# Infusion time for fish oil-containing parenteral emulsions in surgery: A study on omega-3 fatty acid dynamics in rats

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## ABSTRACT

**Background**: To contribute to the design of specialized parenteral nutrition protocols in surgery, we evaluated the dynamics of polyunsaturated fatty acids (PUFA) concentrations in different body pools following the infusion of fish oil-containing lipid emulsion (FOLE) in rats that had undergone surgical central venous catheterization (CVC). **Methods**: After 5-day adaptation in metabolic cages, 78 male Lewis rats (300-450 g) fed a standard diet were sacrificed (baseline control) or had only CVC (surgical control) or also received a 72-hour infusion of a parenteral lipid emulsion with or without fish oil. The catheterized animals were sacrificed 0 (T0), 2 (T2), 6 (T6) and 12 (T12) hours after the infusion ended. Gas chromatography was used to determine the concentrations of eicosapentaenoic (EPA), docosahexaenoic (DHA) and arachidonic (ARA) acids and the n-3:n-6 ratio in plasma, liver and blood leukocytes. Kruskal-Wallis and Wilcoxon tests were applied to plasma and liver data and descriptive analysis to leukocyte data. **Results**: Plasma, liver and leukocytes exhibited almost undetectable EPA and DHA and detectable ARA concentrations at baseline. Immediately after FOLE infusion (T0), these PUFA changed in all pools, resulting in a higher n-3:n-6 ratio compared to rats with no FOLE infusion ($p<0.05$). All these changes decreased over time, with residual effects remaining until T6 in plasma, T12 in liver and only until T2 in leukocytes. **Conclusion**: Our data suggest that n-3 PUFA are cleared early after the end of FOLE infusion, mainly in leukocytes. When FOLE are applied for immunomodulatory purposes in surgery, this should be considered.

## INTRODUCTION

The inflammatory response to surgery is associated with poor prognosis, which can be minimized by the administration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[1-3] These omega-3 (n-3) polyunsaturated fatty acids (PUFA) are associated with anti-inflammatory and tissue reparative properties by serving as intracellular precursors of eicosanoids with low inflammatory potential and/or specialized pro-resolution mediators, as well as modulating cell signaling processes and the activity of transcription factors.[4-6] The availability of n-3 PUFA in cells is central for these modulations to occur and is influenced by the dietary fatty acid composition.[5,7]

Infusion of fish oil-containing lipid emulsions (FOLE) as a source of EPA and DHA is a potential strategy for the adjuvant treatment of surgical patients under parenteral nutrition therapy, with the main purpose of attenuating the harmful consequences of the postoperative immune response.[5] Compared to the infusion of conventional lipid emulsions (LEs), FOLE have been shown to favor a less inflammatory and immunosuppressive profile of circulating cytokines and eicosanoids, as well as to attenuate the negative impact of surgery on leukocyte biology.[8-17] These improvements in immune-inflammatory markers are largely accompanied by better clinical outcomes, but a lack of significant clinical impact has also been reported.[18]

Reasons for the limited clinical benefits after FOLE infusion may include insufficient length of the supplementation for surgical patients to benefit from the biological properties of EPA and DHA. The European and American guidelines in the area of specialized clinical nutrition do not include specific recommendations in this field.[19,20] Aiming to contribute with evidence that guides the future planning of clinical protocols for specialized nutrition, we evaluated the plasma, liver and

leukocyte PUFA profiles at different periods after ending the parenteral infusion of a FOLE. Our intention was to characterize the dynamics of EPA and DHA concentrations after FOLE infusion to know more about the time period over which this should be extended to favor immunological benefits.

## MATERIALS AND METHODS

### *Ethical statement*

All interventional procedures were conducted according to the standards of protection and care of experimental animals of the Research Ethics Committee of the University of São Paulo Medical School (CEP - FMUSP), which approved our experimental protocol (N°: 126/12).

