Immune function and leukocyte sequestration under the influence of parenteral lipid emulsions in healthy humans: a placebo-controlled crossover study

Michelle W Versleijen, Wim J Oyen, Hennie M Roelofs, Sjenet E van Emst–de Vries, Peter H Willems, Jan B Jansen, and Geert J Wanten

ABSTRACT
Background: It remains unclear whether immune modulation by lipids contributes to the high risk of infectious complications that is associated with the use of parenteral nutrition. Although mixed long- and medium-chain triacylglycerol (LCT-MCT)–containing emulsions, but not pure LCT emulsions, activate neutrophils and impair crucial leukocyte functions in vitro, in vivo studies have failed to corroborate these findings.

Objectives: The present investigation was conducted to evaluate the effects of LCT and LCT-MCT on immune function in healthy humans and to assess whether the lack of in vivo effects results from sampling errors due to extravascular sequestration of activated neutrophils.

Design: Saline, LCT-MCT, and LCT emulsions were administered intravenously for 4.5 h to 12 healthy volunteers in a randomized crossover design. Plasma triacylglycerol concentrations were clamped at a clinically relevant concentration of 3–5 mmol/L. Leukocyte population counts and neutrophil activation were assessed before and after infusion. Leukocyte sequestration was evaluated by monitoring the distribution of Technetium-99m–labeled autologous leukocytes during infusions.

Results: Whereas LCT exerted no greater effects than did saline, LCT-MCT significantly decreased lymphocyte counts. However, no evidence for neutrophil activation was found with either lipid. Moreover, the clearance of radiolabeled leukocytes from the liver, spleen, and lungs was not altered by any lipid, which suggested that lipid emulsions do not induce leukocyte sequestration.

Conclusions: Short-term infusion of LCT-MCT (but not LCT) to healthy humans modulates leukocyte population counts but, in clear contrast with the in vitro situation, does not induce neutrophil activation. These disparate findings cannot be explained by MCT-induced leukocyte sequestration.

Am J Clin Nutr 2008;87:539–47.

KEY WORDS Total parenteral nutrition, lipid emulsion, immune modulation, neutrophil activation, leukocyte sequestration, healthy humans

INTRODUCTION
Total parenteral nutrition (TPN) is a life-saving strategy in patients with severe intestinal failure (1). Its associated high rate of infectious complications, however, remains a major drawback. Although many of these problems result from the use of a venous access device, immune modulation by the lipids in TPN may also play a role (2–4).

The source for the most widely used parenteral lipid emulsion, Intralipid, is soybean oil, which mainly contains long-chain triacylglycerols (LCT) and which is especially rich in the polyunsaturated fatty acid linoleic acid, the parent of the n−6 family of polyunsaturated fatty acids (5). Metabolism of linoleic acid yields arachidonic acid, which is converted into eicosanoids that have proinflammatory immunologic properties. The high content of n−6 polyunsaturated fatty acids in soybean oil has thus been considered a drawback, especially in patients with an already imbalanced inflammatory response. Awareness of this drawback sparked the development of new lipids, in which part of the n−6 polyunsaturated fatty acids has been replaced by medium-chain triacylglycerols (MCT) (5). The favorable metabolic profile of MCT, because of their rapid metabolic breakdown and insusceptibility to lipid peroxidation, together with the notion that essential fatty acids can be supplied only by giving LCT, led to the development of mixed LCT- and MCT-containing emulsions (LCT-MCT), such as Lipofundin.

