Prompt inhibition of fMLP-induced Ca^{2+} mobilization by parenteral lipid emulsions in human neutrophils

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Abstract **It remains unclear whether modulation of immune system functions by lipids contributes to the increased infection rate observed in patients treated with parenteral nutrition. We therefore evaluated the effects of lipid emulsions derived from fish oil [very long chain triglycerides (VLCT)], olive oil [long-chain triglycerides- mono-unsaturated fatty acid (LCT-MUFA)], soya oil [long-chain triglycerides (LCT)], or a physical mixture of coconut and soya oil [mixed long- and medium-chain triglycerides (LCT-MCT)] on neutrophil activation. *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) evoked an immediate increase of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{i,av}$) in a suspension of neutrophils. When added 3 min before fMLP, however, all four lipid emulsions reduced the hormone-induced increase in $[Ca^{2+}]_{i,av}$ with the same efficacy but with different potency. Half-maximal inhibition was reached at emulsion concentrations of 0.24 mM VLCT, 0.32 mM LCT-MCT, 0.52 mM LCT, and 0.82 mM LCT-MUFA. Similarly to the lipids, the protein kinase C (PKC) activator PMA markedly reduced the fMLP-induced increase in $[Ca^{2+}]_{i,av}$. PMA inhibition was abolished by the PKC inhibitor staurosporine. In contrast, however, this drug did not interfere with the inhibitory lipid effect, indicating that the lipids act primarily in a PKC-independent manner.** **In summary, this study shows that nutritional lipids can evoke a prompt and significant attenuation of hormone-induced neutrophil stimulation and that the emulsions based on fish oil and a mixture of coconut oil and soya oil are among the most potent ones in this respect.**—Wanten, G., A. Rops, S. E. van Emst-de Vries, T. Naber, and P. H. G. M. Willems. **Prompt inhibition of fMLP-induced Ca^{2+} mobilization by parenteral lipid emulsions in human neutrophils.** ***J. Lipid Res.*** **2002.** 43: **550–556.**

Supplementary key words immune response • lipids • nutrition • emulsion • calcium signaling

Total parenteral nutrition (TPN) implies that all nutritional requirements are met by the administration of an intravenous solution. In the last decades, this technique has established itself as an indispensable strategy to improve the nutritional status of critically ill patients. Unfortunately, the associated risk for infectious complications remains a drawback in which immune-suppressive effects of the lipid component seem to play a role (1–3).

Thus far, the immune-modulatory properties of the most widely used emulsion, containing long-chain triglycerides (LCT), remain unclear (4–13). The data on structurally different emulsions, containing physical mixtures of LCT and medium-chain triglycerides (LCT-MCT) or synthetic structured lipids (SL, with long- and medium-chain fatty acids attached to a glycerol molecule) are limited (14).

We recently observed distinct effects of emulsions containing LCT, LCT-MCT, or structured lipids (SL) on various neutrophil functions (15–18). It was found that MCT, in a for TPN patients physiological triglyceride concentration range (up to 10 mM) directly activate neutrophils, in contrast with LCT or SL. We also found that treatment of neutrophils with MCT mimicked the effects of 12-*O*-tetradecanoylphorbol 13-acetate (PMA), a phorbol ester and potent activator of protein kinase C (PKC), on the increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{i,av}$) evoked by serum-treated zymosan particles (STZ) (19). Here MCT, but not LCT or SL, caused a leftward shift of the dose-response curve for the effect of STZ on $[Ca^{2+}]_{i,av}$, indicating that structurally different lipids distinctively modulate neutrophil activation. We also observed that each emulsion markedly reduced the increase in $[Ca^{2+}]_{i,av}$ in response to *N*-formyl-methionyl-leucyl-phenylalanine (fMLP).

In the present study, we investigate the effects on fMLP-induced Ca^{2+} mobilization in neutrophils of two recently developed lipid emulsions. One is based on fish oil [long-chain triglycerides (VLCT)], rich in very long-chain poly-unsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid; the other is an LCT emulsion based on olive oil [long-chain triglycerides- mono-unsaturated fatty acid (LCT-MUFA)], rich in mono-unsaturated fatty acids,

Abbreviations: CT, long-chain triglycerides; LCT-MCT, mixed long- and medium-chain triglycerides; SL, structured lipids; MUFA, mono-unsaturated fatty acid; STZ, serum-treated zymosan.

