	1.52 ± 0.46
PC, % of total PL	47 ± 8*	55 ± 8†	42 ± 11*	43 ± 9‡	62 ± 8	60 ± 9
PE + PG, μg P/ml BAL	0.24 ± 0.17‖	0.25 ± 0.18	0.17 ± 0.15‖	0.18 ± 0.14	0.43 ± 0.15	0.44 ± 0.17
PE + PG, % of total PL	21 ± 4	15 ± 3†	13 ± 7‡	14 ± 5	18 ± 10	18 ± 8
SPH, μg P/ml BAL	0.16 ± 0.07	0.16 ± 0.07	0.21 ± 0.07	0.19 ± 0.07	0.10 ± 0.1	0.13 ± 0.1
SPH, % of total PL	14 ± 4*	10 ± 3	15 ± 7*	14 ± 5	5 ± 2	5 ± 2
Lyso-PC, μg P/ml BAL	0.03 ± 0.01*	0.065 ± 0.02§	0.04 ± 0.01*	0.03 ± 0.01	0.004 ± 0.002†	0.004 ± 0.002
Lyso-PC, % of total PL	2.6 ± 1.0*	4.0 ± 1.2†	2.9 ± 1.1*	3.0 ± 1.3‡	–	–
Others, μg P/ml BAL	0.17 ± 0.15‖	0.24 ± 0.10	0.22 ± 0.17‖	0.25 ± 0.14	0.40 ± 0.21	0.41 ± 0.25
Others, % of total PL	15 ± 10	15 ± 6	16 ± 13	19 ± 12	16 ± 8	16 ± 8

Definition of abbreviations: ARDS = acute respiratory distress syndrome; BAL = bronchoalveolar lavage; Lyso-PC = lyso-phosphatidylcholine; PAF = platelet-activating factor; PAF-AcH = PAF-acetylhydrolase; PC = phosphatidylcholine; PE= phosphatidylethanolamine; PG = prostaglandins; PL = phospholipids; PLA_2 = phospholipase A_2 = SPH = sphingomyelin.

* Significant difference ($p < 0.01$) compared with control group.

† Significant difference ($p < 0.05$) compared with baseline.

‡ Significant difference ($p < 0.05$) between ARDS groups.

§ Significant difference ($p < 0.01$) compared with baseline.

‖ Significant difference ($p < 0.05$) compared with control group.

After lipid administration, PC concentration increased in the ARDS-Lipid group. It is noteworthy that the increase of PC was positively correlated with that of total phospholipids (Figure 1). In ARDS-Placebo and control groups, there were no significant changes (Table 3).

The percentage and concentration of sphingomyelin and lyso-PC were higher in the ARDS groups compared with the control group. After lipid administration, the percentage and concentration of lyso-PC were elevated in the ARDS-Lipid group, whereas in the ARDS-Placebo and control groups, there were no such differences (Table 3).

PLA_2 and PAF-AcH Activities

PLA_2 activity was significantly higher in the ARDS groups compared with the control group. After lipid administration, a further increase in PLA_2 activity was observed, whereas no significant changes were found in the ARDS-Placebo and control groups (Table 3). The increase of PLA_2 activity after lipid administration was positively correlated with the concentration of lyso-PC (Figure 2).

PAF-AcH activity was higher in the ARDS groups compared with the control group and increased further after lipid administration. On the contrary, in ARDS-Placebo and control groups, there were no changes (Table 3). A significant correlation was found between the PAF-AcH activity and BAL protein concentration (Figure 3).

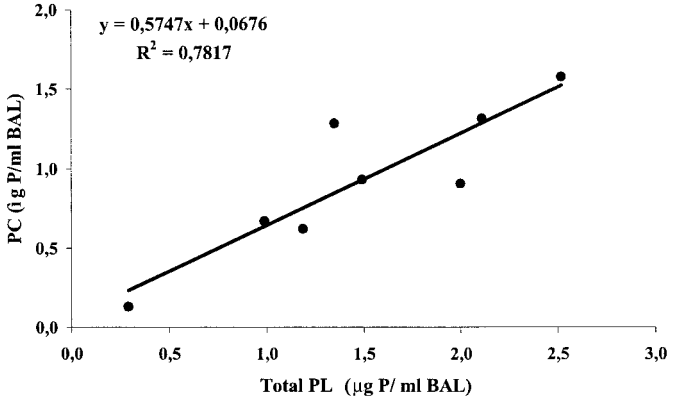

Figure 1. Relationship between total phospholipids (PL) and phosphatidylcholine (PC) in bronchoalveolar lavage fluid (BALF) ($r^2 = 0.78$, $p < 0.001$).