Numerous studies with in vitro, in vivo, and ex vivo designs investigating the immunologic characteristics of parenteral lipids (2–4, 6–13) have yielded inconsistent outcomes, so that a clear picture of the immune-modulating effects of these lipids is still lacking (5, 14–16). Other groups (9, 17) and our group (18–23) previously showed that, in the in vitro setting—ie, in isolated neutrophils—LCT-MCT and pure MCT, in clear contrast to pure LCT, impaired crucial neutrophil functions and induced signs of neutrophil activation. These effects cannot be attributed to functional changes induced by isolation procedures, because similar observations were made when leukocytes in whole blood were exposed to these lipids (24). However, in vivo...
studies of the immune-modulating effects of LCT and LCT-MCT emulsions are sparse. Waitzberg et al (10, 25) showed that LCT-MCT infusion, in contrast to in vitro exposition, did not affect human leukocyte functions, whereas LCT exposition did not have any effect in both settings. Given our in vitro data showing that LCT-MCT increases the expression of CD11b (19), a cell surface adhesion molecule, it may be that, in vivo, capillary adhesion and extravascular migration of activated neutrophils would be encountered. This phenomenon is called leukocyte sequestration, and it results in the disappearance of activated leukocytes from the circulating blood pool from which samples are taken (26, 27). An evaluation of lipid-induced neutrophil modulation in vivo may thus result in false-negative findings because of blood-sampling error.

The present study was conducted to investigate whether short-term infusion of LCT and LCT-MCT distinctly modulates leukocyte functions in vivo. To rule out underlying diseases that may influence immune function, the study was performed in healthy subjects. For the first time, to our knowledge, a possible effect on leukocyte sequestration is taken into account by in vivo monitoring the distribution of autologous technetium 99m-labeled exametazime (Tc-99m-HMPAO)–labeled leukocytes during saline or lipid infusions.

SUBJECTS, MATERIALS, AND METHODS

Subjects

Twelve healthy volunteers [6 F, 6 M; mean age: 30 y (range 19–53 y); mean body mass index (BMI; in kg/m²): 23 (range: 19–30)], none of whom was on medication, were included in the study.

Written informed consent was obtained from all participants. The Ethical Review Board of the Radboud University Nijmegen Medical Center approved the study.

Lipid administration

After an overnight fast, lipid-free control (saline 0.9%) or lipid emulsions containing LCT (Intralipid 20%; Fresenius Kabi, Bad Homburg, Germany) or LCT-MCT (Lipofundin 20%; B Braun Melsungen AG, Melsungen, Germany) were administered intravenously for 4.5 h in a randomized crossover design (blinded for subjects) with a washout period of 2 wk (Figure 1). To stabilize triacylglycerol concentrations at a clinically relevant level over the infusion period, emulsions were infused according to the plasma triacylglycerol clamp technique described by Iriyama et al (28). Because lipid elimination rates are considered to be higher in whites than in Japanese persons, we used infusion rates that, in the Japanese setting, yielded a triacylglycerol clamp of 4 to 5 mmol/L (29) in order to aim at slightly lower concentrations in the white subjects in the present study. To resemble the clinical situation, in which infusion rates are based on caloric needs, we kept equal the weight-based infusion rates for LCT and LCT-MCT. To reach a stable plasma concentration of 3 to 5 mmol/L (priming), lipid emulsions were infused at a rate of 0.5 g · kg body wt⁻¹ · h⁻¹ for the first 10 min and at 0.3 g · kg body wt⁻¹ · h⁻¹ for the following 40 min. Subsequently, this plasma triacylglycerol concentration was clamped by infusion at 0.15 g · kg body wt⁻¹ · h⁻¹ for another 220 min. Blood samples were drawn before and after 3.5 h of infusion.

Laboratory variables

Blood cell counts (specimens collected in EDTA-coated tubes), including an automated leukocyte differentiation, renal function, and sodium, potassium, liver enzyme, total bilirubin, LDH, C-reactive protein, and triacylglycerol concentrations (specimens collected in lithium-heparin tubes), were assessed on an automated analyzer (AdviaTM120, Siemens Medical Solutions, The Hague, Netherlands). Variables were assessed before and after the infusion.

Materials

Reagents were from Sigma Chemicals (St Louis, MO) unless stated otherwise. Hank’s balanced salt solution (HBSS) was purchased from Life Technologies (Paisley, United Kingdom), Percol (p 1.130 g/mL at 20 °C) was obtained from GE Healthcare Biosciences AB (Uppsala, Sweden), and phosphate-buffered saline (PBS) was produced by B Braun Melsungen AG. Isotrophic lysis solution (pH 7.4) contained 155 mmol NH₄CL/L and 10 mmol KHCO₃/L. Neutrophil medium consisted of HBSS supplemented with 0.5% (wt:vol) bovine serum albumin. Phorbol 12-myristate 13-acetate (PMA) was stored as a 0.1 mg/mL stock in dimethyl sulfoxide at room temperature (RT). Serum-treated zymosan (STZ) (10 mg/mL stock) was prepared by dissolving 250 mg zymosan in 30 mL PBS, homogenizing the mixture in a sonicator (for 1 h at 48 kHz), and centrifuging it.