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oleic acid, and linoleic acid. Thus far, a wide range of effects on neutrophil function of various fatty acids have been described, ranging from cell activation to inhibited immunological responses (20–25).

MATERIALS AND METHODS

Blood samples were drawn from healthy volunteers and processed as described below. Serum triglyceride content of these samples was always in the normal range (0.55–1.56 mM l^{-1}), as determined colorimetrically on a Hitachi 747 analyser (Hitachi Ltd., Tokyo, Japan).

Materials

fMLP and 12-*O*-tetradecanoylphorbol 13-acetate PMA were purchased from Sigma Diagnostics (St. Louis, MO). Staurosporine was obtained from Boehringer (Mannheim, Gemany); 4-α-phorbol 12-myristate 13-acetate from LC Services Corporation (Woburn, MA); human serum albumin (HSA) from Behring (Westwood, MA); and Fura-2/AM from Molecular Probes Inc. (Eugene, OR). All other chemicals were of analytical grade. PMA was stored as a 10^{-3} M stock in DMSO at −20°C. fMLP was stored as a 10^{-3} M stock in DMSO at −20°C. HBSS was from Life Technologies (Paisley, Scotland). PBS contained Na^+ 163.9 mM, Cl^- 140.3 mM, HPO_4^{2-}10.9 mM, and H_2PO_{4-} 1.8 mM (pH 7.4). Isotonic lysis solution contained 155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA (pH 7.4). Incubation medium contained HBSS supplemented with 0.5% (w/v) HSA. Percoll (ρ 1.129 g/ml at 20°C) was from Pharmacia Biotech AB (Uppsala, Sweden). LCT emulsion (Intralipid 20%, w/v) was from Pharmacia & Upjohn AB (Stockholm, Sweden). LCT-MCT (Lipofundin 20%, w/v) was from B. Braun Melsungen AG (Melsungen, Germany). Fish oil emulsion (Omegaven 10%, w/v) was from Fresenius (Bad Homburg, Germany), and olive oil emulson (ClinOleic 20%, w/v) was provided by Baxter SA (Lessines, France). For lipid emulsion characteristics see **Tables 1** and **2**. Blood samples were collected in 10 ml Monoject tubes (Sherwood Medical, Ballymoney, N. Ireland) with 143 USP units of lithium heparin.

Cell isolation

Neutrophils were purified from blood anticoagulated with lithium heparin (26). The blood, diluted 1:1 with PBS with 0.4% (w/v) trisodium citrate (pH 7.4), was placed on Percoll (ρ 1.076 g/ml) and centrifuged (700 × *g*, 18 min, 25°C). The pellet was suspended in 50 ml of ice-cold lysis solution for 10 min. After centrifugation (5 min, 400 × *g*, 4°C), the remaining erythrocytes were lysed in fresh lysis solution for another 5 min. Subsequently the cells were washed and resuspended in incubation medium to a final concentration of 2×10^6 cells/ml and kept at room temperature. Cytospin preparations were >97% pure and >99% viable as determined by May-Grünwald-Giemsa and trypan blue staining.

Fluorescence measurements in suspensions of human neutrophils

Neutrophils (2×10^6 cells/ml) were loaded with 5 μM Fura-2/AM for 30 min at 37°C. Excess Fura-2/AM was removed by washing the neutrophils twice with HBSS/0.5% (w/v) HSA and the neutrophils were transferred to a cuvette placed in a Shimadzu RF-5301 spectrofluorophotometer equipped with a magnetic stirrer and a thermostated cuvette holder. The fluorescence emission ratio at 490 nm was monitored as a measure of the average cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{i,av}$) after excitation at 340 and 380 nm.