PAF Levels

The levels of PAF in BAL fluids from the ARDS-Lipid group were 59 ± 42 pg PAF/9 ml BAL and significantly increased after fat administration to 238 ± 49 pg PAF/9 ml BAL fluid ($p < 0.01$). In the ARDS-Placebo group, the levels of PAF were 86 ± 37 pg PAF/9 ml BAL, and they did not change after saline administration (Table 3). In contrast, the levels of PAF were not detectable in the control group.

BAL Cells

The percentage of neutrophils in BAL was significantly higher in the ARDS groups than in the control group. After lipid infusion, a further increase of BAL neutrophils was observed in the ARDS-Lipid group, whereas total BAL cell count was not affected by lipid infusion (Table 4). The percentage of cells stained by Sudan Black B was significantly higher in the ARDS-Lipid group after lipid infusion. The majority of black-staining cells were macrophages (90%), and some of them were neutrophils (Figure 4).

DISCUSSION

In this study, we have shown for the first time that intravenous administration of MCT/LCT to patients with ARDS caused BAL fluid alterations compatible with aggravation of alveolar–capillary membrane permeability, lung tissue inflammation, and lung surfactant abnormalities. The above had an impact on lung mechanics, PVR, and gas exchange. On the other hand, patients with normal lungs who were administered LCT/MCT or patients

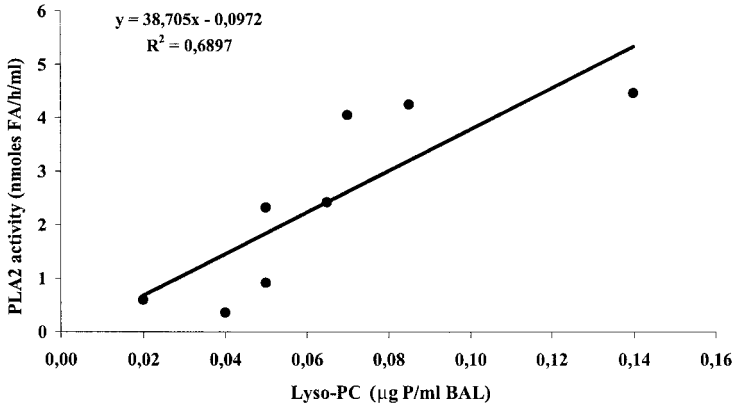

Figure 2. Relationship between lyso-phosphatidylcholine (Lyso-PC) and phospholipase A_2 (PLA_2) in BALF ($r^2 = 0.69$, $p < 0.01$).

with ARDS to whom placebo was administered did not demonstrate any significant changes.

Clinical abnormalities that follow LCT administration have been described by several investigators (13, 14, 22–27). In particular, the administration of LCT, especially at high rates, was reported to cause deterioration in gas exchange and an increase of PVR or even of pulmonary arterial pressure. These changes depended on the rate of fat infusion (22). An important observation of our study is that the rapid infusion of MCT/LCT mixture in patients with ARDS aggravated gas exchange and PVR in the same manner as did the rapid LCT infusion. Therefore, not only the quality of the administered lipid but also the clearance of lipids from the circulation might be an additional critical issue related to lung injury. This fact is supported by the results of Smirniotis and coworkers (27) who suggested that lower infusion rates of MCT/LCT to patients with sepsis and indirect ARDS did not result in deterioration of oxygenation or hemodynamics.

Our group of patients without ARDS did not manifest any clinical abnormalities after the rapid LCT/MCT administration. This indicates that inflammation and especially a preexisting increased permeability of the capillary–alveolar membrane might be a crucial factor for a further deterioration of ARDS caused by lipid infusion. Our data agree with previous studies that patients without ARDS receiving MCT/LCT did not develop any significant alteration in gas exchange or in hemodynamics (25, 26).

Suchner and coworkers (22) found decreased PVR and gas exchange deterioration during rapid fat administration, and this was attributed to an increased ratio of vasodilator (PGI_2)/vasoconstrictor (thromboxane A_2) mediators in plasma. However, in our study, we found increased PVR, which was consistent with the high levels of PAF in the BAL fluid, a locally acting lipid mediator causing vasoconstriction. The increased PAF levels also justify the gas exchange deterioration observed in our study. PAF could affect lungs by different mechanisms; in particular, it could generate interstitial edema (28), induce pulmonary vascular vasoconstriction and bronchoconstriction (29, 30), and finally, it could perpetuate inflammatory reactions (31). In contrast to PVR, systemic vascular resistance has the tendency to decrease. These findings are not inconsistent because direct ARDS is often accompanied by a systematic inflammatory response, which is associated with low resistance and high cardiac output.