FIGURE 1. Time frame of experimental set-up. Saline or lipid emulsions, containing long-chain triacylglycerols or mixed long- and medium-chain triacylglycerols, were administered intravenously for 4.5 h to 12 healthy volunteers (randomized crossover design with a 2-wk wash-out interval). Plasma triacylglycerol concentrations were clamped at a clinically relevant concentration, 3–5 mmol/L) over the infusion period. Blood samples were drawn before and after 3.5 h of infusion. A, neutrophil isolation and activation assessment; B, determination of hematologic and chemical plasma variables; C, leukocyte isolation for labeling with technetium-99m–labeled exametazime (Tc-99m-HMPAO); D, infusion of labeled leukocytes. Imaging of labeled leukocytes was performed during the last hour of saline or lipid infusions.
(850 × g for 5 min at RT). The pellet was suspended in 6 mL human serum, incubated for 45 min in a 37 °C shaking water bath, washed 3 times, and resuspended in PBS to a final concentration of 10 mg/mL. A high-affinity fluorescent intracellular calcium indicator (Fura-2/AM) was from Molecular Probes Inc (Eugene, OR). All antibodies used for the measurement of leukocyte membrane surface antigen expression were purchased from Beckman Coul ter (Miami, FL). Blood samples were collected in 10 mL Monoject tubes with 170 I.U. of lithium heparin (Beliver Industrial Estate, Plymouth, United Kingdom).

**Leukocyte isolation**

Leukocytes were purified from blood anticoagulated with lithium heparin. The blood, diluted 1:1 with PBS, was placed on Percoll (ρ 1.076 g/mL) and centrifuged (700 × g for 20 min at RT). The granulocyte-containing pellet was suspended in 50 mL ice-cold lysis solution, and lysis of erythrocytes was performed on ice for 10–15 min. After centrifugation (400 × g, 5 min, RT), the remaining erythrocytes were lysed on ice in fresh lysis solution for another 5 min. The granulocytes were then washed twice, suspended in medium to the desired final concentration, and kept at room temperature. This method of neutrophil isolation yields >97% pure and >99% viable cell samples as determined by May-Grünwald/Giemsa and trypan blue staining (18).

**Oxygen radical production by isolated neutrophils and whole blood cells**

Oxygen radical production was evaluated by means of luminol-enhanced chemiluminescence, as measured in an automated lum imimeter (LB96V6 Microlumat Plus; EG&G Berthold, Bad Wildberg, Germany), as described previously (30). Briefly, in a 96-well micro plate, 200 μL diluted whole blood (1:100 in HBSS) or isolated neutrophil cell suspension (1 × 10⁶ cells/mL) was added, either with no stimulus or with 20 μL PMA (receptor-independent stimulus, stock 1:20 diluted in HBSS) or STZ (receptor-dependent stimulus). To each well, 20 μL luminol (stock 1:10 diluted in medium) was added. Each measurement was performed in fivefold. Chemiluminescence was monitored every 145 s for 1 h at 37 °C. In this period, stimulus-induced oxygen radical production normally returns to baseline. Luminescence was expressed as relative light units per second. The area under the curve, representing the overall oxygen radical production during 1 h, and luminescence peak time were calculated. Data were analyzed with WINGLOW software (version 1.10; EG&G Berthold, Bad Wildberg, Germany). After subtraction of background signal, the signal intensity in whole blood samples was correlated to the neutrophil population count.