Statistical analysis

Half-maximal inhibitory emulsion concentrations were calculated by means of the nonlinear regression computer program OriginPro 6.1 (OriginLab Corporation, Northampton, MA). In all experiments, the data are expressed as the mean ± SEM. Overall statistical significance was determined by ANOVA. In the case of significance ($P < 0.05$), individual groups were compared

TABLE 1. Composition and characteristics of the different lipid emulsions

	LCT-MCT	VLCT	LCT-MUFA	LCT
Triglyceride Source	Coconut Oil/ Soya Oil (1:1)	Fish Oil	Olive Oil	Soya Oil
Fatty acid (% w/w of total)				
Caproic acid (C6:0)	0.2	-	-	-
Caprylic acid (C8:0)	21.2	-	-	-
Capric acid (C10:0)	19.9	-	-	-
Lauric acid (C12:0)	0.2	-	-	-
Myristic acid (C14:0)	-	5.1	0.2	-
Pentadecanoic acid (C15:0)	-	0.7	-	-
Palmitic acid (C16:0)	7.4	11.7	12.2	11.3
Palmitoleic acid (C16:1)	-	9.2	1.4	-
C16:3 acid	-	2.7	0.3	-
C16:4 acid	-	2.6	0.5	-
Stearic acid (C18:0)	3.2	4.4	2.1	4.5
Oleic acid (C18:1)	14.2	15.1	62.3	21.0
Linoleic acid (C18:2)	29.1	4.4	18.7	53.4
Linolenic acid (C18:3)	4.5	1.8	2.3	9.7
Octadecatetraenoic acid (C18:4)	-	3.7	-	-
Eicosenoic acid (C20:1)	-	1.6	-	-
Dihomo-γ-linolenic acid (C20:3)	-	0.6	-	-
Arachidonic acid (C20:4)	0.2	2.1	-	0.1
Eicosapentaenoic acid (C20:5n-3)	-	19.2	-	-
Heneicosanoic acid (C21:5)	-	1.0	-	-
Docosapentaenoic acid (C22:5)	-	2.1	-	-
Docosahexaenoic acid (C22:6n-3)	-	12.1	-	-

by contrast analysis according to Fisher. *P* values of less than 0.05 were considered significant.

RESULTS

fMLP-induced Ca^{2+} mobilization in human neutrophils

We have previously shown that fMLP induces a transient rise of the fluorescence emission ratio in a suspension of fura-2-loaded human neutrophils (19). This rise, which reflects the effect of fMLP on the average cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{i,av}$), is the result of the stimulated release of Ca^{2+} from the endoplasmic reticulum. To be able to detect possible stimulatory and/or inhibitory effects of the lipid emulsions on fMLP-induced Ca^{2+} mobilization, we first determined the fMLP concentration that caused half-maximal Ca^{2+} mobilization. **Figure 1** shows that fMLP dose dependently increased the peak value of the $[Ca^{2+}]_{i,av}$ transient. Maximal and half-maximal fMLP concentrations were calculated to be 100 nM and 1 nM, respectively. No increase in $[Ca^{2+}]_{i,av}$ was observed following stimulation with 0.1 nM fMLP.

Fish oil and Lipofundin inhibit fMLP-induced Ca^{2+} mobilization more potently than olive oil and Intralipid in human neutrophils

Pretreatment of the cells with 0.1 mM fish oil caused a significant reduction of the increase in $[Ca^{2+}]_{i,av}$ in response to 1 nM fMLP (**Fig. 2A**). On the other hand, Fig. 2B shows that fMLP-induced Ca^{2+} signaling was hardly affected in cells pretreated with the same concentration of olive oil.

In a previous study, we showed that both an emulsion of pure LCT as well as a mixture of LCT and MCT (LCT-MCT) caused inhibition of fMLP-induced Ca^{2+} mobilization in human neutrophils (19). The initial finding that 0.1 mM VLCT markedly inhibited the stimulatory effect of fMLP, whereas the same concentration of LCT-MUFA was