### *Animals and interventional procedures*

Male Lewis rats weighing 300-450 g (n = 78) were purchased from the Multidisciplinary Center for Biological Research (CEMIB) of the Universidade Estadual de Campinas (UNICAMP). All animals remained in individual metabolic cages for 5 days at 20-25°C controlled temperature and day/night cycles of light and feeding AIN-93M *ad libitum* for adaptation to the experimental conditions. After this period, 6 of the animals were sacrificed by cardiac puncture to serve as the baseline control for PUFA concentrations (BC group). The remaining animals (n = 72) were subjected to central venous system access by a previously described surgical technique[21] and to a 24-h infusion (0.25 mL/h) of 6 mL of saline solution (0.9%) for recovery. After this period, 24 of the catheterized animals remained feeding only on the oral AIN-93M diet *ad libitum* to serve as surgical controls (SC group). The other catheterized animals (n = 48) remained feeding on the oral AIN-93M diet *ad libitum* but also received parenteral infusion (72 hours,

4.3 g fat/kg body weight)[22] of a control LE without fish oil (EC group, n = 24) or a FOLE (FO group, n = 24). Parenteral infusion was performed using a gravity infusion pump (Space® perfusor, BBraun, Melsugen, Germany), and the LEs applied were Lipofundin® 20% (BBraun, Melsugen, Germany) containing 50% soybean oil and 50% MCT from coconut oil (EC group) and Lipidem® 20% (BBraun, Melsugen, Germany) containing 40% soybean oil, 50% MCT from coconut oil and 10% fish oil (FOLE group), whose compositions are described in Table 1. Oral dietary intake was measured daily in an analytical balance (Acrimet, H.K. N ° 0211724.0, Barueri, Brazil), during the surgical follow-up (SC group) and lipid emulsion infusion (EC and FO groups). Sacrifice of all the catheterized animals occurred 0 (T0), 2 (T2), 6 (T6) and 12 (T12) hours after the end of the surgical follow-up (SC group) or after the end of the LE infusion period (EC and FO groups). Each group/sacrifice period comprised 6 animals (24 animals of each group / 4 time periods). Sacrifices and surgical procedures were performed under anesthesia by intraperitoneal injection of 80 mg/kg ketamine hydrochloride and 8 mg/kg xylazine hydrochloride. Supplementary Figure S1 illustrates the study design.

***Samples obtained and processed***

At sacrifice, all animals were submitted to blood (cardiac puncture into a 0.2-mL heparin vacuum tube) and liver tissue (ventral incision) sampling. Blood samples were processed to yield plasma (10 min, 1800 rpm, 20°C centrifugation), and mononuclear and polymorphonuclear leukocytes were obtained from blood diluted in saline solution (1:1 v/v) by density gradient centrifugation (30 min, 1800 rpm, room temperature) with 1:1 v/v Histopaque® 1083 or Polymorphpred® 1113, respectively (both from Sigma-Aldrich, USA). All samples were immediately frozen at -80°C until PUFA profile analysis.

***PUFA analysis***

Concentrations of EPA, DHA and arachidonic acid (ARA), as well as the n-3:n-6 ratio (determined as the sum of the absolute values of EPA, DHA and alpha-linoleic acid divided by the sum of the absolute values of linoleic acid and ARA), were assessed in plasma, liver and leukocytes by gas chromatography. We were unable to obtain a sufficient amount of the leukocyte populations for analysis from individual blood samples, so the leukocyte evaluation was performed in duplicates of pools from 6 rats, as determined by a previous pilot study.

Plasma and leukocyte lipids were extracted by a mixture of methanol:chloroform chromatographic solution (2:1, v/v; (methanol from JT Baker, Xalostoc, Edo. De México, México and chloroform from Merck, Darmstadt, Germany) and fatty acids were converted to fatty acid methyl esters (FAMEs) using a modified sodium methoxide solution 0.5 M (Sigma-Aldrich, Steinheim, Germany) in methanol.[23] Therefore, the lipid extracts were dried under nitrogen, and then dissolved in 0.5 mL hexane (Macron Fine Chemicals, Pennsylvania, USA) and transferred into a tube with tight-sealing cap, and mixed with 125 µL sodium methoxide solution (0.5 M) in methanol:chloroform (2:1, v/v). The sample was capped tightly and mixed (vortex for 1 min). Two milliliters of saturated sodium chloride solution (Casa Americana, São Paulo, Brazil) was then added and the sample shaken vigorously for 15 s. After 10 min, 3 mL of the hexane layer was transferred to a vial containing a small amount (250 mg) of anhydrous Na2SO4 (Synth, São Paulo, Brazil). The hexane was evaporated and the sample resuspended in 250 µL of hexane for subsequent GC analysis.