**Leukocyte membrane surface antigen expression on whole blood cells**

The expression of activation molecules on the membrane surface of whole blood leukocytes was evaluated by immunofluorescence staining and subsequent flow-cytometric analysis. Characterization was performed by using antibodies directed against an adhesion molecule of the β₂ integrin family, CD11b; against markers for degranulation of azurophilic (CD63) or specific granulae (CD66b); and against an activation marker, CD62L, that is normally down-regulated during cell activation because of receptor shedding. Immunofluorescence staining was performed according to the “lyse and wash” method. Briefly, 100 μL whole blood was incubated (15 min at RT) with 5 μL of each directly fluorochrome-labeled antibody. The following direct mouse anti-human IgG antibodies were used: CD66b (clone 80H3) and CD62L (clone DREG56) labeled with fluorescein isothiocyanate; CD11b (clone BEAR1) and CD63 (clone CLB-gran12) labeled with phycoerytron (PE); CD45 (clone J33), a leukocyte marker, labeled with PE-Texas red (ECD) and CD14 (clone RMO52); and a monocyte marker, labeled with PE-cyochrome5 (PE-CY5). Next, erythrocytes were lysed (15 min in the dark at RT) by using 155 mmol NH₄Cl/L (pH 7.4). The cell suspension was centrifuged at 500 × g for 5 min at RT. Next, neutrophils were washed in PBS and then fixed in 1% paraformaldehyde and stored at 4 °C until analysis the next day. Staining of cells was determined in multicolor analysis by using a flow cytometer (Epics XL; Beckman Coulter, Hialeah, FL). All measurements were performed on a minimum of 50 000 cells.

**Serum-treated zymosan-induced cytosolic calcium signaling in isolated neutrophils**

Isolated neutrophils (4 × 10⁶/mL) were loaded with 5 μmol Fura-2/AM/mL for 15 min at 37 °C. Excess Fura-2/AM was removed by washing the neutrophils first with medium [HBSS with 0.5% bovine serum albumin (wt:vol)] and second with HBSS. Subsequently, the neutrophils were transferred to a cuvette placed in a spectrofluorophotometer (RF-5301; Shimadzu, Tokyo, Japan) equipped with a magnetic stirrer and a thermostated cuvette holder (37 °C). The fluorescence emission ratio at 490 nm was monitored as a measure of the average cytosolic free Ca²⁺ concentration after excitation at 340 and 380 nm (22).

**Scintigraphy with technetium-99m-labeled exametazime-labeled leukocytes**

**Cell isolation and labeling**

Before the start of saline or lipid emulsion infusion, a 50-mL venous blood sample was drawn into a 60-mL syringe containing 10 mL of a 0.33% solution of methyl cellulose and acid citrate dextrose. All cell isolation and labeling procedures were carried out at RT. After sedimentation for 2 h, the leukocyte-rich plasma was removed and centrifuged at 49 × g for 15 min. The cell pellet was washed twice in 5 mL saline with 1% (wt:vol) human serum albumin and resuspended in 2 mL of saline with 1% human serum albumin, after which 750 MBq Tc-99m-HMPAO was added. After centrifugation (49 × g for 10 min), the pellet containing the radiolabeled leukocytes was resuspended in 2 mL 5% glucose solution, and 3 mL saline with 1% human serum albumin was added. A dose of 100 MBq Tc-99m-labeled autologous leukocytes was administered as an intravenous bolus injection to the volunteer 3.5 h after the start of the saline or lipid infusion (Figure 1).

**Image acquisition and processing**

Immediately after injection of the Tc-99m-labeled autologous leukocytes, anterior and posterior series of 1-min dynamic images of the chest or upper abdomen scans were recorded for 1 h by using a double-head gamma camera equipped with low-energy collimators (ECAM; Siemens, Hofman Estates, IL). The infusion of saline or lipid emulsions was continued for 1 h. A reference tube, containing a known aliquot of the injected dose,
was placed in the midaxial line within the field of view. The observer was blinded to the treatment during analysis of the images. The images were analyzed by drawing regions of interest over the reference tube, the right lung (posterior and anterior), liver (posterior and anterior), and spleen (posterior only), because these organs are the major organs of the reticuloendothelial system and the most probable areas of leukocyte sequestration. From the counts in the regions of interest, the percentages of injected Tc-99m–labeled leukocytes in lung, liver, and spleen were calculated, and time-activity curves were plotted.