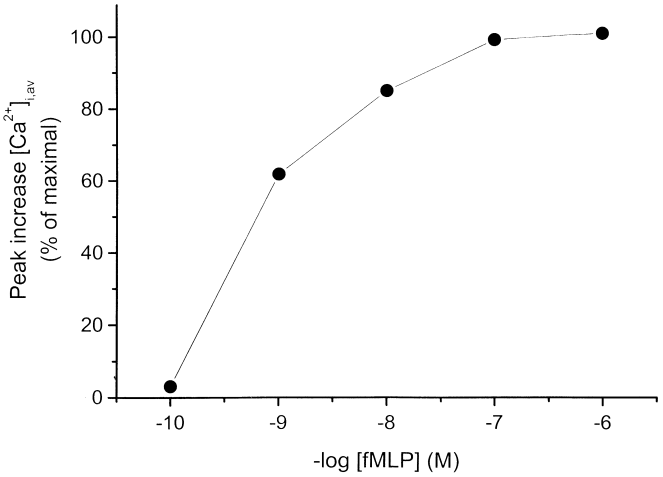

Fig. 1. Dose-response curve for the *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced peak increase in $[Ca^{2+}]_{i,av}$ in a suspension of human neutrophils. Human neutrophils, loaded with the fluorescent Ca^{2+} indicator Fura-2, were transferred to a cuvette placed in a Shimadzu RF-5301 spectrofluorophotometer equipped with a magnetic stirrer and a thermostated (37°C) cuvette holder. The cells were preincubated for 3 min and then stimulated with the indicated concentration of fMLP. The fluorescence emission ratio at 490 nm was monitored as a measure of the average cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{i,av}$) after excitation at 340 and 380 nm. The peak increase in $[Ca^{2+}]_{i,av}$ obtained with 1 μM fMLP is set at 100%, to which all other values are related. The data presented are from a single experiment.

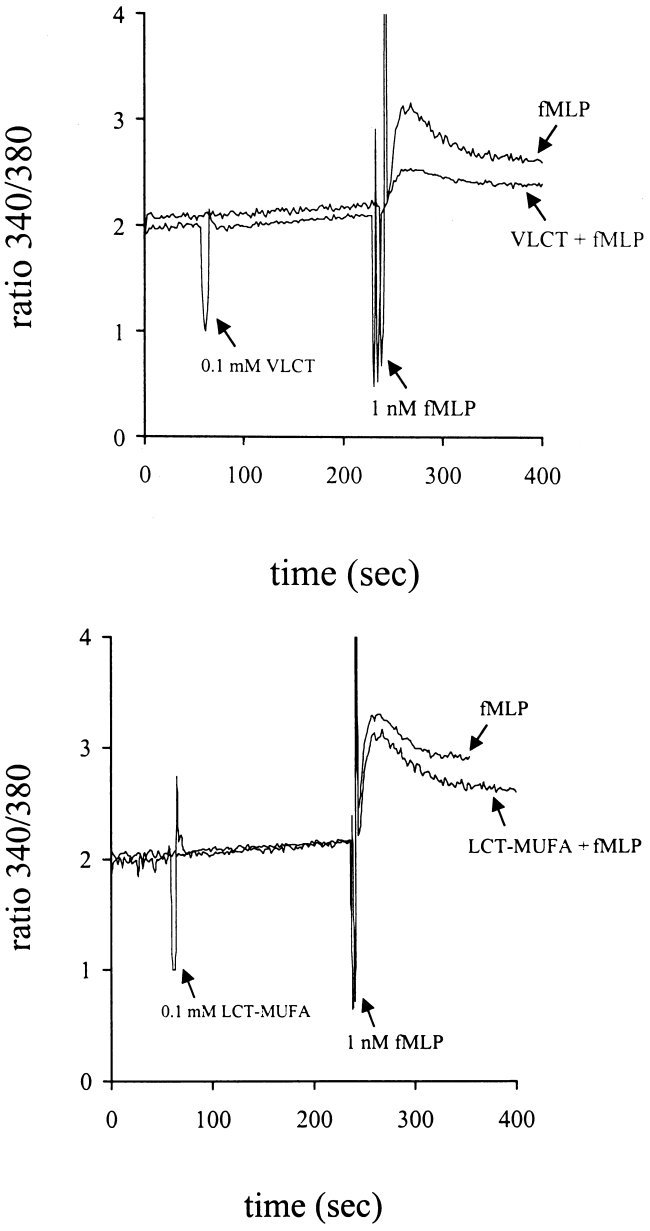

Fig. 2. Effects of the nutritional lipid emulsions very long-chain triglycerides [VLCT (fish oil)] and very long-chain triglycerides-mono-unsaturated fatty acid [LCT-MUFA (olive oil)] on the fMLP-induced increase in $[Ca^{2+}]_{i,av}$ in a suspension of human neutrophils. Fura-2-loaded neutrophils were preincubated with either VLCT (0.1 mM) (A) or LCT-MUFA (0.1 mM) (B) in a medium without serum albumin for 3 min and subsequently stimulated with fMLP (1 nM). Details on $[Ca^{2+}]_{i,av}$ measurement are given in the legend to Fig. 1.