For the lipids extracted from liver, 100 mg of hepatic tissue was added to 500 µL of 0.88% KCl solution (Synth, São Paulo, Brazil) and homogenized in a mini Turrax homogenizer. Subsequently, lipids were extracted from the liver

homogenate according to the protocol of Bligh and Dyer (1959).[24] Briefly, after homogenization, 300 µL of methanol and 900 µL of chloroform were added, and placed under agitation for 1 min. The organic phase was collected and the solvent removed using a rotary evaporator at 40 °C. An aliquot from each total lipid extract was used in a methylation process with methanol:chloroform:chloridric acid (10:1:1 v/v; chloridric acid from Synth, São Paulo, Brazil) for 2 h at 80 °C and then FAMEs were extracted three times using hexane. Then, the organic phase was dried and resuspended in 100 µL of hexane.

The PUFA profile in plasma, liver and leukocytes was then measured using flame-ionization gas chromatography (SHIMADZU, CG-2010, Kyoto, Japan). Samples (2 µL) were injected via an autosampler into a fused-silica capillary column (DB-FFAP capillary column [15 m × 0.100 mm × 0.10 µm] J&W Scientific from Agilent Technologies, Folsom, CA, USA) in a gas chromatography system fitted with a flame ionization detector and eluted with hydrogen at 3.0 mL/min, with a split ratio of 1:150. The injector and detector were heated to 250°C and 260°C, respectively. The column was temperature programmed from 100°C (hold 0.5 min) to 195°C at 25°C/min, then to 205°C (hold 3 min) at 3.0°C/min. Data on PUFA concentrations (mg/L) were obtained by external standardization using commercial reference standards to construct the calibration curve (Sigma®, Sigma-Aldrich, Missouri, USA).

### *Statistical analysis*

For plasma and liver, the analysis of PUFA concentrations across time points was performed in an intragroup approach and compared to the baseline concentrations (BC group) using the Kruskal-Wallis and Wilcoxon tests. Analyses of pooled leukocyte samples resulted in the generation of only one representative result for each group studied, so the analyses of the leukocyte concentrations of

PUFA in leukocytes are only descriptive. The analysis and generation of graphs were performed using the R program (version 3.2.2; R Core Team, Vienna, Austria), considering a significance level of 5%.

## RESULTS

### Oral diet intake

Differences in dietary intake were observed only between the animals from the FO and SC groups sacrificed at T0 (9 [4-16] g *vs*. 20 [14-20] g, respectively; p = 0.019) and between the animals from the EC and SC groups sacrificed at T12 (20 [19-20] g vs. 17 [17-19] g, respectively; p = 0.026), while no significant differences were observed between the animals from the FO and EC groups sacrificed at any time point (Supplementary Table S1).

### PUFA concentrations

According to the analysis of the BC group, Lewis rats exhibited almost undetectable EPA and DHA but detectable to high ARA concentrations in plasma, liver and mononuclear and polymorphonuclear leukocytes at baseline. These PUFA and the n-3:n-6 ratio (data applied for n-3:n-6 ratio calculation are provided in Supplementary Table S2) were sensitive to surgical and nutritional interventions in all pools, as described in the following sections:

#### *Plasma*

EPA concentration was higher in the FO group than in the other groups at T0 and T2 (p = 0.003; Figure 1). The intragroup analysis showed a time-dependent decrease of EPA concentration in the FO group (Table 2).

No differences were observed between groups and time points for DHA concentration ($p > 0.050$; Figure 1).

ARA concentration was higher in both the FO and EC groups than in the BC group at T0 ($p = 0.003$) and was lower in the SC group than in the other groups at all the time points studied ($p \leq 0.005$; Figure 2A). However, ARA concentration was lower in the FO group than in the EC group from T2 ($p = 0.002$; Figure 1). According to the intragroup analysis, ARA concentration was higher at T0 than at the other time points studied in both FO and EC groups ($p = 0.002$), but in the FO group only these concentrations decreased in a time-dependent fashion (Table 2).