Statistical analysis

Overall differences among treatments were analyzed with the nonparametric Friedman’s test. When significant to a level of $P \leq 0.05$, differences between treatment pairs were analyzed further by Wilcoxon’s signed-rank test (2-tailed) with Bonferroni correction for multiple comparisons. A corrected $P$ value $\leq 0.05$ was considered significant. Differences in preinfusion and postinfusion levels were also analyzed by means of Wilcoxon’s signed-rank test. Values are expressed as means $\pm$ SEMs unless stated otherwise. All statistical analyses were performed with the use of SPSS software (version 12.0; SPSS Inc, Chicago, IL).

RESULTS

Laboratory variables

In all subjects, serum urea, creatinine, sodium, potassium, liver enzymes, total bilirubin, LDH, and C-reactive protein were within normal ranges. They remained unaffected by either saline or lipid infusion (data not shown).

Triacylglycerol concentration

The infusion of both lipid emulsions, according to the plasma triacylglycerol clamp technique, significantly increased plasma triacylglycerol concentrations from 1 mmol/L to 3–4 mmol/L. There were no significant differences between serum triacylglycerol concentrations after LCT and LCT-MCT infusion (Table 1).

Leukocyte counts

Preinfusion (baseline) cell counts did not differ significantly between treatments. A significant increase in total leukocyte and absolute neutrophil counts was observed after all 3 infusions. These increases appeared highest after LCT-MCT administration, but the differences from saline and LCT were not significant (Table 2 and Figure 2). In contrast, the absolute number of lymphocytes remained unaltered after infusion of saline or LCT but decreased significantly after infusion of LCT-MCT. Absolute monocyte counts did not change on infusion of saline or lipids.

Oxygen radical production

In isolated neutrophils and whole blood cells, the relative change in spontaneous (unstimulated) and PMA- or STZ-induced overall oxygen radical production, as presented by the ratio of the area under the curve after infusion to that before infusion, did not differ significantly between treatments (Table 3). Chemiluminescence in unstimulated whole blood samples was within the background range. Absolute changes in PMA- or STZ-induced chemiluminescence peak time were minimal and did not differ significantly between groups (Table 4).

Serum-treated zymosan–induced intracellular calcium signaling in neutrophils

Stimulation of a suspension of neutrophils loaded with the cytosolic $Ca^{2+}$ indicator fura-2 with STZ typically results in an

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**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Before infusion</th>
<th>After infusion</th>
<th>After − before</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>1.02 $\pm$ 0.08</td>
<td>0.99 $\pm$ 0.09</td>
<td>$-0.04$ $\pm$ 0.05</td>
</tr>
<tr>
<td>LCT</td>
<td>0.98 $\pm$ 0.08</td>
<td>3.81 $\pm$ 0.31$^2$</td>
<td>2.83 $\pm$ 0.28$^3$</td>
</tr>
<tr>
<td>LCT-MCT</td>
<td>0.96 $\pm$ 0.09</td>
<td>3.12 $\pm$ 0.32$^2$</td>
<td>2.15 $\pm$ 0.26$^3$</td>
</tr>
</tbody>
</table>

$^1$ All values are $\bar{x} \pm$ SEM. LCT, long-chain triacylglycerols; LCT-MCT, mixture of LCT and medium-chain triacylglycerols. Plasma triacylglycerol concentrations before and after a 3.5-h intravenous infusion of saline or lipid emulsion to healthy humans ($n = 12$). Preinfusion triacylglycerol concentrations did not differ significantly between treatments. There were no significant differences between LCT and LCT-MCT infusion.

$^2$ Significant changes from preinfusion concentrations, $P < 0.05$ (Wilcoxon’s signed-rank test).

$^3$ Significant differences in absolute increment (after minus before) between lipids and saline, $P < 0.05$ (Wilcoxon’s signed-rank test with Bonferroni correction).

**Table 2**

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Before infusion</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>$5.1 \pm 0.33$</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>$2.68 \pm 0.19$</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>$1.83 \pm 0.22$</td>
</tr>
<tr>
<td>Monocytes</td>
<td>$0.43 \pm 0.06$</td>
</tr>
</tbody>
</table>

$^1$ All values are $\bar{x} \pm$ SEM. LCT, long-chain triacylglycerols; LCT-MCT, mixture of LCT and medium-chain triacylglycerols. Absolute plasma leukocyte counts before and after a 3.5-h intravenous infusion saline or lipid emulsion to healthy humans ($n = 12$). Preinfusion cell counts did not differ significantly between treatments for all leukocyte subsets.