The production of PAF within inflammatory cells is catalyzed by PLA_2. PLA_2 can affect pulmonary function directly by hydrolyzing lung surfactant phospholipids, thus increasing surface tension (32, 33). Therefore, the increased activity of PLA_2 in the BAL fluid of patients with ARDS administered LCT/MCT could represent an additional important factor in the aggravation of lung injury after intravenous lipid administration. Indirectly, PLA_2 could affect lung parenchyma through the production of biologically active molecules such as PAF, eicosanoids, and lyso-PC. PC, the main component of surfactant phospholipids, can produce the toxic agent lyso-PC through the action of PLA_2. After lipid administration, the concentration of total phospholipids, in particular that of PC, increased in our patients with ARDS. A possible reason for this increase might be a diffusion of fat emulsion, containing a significant amount of PC, through the more permeable alveolar membrane. The positive correlation of lyso-PC and PLA_2 in this study probably highlights another role of PLA_2 in the deterioration of lung injury through the production of lyso-PC.

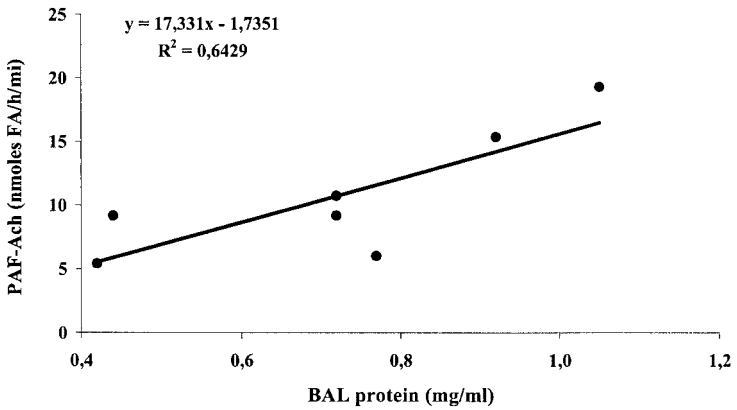

Figure 3. Relationship between total protein and platelet-activating factor–acetylhydrolase (PAF-AcH) in BALF. ($r^2 = 0.64$, $p < 0.01$).

TABLE 4. ABSOLUTE NUMBER AND DIFFERENTIAL CELL COUNT OF BRONCHOALVEOLAR LAVAGE

	ARDS-Lipids Group		ARDS-Placebo Group		Control Group	
	Before	After	Before	After	Before	After
Total cell count, $\times 10^3$/ml	290 ± 50*	320 ± 55	380 ± 90*	390 ± 80	230 ± 42	240 ± 46
Alveolar macrophages, %	45 ± 5†	39 ± 6	49 ± 12†	53 ± 11	70 ± 10	72 ± 11
Neutrophils, %	35 ± 4†	45 ± 5‡	30 ± 8†	31 ± 8§	14 ± 4	12 ± 3
Eosinophils, %	8 ± 2	7 ± 3	2 ± 2	4 ± 5	5 ± 2	5 ± 2
Lymphocytes, %	12 ± 2	9 ± 3	14 ± 6	7 ± 6	10 ± 3	11 ± 3
Sudan Black B staining, %	18 ± 8*	63 ± 16‡	11 ± 5*	9 ± 6§	8 ± 6	9 ± 5

Definition of abbreviation: ARDS = acute respiratory distress syndrome.
* Significant difference ($p < 0.05$) compared with control group.
† Significant difference ($p < 0.01$) compared with control group.
‡ Significant difference ($p < 0.01$) compared with baseline.
§ Significant difference ($p < 0.05$) between ARDS groups.

Besides PLA_2, PAF-AcH activity was also increased in our patients with ARDS after lipid administration. PAF-AcH from plasma, where high levels of the enzyme exist (12), may contaminate BAL fluid because of the increased permeability of the alveolar–capillary membrane. This interpretation is supported by a considerable positive correlation between BAL protein, a permeability marker, and PAF-AcH activity in BAL. Furthermore, if PAF-AcH were produced locally, we would expect the levels of PAF in BAL fluid to be lower than what we found. Thus, the concomitant increase of PAF-AcH and PAF in BAL fluid also supports the interpretation of BAL contamination, at least in part, with plasma PAF-AcH. It is noteworthy that PLA_2, which has a low plasma concentration, did not correlate with BAL protein.