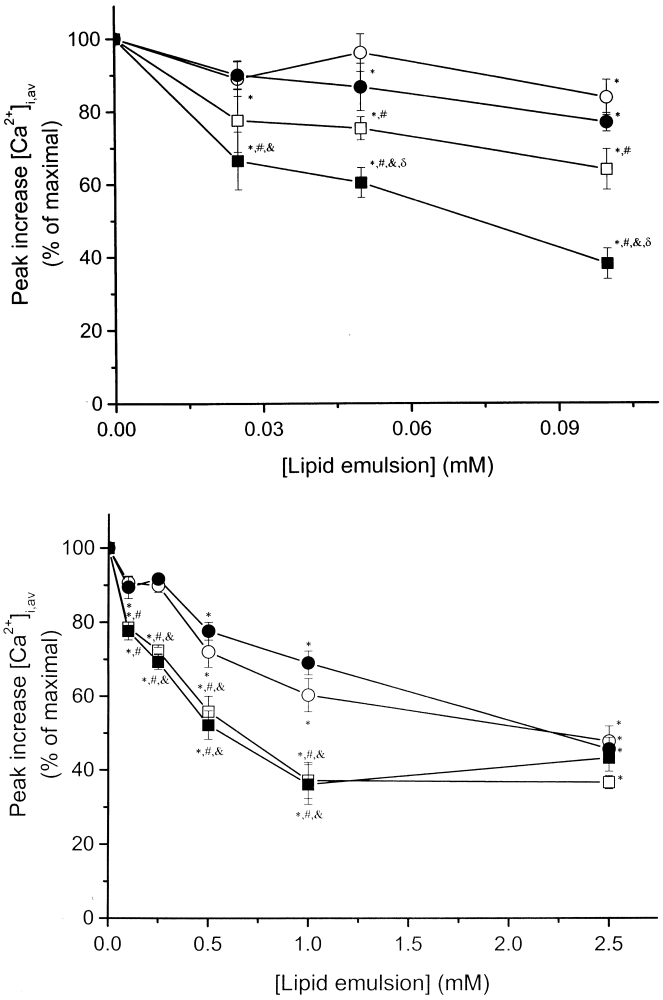

Fig. 3. Dose dependence of the effects of VLCT, LCT-MUFA, long-chain triglycerides [LCT (soya oil)], and mixed long- and medium-chain triglycerides [LCT-MCT (mixture of coconut oil and soya oil)] on the fMLP-induced peak increase in $[Ca^{2+}]_{i,av}$ in the absence (A) and presence (B) of serum albumin. Neutrophils loaded with Fura-2 were preincubated in the presence of the indicated concentrations of VLCT (closed squares), LCT-MCT (open squares), LCT (closed circles) and LCT-MUFA (open circles) in a medium without (A) or with (B) serum albumin for 3 min, after which the cells were stimulated with 1 nM fMLP. $[Ca^{2+}]_{i,av}$ was monitored as described in the legend to Fig. 1. The peak increase in $[Ca^{2+}]_{i,av}$ obtained in the absence of lipid emulsion (untreated control) is set at 100%, to which all other values are related. The data represent mean ± SEM of at least four measurements. *Significantly different from untreated control ($P < 0.01$); #Significantly different from corresponding LCT-MUFA ($P < 0.01$); &Significantly different from corresponding LCT ($P < 0.01$); δSignificantly different from corresponding LCT-MCT ($P < 0.01$).

virtually without effect, urged us to construct dose-inhibition curves for the four different lipid emulsions. **Figure 3A** shows that, in the absence of HSA in the medium, VLCT inhibited the fMLP-induced $[Ca^{2+}]_{i,av}$ rise much more efficaciously than the other three lipid emulsions. At a concentration of 0.1 mM, VLCT, LCT-MCT, LCT-MUFA, and LCT inhibited the effect of fMLP by 60%, 35%, 20%, and 15%, respectively. In all four cases, the effect was statistically significant. At a lower concentration of 0.025 mM, however, only VLCT and LCT-MCT caused a significant inhibition of the fMLP-induced $[Ca^{2+}]_{i,av}$ rise by 35% and 20%, respectively. Nonlinear curve fitting revealed that 0.031 mM VLCT was required for half-maximal inhibition of the response to fMLP.