$^2$ Significant changes from preinfusion cell counts, $P \leq 0.05$ (Wilcoxon’s signed-rank test). For differences in treatment effects between groups, see Figure 2.
biphasic increase in the fura-2 fluorescence emission ratio. This reflects a biphasic change of the average cytosolic free \( \text{Ca}^{2+} \) concentration of all cells in suspension, consisting of an initial slow phase followed by a second fast phase and finally resulting in a plateau phase that is maintained for a prolonged time (Figure 3). In previous studies, we showed that in vitro preinfusion treatment of isolated neutrophils with LCT-MCT or pure MCT, rather than pure LCT, sensitizes the neutrophils to stimulation by STZ in a PMA-like manner; the rate of \( \text{Ca}^{2+} \) rise during the first slow phase is increased and the second fast phase is abolished (22) (Figure 3D–F). The present study shows that intravenous infusion of LCT-MCT failed to alter the biphasic shape of the STZ-induced increase in average cytosolic free \( \text{Ca}^{2+} \) concentration (Figure 3A–C). However, if neutrophils from subjects infused with either saline, LCT-MCT, or LCT were treated ex vivo with LCT-MCT for 3 min before infusion, normal sensitization to STZ stimulation was observed (Figure 3D–F). As expected, ex vivo preinfusion treatment of these cells with LCT did not affect the biphasic shape of the intracellular calcium response.

### Expression of membrane surface antigens

The modulation of the expression of membrane surface activation, adhesion, and degranulation markers on whole blood neutrophils and monocytes did not differ significantly between treatments, except for a slight but significantly \((P = 0.024)\) higher CD62L expression after LCT-MCT infusion than after saline infusion (Table 5).

### Scintigraphy with Tc-99m–labeled exametazime-labeled leukocytes

The clearance of autologous Tc-99m-HMPAO–labeled leukocytes from the pulmonary, hepatic, and splenic circulations

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**TABLE 3**

The ratio of neutrophil oxygen radical production after to that before intravenous saline or lipid infusion\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Saline infusion</th>
<th>LCT infusion</th>
<th>LCT-MCT infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolated neutrophils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>1.12 ± 0.19(^2)</td>
<td>1.38 ± 0.22</td>
<td>1.94 ± 0.91</td>
</tr>
<tr>
<td>PMA</td>
<td>1.03 ± 0.04</td>
<td>1.08 ± 0.06</td>
<td>1.09 ± 0.07</td>
</tr>
<tr>
<td>STZ</td>
<td>1.17 ± 0.10</td>
<td>1.30 ± 0.11</td>
<td>1.32 ± 0.13</td>
</tr>
<tr>
<td><strong>Whole blood cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>Within background range</td>
<td>Within background range</td>
<td>Within background range</td>
</tr>
<tr>
<td>PMA</td>
<td>1.27 ± 0.11</td>
<td>1.44 ± 0.09</td>
<td>1.38 ± 0.05</td>
</tr>
<tr>
<td>STZ</td>
<td>1.28 ± 0.14</td>
<td>1.60 ± 0.12</td>
<td>1.49 ± 0.09</td>
</tr>
</tbody>
</table>

\(^1\) LCT; long-chain triacylglycerols; LCT-MCT; mixture of LCT and medium-chain triacylglycerols; PMA, phorbol 12-myristate 13-acetate; STZ, serum-treated zymosan. Effect of a 3.5-h intravenous infusion of saline or lipid emulsion to healthy humans \((n = 12)\) on the unstimulated and PMA- or STZ-induced overall oxygen radical production (after 60 min), as measured with luminol-enhanced chemiluminescence, in isolated neutrophils and whole blood cells, depicted as the ratios of after to before infusion. There were no significant differences between treatments, \(P \leq 0.05\) (Friedman’s test).

