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Parenteral Administration of Medium- but Not Long-Chain Lipid Emulsions May Increase the Risk for Infections by *Candida albicans*

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Intravenous administration to volunteers of an emulsion of medium-chain lipids, but not of an emulsion of pure long-chain lipids or a placebo, increased the growth of *Candida albicans* in serum and modulated *Candida*-induced cytokine production by mononuclear cells in a way suggesting that medium-chain, but not long-chain, triglycerides increase the risk for infections by *Candida*.

The provision of total parenteral nutrition (TPN) is an indispensable strategy to improve the nutritional status of critically ill patients. However, altered immune responses by the TPN lipid component may contribute to the increased rate of infectious complications for these patients (43). It remains unclear whether structurally different lipid emulsions containing either pure long-chain triglycerides (LCT) or mixed long- and medium-chain triglycerides (LCT-MCT) exert distinct immune-modulating effects (1, 3, 4, 7, 8, 10, 12–15, 18, 25, 27, 28, 32, 33, 35–37, 40, 42, 43, 44). Recently, we found that LCT-MCT, unlike LCT, increase in vitro oxygen radical production and adhesion of neutrophils but decrease cellular motility and killing of *Candida albicans* (20, 45–48). This observation is important because clinical studies indicate that 5% of all patients receiving TPN develop candidemia, with significant mortality and morbidity (38).

In the present study, we exposed LCT and LCT-MCT to the metabolisms and immune systems of healthy volunteers. We investigated the effects of lipids on two pathogenetic aspects of *Candida* infections: yeast growth and the balance of proinflammatory (gamma interferon [γ-IFN], tumor necrosis factor alpha [TNF-α], interleukin-1β [IL-1β], and IL-6) and anti-inflammatory (IL-10) cytokines (2, 19, 29, 30, 34, 41).

Emulsions containing LCT, LCT-MCT, or saline were administered during 4 h to eight volunteers in a study with a crossover design and a 1-week washout period. Blood samples were taken before and after 4 h of lipid or placebo administration and analyzed as described below. In order to stabilize plasma triglyceride concentrations at a clinically relevant concentration of 3 to 5 mmol/liter, emulsions (overall, ca. 220 ml) were infused according to a triglyceride-clamp schedule (16, 17). For emulsion characteristics, see Table 1.

Leukocytes were isolated from 20 ml of blood anticoagulated with lithium-heparin (6, 21, 45). Peripheral blood mononuclear cells (PBMC) were removed and suspended in medium (RPMI 1640 DM; Flow Laboratories, Irvine, United Kingdom). Heat-killed *C. albicans* (strain UC820; final concentration, 10^7 CFU/ml) was used for ex vivo PBMC stimulation in the cytokine assays. After PBMC isolation, cell numbers were adjusted (5×10^6/ml) and cell suspension samples were incubated with *Candida* (24 h, 37°C). After incubation, the supernatants were frozen (−20°C) until assayed. IL-1β and TNF-α (both in nanograms per milliliter) in supernatants were measured by radioimmunoassays as described previously (23). Detection limits of the assay were 20 pg/ml for TNF-α and 40 pg/ml for IL-1β. Interassay variation was less than 15%, and intra-assay variation was less than 10%. IL-6, IL-8, IL-10, and IFN-γ concentrations were determined in duplicate with commercially available enzyme-linked immunosorbent assay kits (Pelikine Compact human enzyme-linked immunosorbent assay; CLB, Amsterdam, The Netherlands).

After inoculation and overnight culturing, *C. albicans* was suspended at 10^6 CFU/ml. The *Candida* suspension was incubated (24 h, 37°C) with serum samples and Sabouraud medium. After incubation, samples were plated onto Sabouraud

TABLE 1. Characteristics of lipid emulsions according to manufacturers

Component or characteristic	LCT	LCT-MCT
Fractionated soybean oil (g/liter)	200	100
MCT (g/liter)	0	100
Fatty acids (% [wt/wt] of total)		
Caproic acid ($C_{6:0}$)		0.5
Caprylic acid ($C_{8:0}$)		28.5
Capric acid ($C_{10:0}$)		20
Lauric acid ($C_{12:0}$)		1
Palmitic acid ($C_{16:0}$)	9	6.5
Stearic acid ($C_{18:0}$)	5	2
Oleic acid ($C_{18:1}$)	25	11
Linoleic cid ($C_{18:2}$)	55	26
Linolenic acid ($C_{18:3}$)	8	4
Arachidonic acid ($C_{20:4}$)	1	0.5
Mean molecular weight of triglycerides	865	634
Fractionated egg phospholipids (g/liter)	12	12
Glycerol (g/liter)	22.5	25
pH	8.0	8.0