In the presence of 0.5% (w/v) HSA, the inhibitory effect of 0.1 mM VLCT was significantly lowered to no more than 20% (Fig. 3B). At the same concentration, however, the inhibitory effect of the other three lipid emulsions was only slightly reduced. All four lipid emulsions maximally inhibited the fMLP response by 55% on average. With VLCT and LCT-MCT this value was reached at a concentration of 1 mM, whereas with LCT-MUFA and LCT, a concentration of 2.5 mM was required to obtain the maximal effect. Half-maximal inhibition was calculated to be reached at 0.24 mM (VLCT), 0.32 mM (LCT-MCT), 0.52 mM (LCT), and 0.82 mM (LCT-MUFA). These values show that LCT-MCT and VLCT inhibit fMLP-induced Ca^{2+} mobilization 1.6 to 3.4 times more potently than LCT and LCT-MUFA in human neutrophils incubated in the presence of serum albumin.

PKC is not involved in inhibition of fMLP-induced Ca^{2+} mobilization by lipid emulsions in human neutrophils

Phorbol esters, such as PMA, are widely used to study the role of PKC. Pretreatment of the neutrophils with PMA resulted in complete inhibition of the increase in $[Ca^{2+}]_{i,av}$ in response to 10 nM fMLP (**Fig. 4A**). This inhibitory effect of PMA was abolished by the putative PKC inhibitor staurosporine. These findings are indicative of the potential of PKC to function as an internal regulator of fMLP-induced transmembrane signaling. In contrast to PMA, the inactive phorbol ester 4-α-phorbol 12-myristate 13-acetate did not change the response to fMLP (data not shown). Figure 4B shows that staurosporine was unable to reverse the inhibitory effect of LCT-MCT (2.5 mM). A similar observation was reached with the other three lipid emulsions (data not shown). This demonstrates that the lipid emulsions, when added at a clinically relevant concentration of 2.5 mM, do not exert their inhibitory effect through PKC.

DISCUSSION

Clinical and experimental studies suggest that altered immune responses by lipids contribute to the increased rate of infectious complications in patients treated with intravenous (parenteral) nutrition (1–3). On the other hand, modulation of inflammatory responses by nutritional lipids offers therapeutic strategies in the treatment of various diseases, ranging from vasculopathy to inflammatory bowel disease and cancer (27–30). However, our understanding of the underlying mechanisms behind these observations remains limited.

The present work shows that short-term (3 min) treatment with nutritional lipid emulsions can markedly affect the activation of human neutrophils with the bacterial