The n-3:n-6 ratio was higher in the FO group than in the other groups at T0 and T2 ($p \leq 0.005$; Figure 1). According to the intragroup analysis, the n-3:n-6 ratio was lower in the FO group at T0 than at T2 ($p = 0.002$), but higher at both T0 and T2 than at T6 and T12 ($p \leq 0.003$).

***Liver***

EPA concentration was higher in the FO group than in the other groups at all the time points studied ($p = 0.002$; Figure 2). The intragroup comparative analysis showed a time-dependent decrease of EPA concentration in the FO group, except between T2 and T6 (Table 2).

DHA concentration was higher in both EC and FO groups than in the BC and SC groups, but this effect was observed only at T0 for the EC group ($p = 0.002$) and at all the time points studied for the FO group ($p = 0.002$; Figure 2). Furthermore, DHA concentration was higher in the FO group than in the EC group at T2, T6 and T12 ($p \leq 0.050$; Figure 2). The intragroup comparative analysis showed a time-dependent decrease of DHA concentration in the EC group ($p \leq 0.005$) and in the FO group, except between T2 and T6 for FO (Table 2).

ARA concentration was higher in both FO and EC groups than in the BC group at T0 ($p = 0.003$) and lower in the SC group than in the other groups at all the time points studied ($p \leq 0.005$; Figure 2). However, compared to the EC group, ARA concentration was higher in the FO group at T0, T2 and T6, but lower at T12 ($p \leq 0,005$; Figure 2). According to the intragroup analysis, the ARA concentration was higher at T0 than at the other time points studied in both FO and EC groups ($p = 0.002$); however, in the FO group only the ARA concentration decreased in a time-dependent fashion, except between T2 and T6 (Table 2). In addition, the SC group had a higher ARA concentration at T2 than at T0 ($p = 0.041$).

The n-3:n-6 ratio was higher at T0, T2 and T6 in the EC group ($p = 0.003$) and at all the time points studied in the FO group ($p = 0.003$), compared to the BC and SC groups (Figure 2). However, this ratio was significantly higher in the FO group than in EC group from T2 ($p = 0.002$; Figure 2). According to the intragroup analysis, the n-3:n-6 ratio decreased significantly and in a time-dependent manner in both EC and FO groups, except between T2 and T6 for FO (Table 2).

***Leukocytes (descriptive data)***

In leukocytes, there was a total absence of effects on the PUFA profile at T6. Furthermore, descriptive differences of n-3 PUFA concentrations were observed at T0 and T2, being no longer present after these time points. Therefore, the T12 time point was not examined for this body pool.

*Mononuclear cells*

Compared to the BC group, EPA concentration was low in the SC and FO groups and high in the EC group at T0; however, EPA concentration was high in the FO group compared to the SC and EC groups at T2 (Figure 3).

Compared to the BC group, DHA concentration was high in SC, EC and FO groups at T0 (Figure 3).

Compared to the BC group, ARA concentration was low SC, EC and FO groups but showed different dynamics: early for the FO group (from T0) than for the SC and EC groups (from T2). In addition, ARA concentration was high in the SC group at T0 and T2, in comparison to the other groups (Figure 3).

Compared to the BC group, the n-3:n-6 ratio was high in the SC, EC and FO groups at T0; however, the n-3:n-6 ratio was high in the FO group compared to the other groups at T0 and T2 (Figure 3).

*Polymorphonuclear cells*

No differences were observed between groups and time points for EPA concentration (Figure 4).

Compared to the BC group, DHA concentration was high in the SC and FO groups at T0, but this effect was greater in the FO group (Figure 4).

Compared to the BC group, ARA concentration was low in SC, EC and FO groups but with different dynamics: earlier for the SC and FO groups (from T0) than for the EC group (from T2).

Compared to the BC group, the n-3:n-6 ratio was high in the SC group at T0; however, at the same time point (T0) this ratio was high in the FO group compared to the other groups.

## DISCUSSION

Despite an initial concern on the antiplatelet aggregation properties of some omega-3-derived metabolites, the parenteral supply of FOLE has been shown to

be clinically safe in surgical patients.[25] Here, we confirmed a favorable modulation of PUFA concentrations in rats after this practice, marked by a higher n-3:n-6 ratio in the plasma, liver and blood leukocytes. In addition, we show for the first time that this effect was labile in all these body polls (mainly in leukocytes), with a rapid progressive loss of n-3 PUFA once infusion of FOLE ceased.