\(^2\) \(\bar{x} \pm \text{SEM}\) (all such values).
was not slower during any lipid infusion than during saline infusion, which suggested that these emulsions do not induce extravascular or capillary leukocyte sequestration in these organs (Figure 4). During the infusion of LCT-MCT, the total uptake of radiolabeled leukocytes in the spleen (area under the curve in Figure 4) tended to be even lower than that with saline ($P = 0.06$) but not lower than that with LCT ($P = 0.27$). There were no significant differences between saline and LCT.

**DISCUSSION**

The main finding in the present study is that, in the absence of underlying diseases and in a clinically relevant plasma triacylglycerol concentration range, there are no indications for significant modulation of neutrophil functions and activation during short-term infusion of LCT and LCT-MCT emulsions. Second, lipid-induced leukocyte activation and subsequent sequestration leading to blood sampling error is not a mechanism that could explain any discrepancies between results of investigations with different (in vitro versus in vivo) designs.

We have shown in earlier studies that MCT-containing lipids (pure MCT or LCT-MCT), in contrast to pure LCT emulsions, modulate cell functions of isolated and whole blood neutrophils obtained from healthy humans in vitro (18, 19, 21, 22, 24, 31). However, whereas in vitro exposure to MCT increases the stimulus-induced oxygen radical production (18), that response was not distinctly altered by any intravenous lipid infusion (Tables 3 and 4). In addition, MCT, which sensitize the zymosan-stimulated oxygen radical production in a protein kinase C–dependent manner in vitro (22), did not alter the biphasic morphology of this response after intravenous lipid infusion (Figure 3A–C). The finding that ex vivo treatment with LCT-MCT of cells that were isolated before and after infusion

![Image](https://academic.oup.com/ajcn/article-abstract/87/3/539/4633427/122x134 to 482x314)
TABLE 5
Neutrophil membrane surface antigen expression before and after intravenous saline or lipid infusion.

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>LCT</th>
<th>LCT-MCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b</td>
<td>0.68 ± 0.10(^2)</td>
<td>0.79 ± 0.11</td>
<td>0.81 ± 0.09</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>0.90 ± 0.09</td>
<td>1.06 ± 0.14</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>CD66b</td>
<td>0.72 ± 0.07</td>
<td>0.94 ± 0.10</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td>CD63</td>
<td>&lt;detection limit</td>
<td>&lt;detection limit</td>
<td>&lt;detection limit</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>&lt;detection limit</td>
<td>&lt;detection limit</td>
<td>&lt;detection limit</td>
</tr>
<tr>
<td>CD62L</td>
<td>1.05 ± 0.09</td>
<td>1.15 ± 0.11</td>
<td>1.39 ± 0.12</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.94 ± 0.06</td>
<td>1.09 ± 0.10</td>
<td>1.43 ± 0.15(^3)</td>
</tr>
</tbody>
</table>

\(^1\) LCT, long-chain triacylglycerols; LCT-MCT, mixture of LCT and medium-chain triacylglycerols. Effect of a 3.5-h intravenous infusion of saline or lipid emulsion to healthy humans (n = 12) on the expression of membrane surface antigens [adhesion (CD11b) and degranulation (CD66b and CD63) and activation markers (CD62L)] on whole blood neutrophils and monocytes, depicted as the ratios of after to before infusion. Because monocytes do not contain granules, the expression of CD63 and CD66b was evaluated only in granulocytes. There were no significant differences between LCT and LCT-MCT.

\(^2\) ± SEM (all such values).

\(^3\) A significant difference of lipids with saline, P ≤ 0.05 (Wilcoxon’s signed-rank test with Bonferroni correction).

For the first time (to our knowledge) in this field of research, the hypothesis that extravascular or capillary sequestration of activated leukocytes plays a role in the observed absence of (measurable) neutrophil activation after lipid exposure in vivo was tested. The fact that the clearance of radiolabeled leukocytes from pulmonary, hepatic, and splenic circulations, representing the major organs of the reticuloendothelial system, was not delayed during any lipid infusion makes it unlikely that, in vivo, neutrophils are activated by LCT-MCT or that sampling error due to leukocyte sequestration does occur.