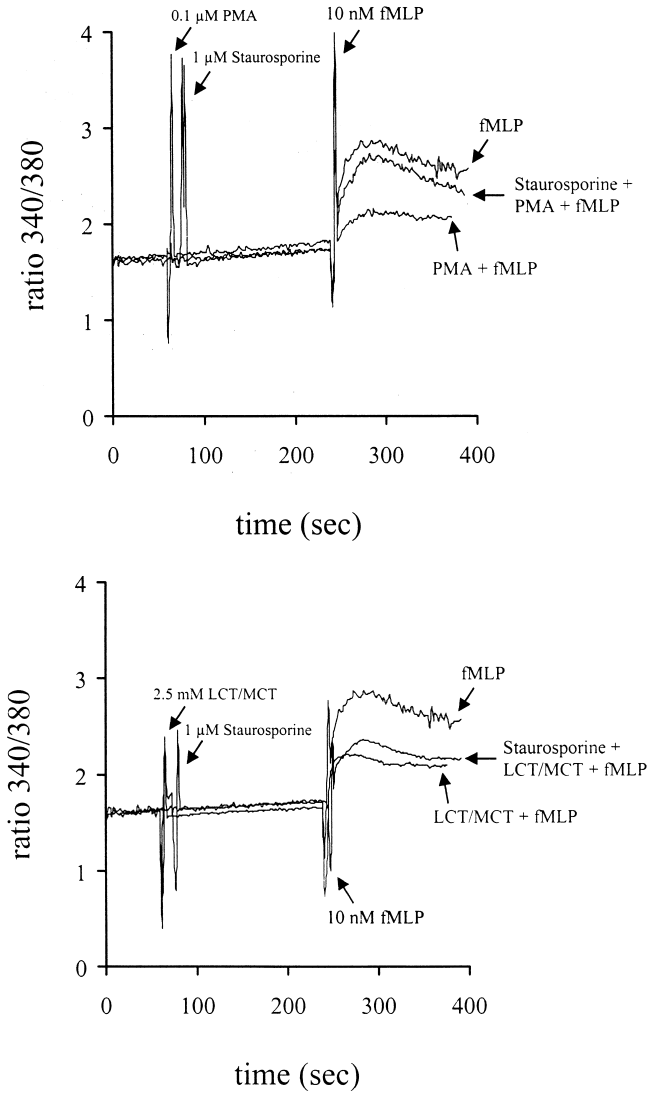

Fig. 4. Effect of staurosporine on the fMLP-induced peak increase in $[Ca^{2+}]_{i,av}$ in human neutrophils pretreated with PMA (A) or LCT-MCT (B). Fura-2-loaded neutrophils were preincubated in the absence or presence of 0.1 μM PMA (A) or 2.5 mM LCT-MCT (B) for 3 min. Where indicated, 1 μM staurosporine was added at 30 s after PMA (A) or LCT-MCT (B). After 3 min, the cells were stimulated with 10 nM fMLP. Details on $[Ca^{2+}]_{i,av}$ measurement are given in the legend to Fig. 1.

tripeptide fMLP. The extent of neutrophil activation was expressed as function of the peak height of the fMLP-induced increase of the average cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{i,av}$) in a suspension of cells loaded with the fluorescent Ca^{2+} indicator fura-2. Four structurally different lipid emulsions were included in this study, namely Omegaven (fish oil), ClinOleic (olive oil), Lipofundin (a mixture of coconut oil and soya oil), and Intralipid (soya oil). In the presence of 0.5% (w/v) HSA, each of these lipid emulsions maximally reduced the fMLP response by 50% to 60%. The inhibitory effect of the lipid emulsions was dose-dependent and the order of potency was VLCT, being the most potent emulsion, followed by LCT-MCT,

TABLE 2. Composition and characteristics of the different lipid emulsions[a]

	LCT-MCT	VLCT	LCT-MUFA	LCT
Mean molecular weight	634	882	873	865
Egg phospholipids (g/l)	12	12	12	12
Glycerol (g/l)	25	25	22.5	22.5
pH	8.0	7.5–8.7	7–8	8.0

[a] Data provided by manufacturer.

LCT, and LCT-MUFA, being the least potent emulsion (half-maximally inhibitory concentrations of 0.24 mM, 0.32 mM, 0.52 mM, and 0.82 mM, respectively). In the absence of HSA, however, VLCT inhibited the fMLP response with the same efficacy but 8-fold more potently (half-maximally inhibitory concentrations of 0.031 mM and 0.24 mM in the absence and presence of HSA, respectively). In contrast, the percentage inhibition observed with 0.1 mM LCT and 0.1 mM LCT-MUFA was similar under both conditions, whereas the percentage inhibition observed with 0.1 mM LCT-MCT was only slightly increased in the absence of serum albumin. Taken together, this suggests that VLCT contains an inhibitory active component that is effectively bound by HSA. LCT-MCT contains less of this component, whereas LCT and LCT-MUFA contain only little of it.