In our study, Lewis rats were fed a standard diet that has soybean oil as the fat source and thus has a relatively low n-3:n-6 PUFA ratio (1:7), very similar to the human Western dietary pattern.[26,27] At baseline (BC group), these animals exhibited almost undetectable EPA and DHA but readily detectable ARA concentrations in liver, plasma and leukocytes. Other studies report detectable amounts of EPA and DHA in plasma and liver phospholipids from animals fed the same standard diet.[28-30] However, we have repeated the chromatographic analyzes on the baseline samples and confirmed our findings.

Given the dynamics of human body, cell incorporation of PUFA occurs in sync with the cellular uptake of its plasma circulating content. Indeed, incorporation rate is at least partly dependent upon rate of turnover of the pool being studied. By studying different body pools, we were able to verify the plasma clearance of PUFA along with its cell incorporation (liver and peripheral leukocytes).

ARA, EPA and DHA were quite sensitive to FOLE infusion, and we observed a higher n-3:n-6 ratio in all pools immediately upon cessation of infusion, compared to the ratio in animals that did not receive the FOLE intervention. This effect decreased over time for all pools, but the n-3:n-6 PUFA ratio remained elevated in liver at 12 hours, in plasma and mononuclear leukocytes at 2 hours, and was no longer detectable in polymorphonuclear leukocytes at 2 hours. Therefore, leukocytes did not retain n-3 PUFA as well as the liver. This finding

suggests that leukocytes use n-3 PUFA at very high rates, making their bioavailability short in these cells.

Particularly, despite the FOLE infusion have provided large amounts of EPA and DHA for FO, EPA concentrations in this group were undetectable in mononuclear and polymorphonuclear leukocytes, but high in liver (vs. baseline) at T0. This finding suggests a high rate of EPA use by leukocytes in the FO group from the surgery to the end of FOLE infusion (4 days). On the other hand, DHA concentrations at T0 were high in leukocytes and liver from the FO group. This may be explained by the relevance of the high unsaturation grade of DHA for leukocytes, which can increase the fluidity of cell membranes required for invagination and phagocytosis. Therefore, our data suggest that EPA was applied for the syntheses of other molecules (i.e. inflammatory mediators) in leukocytes, while DHA was used as part of their cell membranes.

By using EPA for the synthesis of inflammatory mediators, leukocytes can generate eicosanoids with low pro-inflammatory potential. Several studies have largely reported that changes in PUFAs concentrations following FOLE infusion occurs and can be accompanied by better profiles of inflammatory mediators. We did not intend to confirm previous findings in this field, but to evaluate how long these well stablished changes in cell PUFAs profiles remain to support cell immunomodulation and favor clinical outcomes. As far as we know, no other study has evaluated the cell dynamics of PUFAs induced by FOLE infusion after their incorporation in cells. Therefore, our findings are pioneering in showing that cellular incorporation of PUFAs after FOLE infusion, targeted for immunomodulation, is lost in few hours when it ends, especially in leukocytes.

Since the postoperative response can be longer than a few hours, our observations in leukocytes raise the question of whether the FOLE infusion should

be applied to enrich these cells with n-3 PUFA before elective surgeries, for immunomodulation. However, surgical patients often have pathologies involving immunologic disturbances (i.e., cancer, inflammatory bowel diseases) that may be attenuated by preoperative FOLE infusion. Accordingly, a classic retrospective study involving 256 surgical patients reported a larger clinical benefit in infusing FOLE perioperatively compared with just postoperatively.[31] Our data cannot exclude the relevance in supplying FOLE in the preoperative period, but point out to the potential need in extending this intervention during the entire period of the postoperative immune-inflammatory response to achieve clinical benefits.