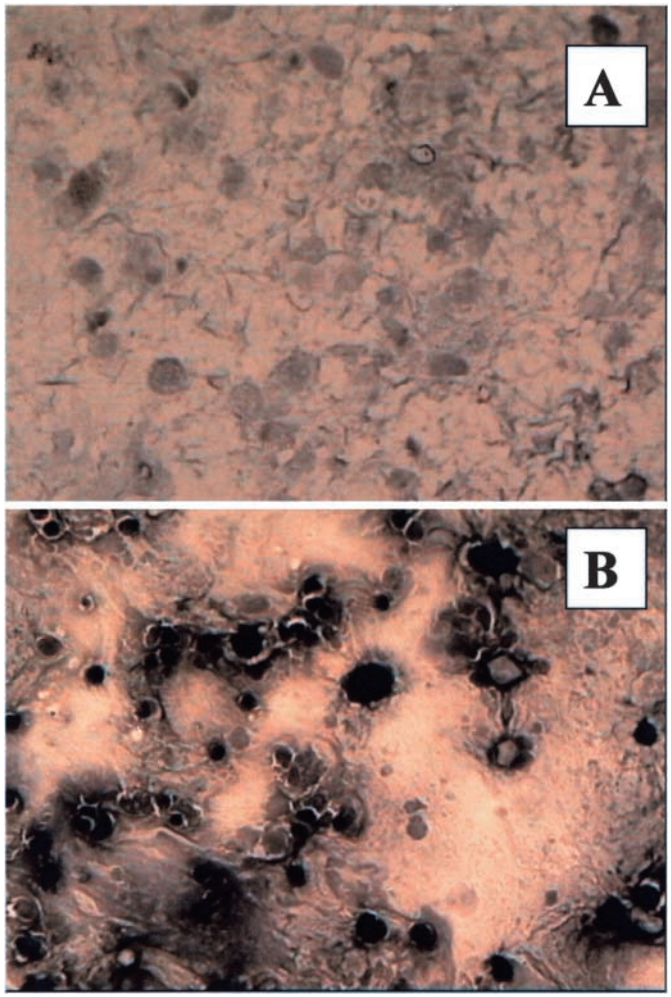

Figure 4. BALF cells stained by Sudan Black B from a representative of the acute respiratory distress syndrome (ARDS)-Lipid group. (*A*) Before and (*B*) after the parenteral administration of long-chain triglycerides/medium-chain triglycerides (LCT/MCT), approximately 80% of cells were stained, approximately 90% of the stained cells are macrophages, and approximately 10% neutrophils. Original magnification ×400.

In our study, BAL protein was used as a marker of alveolar–capillary membrane damage. The increased BAL protein in patients with ARDS after lipid administration implies a further deterioration of alveolar–capillary barrier properties. Apart from this, the increased concentration of protein in the alveolar space is known to disturb the biophysical properties of lung surfactant (5).

It has been reported previously that intravenous administration of triglycerides, which are the main components of the LCT/MCT emulsion, increases pulmonary vascular permeability in laboratory animal models through the activation of neutrophils (34, 35). Activated neutrophils and macrophages release cytotoxic agents, such as PAF and PLA_2. PAF binding to cell surface receptors could regulate recruitment, further activation, and adhesion of neutrophils (36), leading thus to a perpetuation of inflammation.

Another interesting finding of our study was the fact that a high percentage of BAL cells, mainly macrophages from our patients with ARDS, contained lipid droplets after lipid administration, as indicated by the Sudan Black B stain. This picture is consistent with the fat embolism syndrome (37). Therefore, LCT/MCT administration could induce a fat embolism–like syndrome as concerns the lung involvement. This point of view is supported by a case report on fat embolism–like syndrome occurring during lipid-rich parenteral nutrition (38).

Alveolar macrophages are the main scavengers in lung tissue and phagocytose the lipid particles. Therefore, the Sudan Black–stained macrophages in our patients with ARDS are possibly alveolar macrophages, which have phagocytosed lipid passing from the circulation through the permeable alveolar–capillary membrane, or even damaged surfactant. However, it has been reported elsewhere that the early expansion of the macrophage population observed in acute lung injury is probably due to an influx of monocytes from the vascular component (39). Therefore, we cannot exclude the possibility that a small number of lipid-containing macrophages retrieved in BAL could derive from the circulating monocytes.

In conclusion, this study indicates that the administration of

MCT/LCT to patients with early direct ARDS causes alterations in lung function and hemodynamics. Inflammatory cells and PLA_2 possibly play a role in deteriorating lung injury. Neutrophils or macrophages activated by lipids in the intracapillary or alveolar space release enzymes and inflammatory mediators, such as PLA_2 and PAF. These enhance the alveolar–capillary membrane permeability, tissue inflammation, and surfactant damage. The preexisting inflammation probably is required for the development of lung injury by lipids.

Conflict of Interest Statement: M.E.L. has no declared conflict of interest; S.L. has no declared conflict of interest; C.N. has no declared conflict of interest; V.G. has no declared conflict of interest; G.N. has no declared conflict of interest.

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