With respect to the effects of lipids on leukocyte population counts, we found that the infusion of LCT-MCT but not of LCT or saline significantly decreased absolute lymphocyte numbers. Although, all lymphocyte numbers remained within normal ranges (Table 2), this decrease is remarkable and may be relevant in patients with an already imbalanced immune response. This issue should be addressed in future studies of the effect of LCT-MCT on the cell numbers and the function of specific lymphocyte subsets, such as B lymphocytes, helper and cytotoxic T lymphocytes, and natural killer cells. The increase in lymphocyte and neutrophil counts, which were observed after all infusions, was most pronounced (although not significantly so) after the LCT-MCT infusion (Table 2 and Figure 2). Because absolute leukocyte counts also increased after saline infusion, saline should be regarded as a lipid-free control rather than as a true placebo. It is also not unlikely that procedure-related stress may play a role. Pure LCT exerted no greater effect than did saline, and thus it is unlikely that emulsion components other than lipids, such as the emulsifier or antioxidants, are responsible for the observed MCT effects.

Because leukocyte sequestration does not explain the contrasting results of in vitro and in vivo studies, other explanations should be considered. First, it is possible that, in vivo, the plasma concentrations of MCT are too low to exert the neutrophil activating effects that were observed in vitro. As in clinical practice, infusion rates of LCT-MCT and LCT emulsions were weight-based, which resulted in a higher molar infusion rate during LCT-MCT infusion than during LCT infusion. Despite this higher molar infusion rate, the plasma triacylglycerol concentration was not higher after LCT-MCT infusion (3.12 mmol/L) than after LCT infusion (3.81 mmol/L) (Table 1). Because MCT are cleared from the plasma and metabolized at much higher rates than are LCT (34, 35), triacylglycerol clamping with LCT-MCT may result in a relative accumulation of plasma LCT and low
plasma MCT concentrations. Second, plasma may contain components that inhibit or neutralize any immune-modulating effects of MCT in vivo. The observation that the potency of MCT to up-regulate the expression of adhesion and degranulation markers is highest in isolated cells (19), lower in whole blood cells (24), and absent in the present in vivo setting supports this hypothesis.

Most in vivo studies of immune modulation by LCT and LCT-MCT in humans have been performed in critically ill, or malnourished persons or in trauma or postoperative patients (7, 8, 36–39). As mentioned, results to date have been very disparate, most probably as a result of widely varied clinical conditions, medication use, and experimental settings. The present study, therefore, was performed in a more homogeneous group. It can be argued that short-term administration of parenteral lipid emulsions to healthy subjects does not resemble clinical practice, because the incorporation of fatty acids into cell membranes takes more time to become significant in healthy persons than in those who are not healthy. However, it should be also considered that, in all of these in vitro studies, effects on neutrophil functions were observed within 1 h of lipid exposure and also that a 48-h infusion had comparable results (10, 18, 19). Eventually, larger randomized trials in patients receiving long-term TPN and patients with specific acute conditions requiring short-term TPN will probably be indispensable to clear the dust in this field. It is unlikely, however, that the many practical problems concerning the performance of such studies in the past will change in the near future.

We thank Marij Leenders (Central Hematology Laboratory) for technical assistance during flow cytometry analysis and Karin van Hoven-van Loo for supervision of the participants during infusions. The authors’ responsibilities were as follows—MWV: participant recruitment, administration of emulsions, cell isolation and neutrophil activation assays, analysis of leukocyte scan images, statistical analysis of data, and writing the draft of the manuscript; WJO: study design, performance of technetium-99m-labeled leukocyte scans, analysis of the leukocyte scan images, and critical revision of the manuscript; HMR: cell isolation and neutrophil activation assays; SEVe-DV: intracellular calcium spectrophotometry; PHW: interpretation of spectrophotometry data and critical revision of the manuscript; JB: critical revision of the manuscript; GJW: study design, interpretation of data, and writing the draft of the manuscript; and all authors: final manuscript preparation. GJW has received speaking fees from Fresenius Kabi (Bad Homburg, Germany) and Baxter (Maurepas, France). None of the other authors had a personal or financial conflict of interest.

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