Infusion protocols of studies evaluating the same FOLE as used here in surgical patients ranged from 4 to 5 postoperative days.[14-17,32,33] In these studies decreased length of hospital stay (LOS) and infection rates are usually more frequent when this FOLE was infused for 5 than for 4 postoperative days, but different effects on LOS also were observed between those studies applying identical infusion protocols.[14,16,17,32] This intriguing effect on LOS may be a reflection of the highly heterogeneous dynamics of the immune response, with postoperative humoral and cellular immune responses being subject to different factors influencing their course. One well-known factor is the type of surgical procedure applied (conventional or laparoscopic), but others are also potentially relevant, such as age, underlying disease and stage of its development.[34]

The infusion protocol used in our study was based on the study of Hagi et al.[22], which reported that the concentrations of n-3 PUFA in splenocytes nearly reached a plateau after a 3-days of infusion of FOLE with different n-3:n-6 PUFA ratios in rats. These authors depleted n-3 and n-6 PUFAs prior to FOLE infusion, while we offered an oral diet having both n-3 and n-6 PUFAs before and during such infusion, to avoid bias from nutritional deficits. Recently, we found higher

concentrations of n-3 PUFAs 48 hours than 72 hours after FOLE infusion in nutritionally non-depleted rats (*in press*). This may explain why we find undetectable EPA concentration in monocytes immediately after 72 hours of FOLE infusion and reinforce the hypothesis that this n-3 PUFA may have be used for the synthesis of inflammatory modulators in the FO group.

Our strengths points are the use of controls for the background diet (BC group) and for the surgical procedure (SC group), along with the inclusion of multiple time points. The main methodological limitation was the need to pool leukocytes for analysis due to the small blood volume available. However, the use of genetically similar animals (isogenic) enabled a more uniform response between them, reducing the inherent bias of analyses in pooled samples. Furthermore, although this was not our study aim, our findings in leukocytes make a coherent biological sense (see Supplementary Text). Differences between the metabolism of Lewis rats and humans also cannot be disregarded.

Although several meta-analysis have confirmed that parenteral infusion of FOLE may improve clinical outcomes in surgical patients, the optimal infusion length to achieve these benefits remains unknown.[35-39] Our data suggest that the bioavailability of n-3 PUFA in leukocytes after this practice is highly labile, and therefore, the supply of n-3 PUFA should cover the entire period of the postoperative immune-inflammatory response dynamic. Studies on this dynamic may aid in the future design of customized protocols for parenteral FOLE infusion, according to the type of surgical intervention and the specific clinical condition of the patient.

## REFERENCES

1. Jing Yu, Lian Liu,Yue Zhang, Jia Wei, and Fan Yang. Effects of omega-3 fatty acids on patients undergoing surgery for gastrointestinal malignancy: a systematic review and meta-analysis. *BMC Cancer*. 2017;17:271.
2. Klek S, Waitzberg DL. Intravenous lipids in adult surgical patients. *World Rev Nutr Diet.* 2015;112:115-9.
3. McCarthy MS, Morgan BB, Heineman JT, Martindale RG. Nutritional armor for the injured warfighter: omega-3 fatty acids in surgery, trauma, and intensive care. *Mil Med.* 2014;179:88-94.
4. Calder PC. Functional Roles of Fatty Acids and Their Effects on Human Health. *JPEN J Parenter Enteral Nutr.* 2015;39:18S-32S.
5. Waitzberg DL, Torrinhas RS. Fish oil lipid emulsions and immune response: what clinicians need to know. *Nutr Clin Pract*. 2009;24:487-99.
6. Dalli J, Ramon S, Norris PC, Colas RA, Serhan CN. Novel proresolving and tissue-regenerative resolvin and protectin sulfido-conjugated pathways. *FASEB J.* 2015;29:2120-36.
7. Healy DA, Wallace FA, Miles, EA, Calder, PC, Newsholme P. Effect of low-to-moderate amounts of dietary fish oil on neutrophil lipid composition ad function. *Lipids.* 2000;35:763-8.
8. Weiss G, Meyer F, Matthies B, Pross M, Koenig W, Lippert H. Immunomodulation by perioperative administration of n-3 fatty acids. *Br J Nutr.* 2002;87:S89-94.
9. Liang B, Wang S, Ye Ying-Jiang, Yang Xiao-Dong, Wang You-Li, Qu J, Xie Qi-Wei, Yin Mu-Jun Impact of postoperative omega-3 fatty acid-supplemented parenteral nutrition on clinical outcomes and immunomodulations in colorectal cancer patients. *World J Gastroenterol.* 2008;14:2434-9.