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TABLE 2. Effects of lipid administration on triglyceride concentrations

Infusion	Triglyceride concn (25th, 75th percentiles)[a]		
	Before infusion	After infusion	After − before infusion
Placebo	1.48 (1.04, 1.94)	1.06 (0.70, 1.74)	−0.11 (−0.50, 0.10)
LCT	0.97 (0.79, 1.46)	3.15 (2.64, 3.89)[b]	1.67 (0.96, 2.36)[c]
LCT-MCT	1.28 (0.80, 1.44)	3.54 (2.24, 4.47)[b]	2.43 (1.41, 5.04)[c]

[a] Triglyceride concentrations (in millimoles per liter) in subjects before and after a 4-h infusion of lipids or the placebo.
[b] Significant change versus values for the preinfusion concentration.
[c] Significant change versus values for the preinfusion concentration and the concentration with the placebo.

agar plates and incubated (29°C) for 8 and 24 h. The colonies were counted (numbers of CFU per milliliter), and growth rates were expressed as ratios of numbers of CFU in samples after lipid administration to those before lipid administration.

Results are expressed as medians (with 25th and 75th percentiles). The statistical significance of treatment effects was determined by analysis of variance with Bonferroni correction for multiple comparisons and by Tukey's posttest.

Infusion of LCT and LCT-MCT equally increased triglyceride concentrations (Table 2). However, ex vivo cytokine production by PBMC was distinctly influenced by lipid treatment (Table 3). *Candida*-induced production of TNF-α, IL-1β, and IL-10 increased after LCT-MCT exposure. Compared with the placebo, LCT showed no effect, although this might be due to the sample size. *Candida*-induced IFN-γ production tended to decrease, but values did not reach statistical significance. Infusion of LCT or placebo, with this limited sample size, did not influence the production of any cytokine. With LCT-MCT, we observed a significantly increased rate of growth of *Candida* after 8 and 24 h compared with rates with the placebo and LCT (Fig. 1).

Infections by *C. albicans* pose a threat to the use of NADP (9, 38). Outcomes probably depend on yeast growth rates as well as counteractive responses of the innate and adaptive immune systems (30, 31). It appears that the balance of pro- and anti-inflammatory cytokines is altered by LCT-MCT in a way that is known to deactivate innate immunity (30, 31). Also, our results indicate that LCT-MCT, but not LCT, favors the development of *Candida* infections by enhancing yeast growth rates. Microscopic evaluation (data not shown) revealed the formation of pseudohyphae after lipid administration, indicating that growth rates are underestimated even in our experimental setting. Importantly, inhibitory effects of serum on yeast growth due to iron deprivation were ruled out, as addition of $FeCl_3$ (10 μmol/liter) to serum did not affect test results (data not shown).

Our ex vivo findings support in vitro work where LCT-MCT, but not LCT, impaired neutrophil killing of *Candida* (48). Previous in vitro studies with LCT showed that *Candida* grows better in a lipid-rich environment (5, 11, 24, 36). We did not find a growth-enhancing effect on *Candida* for parenteral LCT, suggesting that with its metabolic breakdown, the effects of LCT on candidal growth disappear, in contrast with what occurs with MCT.

The relative importance of the findings of increased levels of TNF-α, IL-1β, and (to a lesser extent) IL-8 production with MCT, which could be considered protective, remains unclear and probably can be evaluated only in a clinical study.

The altered balance of *Candida*-induced cytokine production by PBMC, with increased production of IL-10 (by Th2 lymphocytes) and unchanged or decreased production of IFN-γ (by Th1 lymphocytes), results in a decreased IFN-γ/IL-10 ratio. Such an imbalance in Th1 and Th2 responses is considered a major risk factor for the development of fungal infections (30, 34, 41). On the other hand, it is also possible that the IL-10 measured in our experiments was produced by monocytes.