The data presented show that VLCT and, to a lesser extent, LCT-MCT inhibit fMLP-induced neutrophil activation more potently than LCT and LCT-MUFA. Close comparison of the fatty acid composition of the triglycerides present in these emulsions (Table 1) reveals that the most potent inhibitor VLCT differs from the other three emulsions in that it contains relatively much of the n-3 polyunsaturated fatty acids eicosapentaenoic acid (n-3 EPA) (20:5, v/v) and n-3 polyunsaturated fatty acids docosahexaenoic acid (n-3 DHA) (22:6, v/v). Furthermore, it contains considerably less oleic acid (18:1) than LCT-MUFA. Because the latter emulsion is the least potent inhibitor, it can be concluded that the DHA- and/or EPA-containing triglycerides are responsible for the higher potency of the VLCT emulsion. The second potent inhibitor LCT-MCT differs from the other three emulsions in that it contains relatively much of the MCT caprylic acid (8:0, v/v) and capric acid (10:0, v/v). It contains markedly less oleic acid (18:1, v/v) than LCT-MUFA and a moderate amount of linoleic acid (18:2, v/v), as compared with the second least potent inhibitor LCT. This demonstrates that the PUFA-containing triglycerides are not responsible for the higher potency of the LCT-MCT emulsion. Finally, the second least potent inhibitor LCT differs from the least potent inhibitor LCT-MUFA in that it contains considerably more linoleic acid (18:2, v/v). Taken together these findings show that triglycerides containing EPA and/or DHA inhibit fMLP-induced neutrophil activation more potently than those containing MCT and much more potently than those containing linoleic acid or oleic acid. Detailed analysis using pure triglycerides may show whether triglycerides containing EPA and/or DHA are indeed more potent

in inhibiting the fMLP response than those containing MCT or linoleic acid.

In a broader context, the present findings contribute to our understanding of the results of previous clinical studies in which fish oil preparations were effective in the treatment of inflammatory bowel disease, as well as in attenuating inflammatory responses in cancer patients (29–33).

We have previously shown that lipid emulsions containing MCT, including Lipofundin, completely mimicked the effects of PMA on the STZ-induced increase in $[Ca^{2+}]_{i,av}$ (19). Conversely, lipid emulsions that did not contain MCTs, including Intralipid, lowered the plateau $[Ca^{2+}]_{i,av}$ but had no effect on the kinetics of the STZ-induced increase in $[Ca^{2+}]_{i,av}$. This suggested that emulsions containing MCT act through PKC to change the kinetics, but not the final height, of the STZ-induced increase in $[Ca^{2+}]_{i,av}$. Here we show that the same lipid emulsions, when added at a clinically relevant concentration of 2.5 mM, also markedly reduce the fMLP-induced increase in $[Ca^{2+}]_{i,av}$. Inhibition was not reversed by staurosporine, demonstrating that PKC is not primarily involved in the mechanism of action of these emulsions. In contrast, this drug readily reversed the inhibitory effect of PMA.

The mechanism(s) underlying the prompt inhibitory action of these triglyceride species might involve *i*) binding of fMLP, thereby decreasing the effective concentration of the hormone, *ii*) activation of intracellular mechanism(s), resulting in receptor desensitization, or *iii*) changes in membrane properties, leading to impaired signal transduction. In our previous study, we showed that LCT and LCT-MCT, when added at 2.5 mM, reduced the maximal response to fMLP by 40% and 57%, respectively, without changing the half-maximally stimulatory concentration of the hormone (EC_{50} values of 0.79 nM, 0.63 nM, and 0.79 nM for untreated and LCT- and LCT-MCT-treated cells, respectively). This shows that the lipid emulsions do not act by lowering the effective concentration of fMLP. In contrast, PMA evoked both a rightward shift of the dose-response curve for the effect of fMLP (EC_{50} of 12.6 nM) and a 50% reduction of the maximal effect of the hormone. Here we show that staurosporine readily reversed the inhibitory effect of PMA. This indicates that PMA did not act by reducing the effective concentration of fMLP, but by reducing the sensitivity of the fMLP receptor (rightward shift dose-response curve) and the signal transduction efficiency (reduction maximal effect of fMLP). Staurosporine did not reverse the inhibitory effect of the lipid emulsions. This suggests that the lipid emulsions act by interfering with the signal transduction efficiency.

In summary, this article shows that acute addition of lipid emulsions, at a clinically relevant concentration of 2.5 mM, can significantly reduce hormone-induced neutrophil stimulation. On the other hand, when added at a final concentration of 1 mM, LCT-MUFA, and LCT emulsions affect the response to fMLP considerably less than VLCT and MCT-containing emulsions. Future studies employing neutrophils that are obtained from subjects receiving total parenteral infusion will learn whether and, if so, how fMLP-induced Ca^{2+} signaling is affected under conditions of long-term exposure to nutritional lipid emulsions.

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