10. Schauder P, Röhn U, Schäfer G, Korff G, Schenk HD. Impact of fish oil enriched total parenteral nutrition on DNA synthesis, cytokine release and receptor expression by lymphocytes in the postoperative period. *Br J Nutr.* 2002;87:S103–10.
11. Cury-Boaventura MF, Torrinhas RS, Godoy AB, Curi R, Waitzberg DL. Human Leukocyte Death After a Preoperative Infusion of Medium/Long-Chain Triglyceride and Fish Oil Parenteral Emulsions: A Randomized Study in Gastrointestinal Cancer Patients. *JPEN J Parenter Enteral Nutr.* 2012;6:677-84.
12. Long H, Yang H, Lin Y, Situ D, Liu W. Fish oil-supplemented parenteral nutrition in patients following esophageal cancer surgery: effect on inflammation and immune function. *Nutr Cancer.* 2013;65:71-5.
13. de Miranda Torrinhas RS, Santana R, Garcia T, Cury-Boaventura MF, Sales MM, Curi R, Waitzberg DL. Parenteral fish oil as a pharmacological agent to modulate post-operative immune response: a randomized, double-blind, and controlled clinical trial in patients with gastrointestinal cancer. *Clin Nutr.* 2013;32:503-10.
14. Wichmann MW, Thul P, Czarnetzki HD, Morlion BJ, Kemen M, Jauch KW. Evaluation of clinical safety and beneficial effects of a fish oil containing lipid emulsion (Lipoplus, MLF541): data from a prospective, randomized, multicenter trial. *Crit Care Med.* 2007;35:700-6.
15. Köller M, Senkal M, Kemen M, König W, Zumtobel V, Muhr G. Impact of omega-3 fatty acid enriched TPN on leukotriene synthesis by leukocytes after major surgery. *Clin Nutr.* 2003;22:59-64.
16. Zhang CH, Li N, Wang XY, Li GL, Fan CG, Li JS. Influence of Lipoplus fat emulsion on postoperative nutritional status and early inflammatory

response in patients with gastrointestinal malignancies. *Zhonghua Wei Chang Wai Ke Za Zhi.* 2012;15:448-51.

17. Wang J, Yu JC, Kang WM, Ma ZQ. Superiority of a fish oil-enriched emulsion to medium-chain triacylglycerols/long-chain triacylglycerols in gastrointestinal surgery patients: a randomized clinical trial. *Nutrition.* 2012;28:623-9.
18. Honeywell S, Zelig R, Rigassio Radler D1. Impact of Intravenous Lipid Emulsions Containing Fish Oil on Clinical Outcomes in Critically Ill Surgical Patients: A Literature Review. *Nutr Clin Pract.* 2019;34:112-122.
19. McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, McCarthy MS, Davanos E, Rice TW, Cresci GA, Gervasio JM, Sacks GS, Roberts PR, Compher C; Society of Critical Care Medicine; American Society for Parenteral and Enteral Nutrition. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.).*JPEN J Parenter Enteral Nutr.* 2016;40:159-211.
20. Braga M, Ljungqvist O, Soeters P, Fearon K, Weimann A, Bozzetti F; ESPEN. ESPEN Guidelines on Parenteral Nutrition: surgery. *Clin Nutr.* 2009;28:378-86.
21. Yamaguchi N, Lima-Gonçalves E, Waitzberg DL, Diniz Filho AM, Goffi FS. Effects of parenteral nutrition on tumor growth: experimental aspects. *Rev Hosp Clin Fac Med Sao Paulo.* 1990;45:115-22.
22. Hagi A, Nakayama M, Shinzaki W, Haji S, Ohyanagi H. Effects of the omega-6:omega-3 fatty acid ratio of fat emulsions on the fatty acid

composition in cell membranes and the anti-inflammatory action. *JPEN J Parenter Enteral Nutr.* 2010;34:263-70.