IFN-γ activates phagocytic cells to kill *Candida*, whereas IL-10 has been shown to inhibit proinflammatory cytokine production and to aggravate the course of disseminated candidiasis (22, 26, 39). The influence of LCT-MCT on the production of IFN-γ (decreased production) and monokines (increased production), in combination with the lack of effects of LCT, suggests that MCT have differential effects on T cells and

TABLE 3. Effects of lipid administration on cytokine production by PBMC

Stimulus	Cytokine	Concn (mmol/liter) (25th, 75th percentiles) with[a]:			ANOVA[b] result
		Placebo	LCT	LCT-MCT	
Candida	IFN-γ	1.01 (0.41, 1.66)	1.00 (0.76, 1.24)	0.38 (0.12, 0.71)	0.11
	IL-10	0.82 (0.58, 0.96)	0.76 (0.61, 0.95)	1.87 (1.08, 2.90)[c,d]	0.03
	TNF-α	0.69 (0.38, 0.96)	1.24 (0.96, 1.53)	2.01 (1.49, 5.34)[c]	0.04
	IL-1β	0.64 (0.44, 0.78)	1.07 (0.78, 1.21)	1.75 (1.42, 2.58)[c,d]	0.01
	IL-6	0.74 (0.54, 1.36)	0.81 (0.60, 1.28)	1.86 (0.94, 3.74)	0.16
	IL-8	0.83 (0.71, 0.97)	1.09 (0.93, 1.24)	1.35 (0.87, 1.82)	0.38

[a] Effects of lipids or a placebo on the ratio of cytokine production by PBMC before the infusion to that after the infusion.
[b] ANOVA, analysis of variance.
[c] , significant effect versus the effect with the placebo.
[d] , significant effect of LCT-MCT versus the effect with LCT.

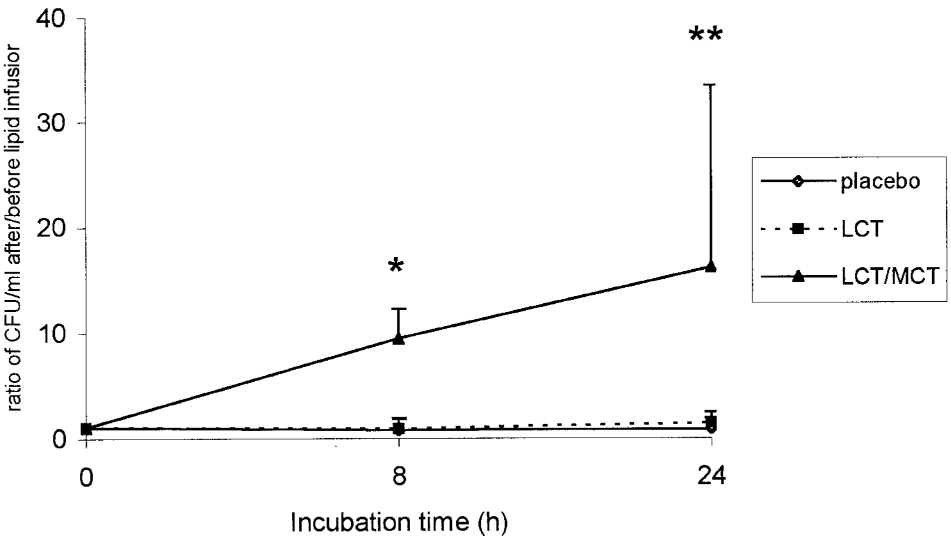

FIG. 1. Effects of lipid emulsions or the placebo after 8- and 24-h incubations on the growth of samples of *C. albicans* in cell-free serum. Results are ratios of growth obtained after lipid infusion to growth obtained before lipid infusion. *, significance ($P \leq 0.01$) of treatment effects relative to results with the placebo and LCT; **, significance ($P \leq 0.02$) of treatment effects relative to results with the placebo and LCT.

monocytes. LCT exerted no effects on cytokine production compared with the placebo, making it improbable that components other than the lipids, e.g., an emulsifier and antioxidants, are responsible for the emulsion effects. Finally, it has to be kept in mind that definite proof for the effects of lipids on the susceptibility to fungal infections of humans can be obtained only in large-scale clinical studies.

In conclusion, the results of our study suggest that parenteral MCT, contrary to pure LCT, increase susceptibility to infections with *C. albicans* by increasing candidal growth rates and by having a detrimental effect on antifungal immune responses.

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