23. Huang Z, Wang B, Crenshaw AA. A simple method for the analysis of trans fatty acid with GC–MS and ATTM-Silar-90 capillary column. Food Chem. 2006;98:593-8.
24. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 1959; 37:911-7.
25. Heller AR, Fischer S, Rössel T, Geiger S, Siegert G, Ragaller M, Zimmermann T, Koch T. Impact of n-3 fatty acid supplemented parenteral nutrition on haemostasis patterns after major abdominal surgery. Br J Nutr. 2002 Jan;87 Suppl 1:S95-101.
26. Waitzberg DL, Torrinhas RS, Jacintho TM. New parenteral lipid emulsions for clinical use. JPEN J Parent Enteral Nutr. 2006;30:351-67.
27. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother.* 2002;56:365-79.
28. Mills JD, Bailes JE, Sedney CL, Hutchins H, Sears B. Omega-3 fatty acid supplementation and reduction of traumatic axonal injury in a rodent head injury model. J Neurosurg. 2011;114:77-84.
29. Kotkat HM, Rady AA, Nemcsok J. Effect of dietary fish oil (active EPA-30) on liver phospholipids in young and aged rats. Comp Biochem Physiol A Mol Integr Physiol. 1999;122:283-9.
30. Leng S, Winter T, Aukema HM. Dietary ALA, EPA and DHA have distinct effects on oxylipin profiles in female and male rat kidney, liver and serum. J Nutr Biochem. 2018;57:228-37.

31. Tsekos E, Reuter C, Stehle P, Boeden G. Perioperative administration of parenteral fish oil supplements in a routine clinical setting improves patient outcome after major abdominal surgery. *Clin Nutr.* 2004;23:325-30.
32. Berger MM, Tappy L, Revelly JP, Koletzko BV, Gepert J, Corpataux JM, Cayeux MC, Chiolero RL. Fish oil after abdominal aorta aneurysm surgery. *Eur J Clin Nutr.* 2008;62:1116-22.
33. Senkal M, Geier B, Hannemann M, Deska T, Linseisen J, Wolfram G, Adolph M. Supplementation of omega-3 fatty acids in parenteral nutrition beneficially alters phospholipid fatty acid pattern. *JPEN J Parenter Enteral Nutr.* 2007;31:12-7.
34. Ordemann J, Jacobi CA, Schwenk W, Stösslein R, Müller JM. Cellular and humoral inflammatory response after laparoscopic and conventional colorectal resections. *Surg Endosc.* 2001;15:600-8.
35. Chen B, Zhou Y, Yang P, Wan HW, Wu XT. Safety and efficacy of fish oil-enriched parenteral nutrition regimen on postoperative patients undergoing major abdominal surgery: a meta-analysis of randomized controlled trials. *JPEN J Parenter Enteral Nutr.* 2010;34:387-94.
36. Zhao Y, Wang C. Effect of ω-3 polyunsaturated fatty acid-supplemented parenteral nutrition on inflammatory and immune function in postoperative patients with gastrointestinal malignancy: A meta-analysis of randomized control trials in China. *Medicine (Baltimore)*. 2018;97:e0472.
37. Langlois PL, Hardy G, Manzanares W. Omega-3 polyunsaturated fatty acids in cardiac surgery patients: An updated systematic review and meta-analysis. *Clin Nutr*. 2017;36:737-46.

38. Li NN, Zhou Y, Qin XP, Chen Y, He D, Feng JY, Wu XT. Does intravenous fish oil benefit patients post-surgery? A meta-analysis of randomised controlled trials. *Clin Nutr*. 2014;33:226-39.
39. Wei C, Hua J, Bin C, Klassen K. Impact of lipid emulsion containing fish oil on outcomes of surgical patients: systematic review of randomized controlled trials from Europe and Asia. *Nutrition*. 2010;26:474-81.

Table 1. Fatty acid composition (% of total fatty acids) of the control (Lipofundin® 20%) and fish oil-containing (Lipidem® 20%) parenteral lipid emulsions studied

| **Fatty Acids** | Lipofundin® 20% | Lipidem® 20% |
|---|---|---|
| Caprylic acid (C8:0) | 25.6 | 26.1 |
| Capric acid (C10:0) | 19.3 | 19.7 |
| Palmitic acid (C16:0) | 7.2 | 6.1 |
| Stearic acid (C18:0) | 2.8 | 2.6 |
| Oleic acid (C18:1n-9) | 13.0 | 11.4 |
| Linoleic acid (C18:2n-6) | 26.8 | 21.9 |
| Alpha-linolenic acid (C18:3n-3) | 3.4 | 2.8 |
| Stearidonic acid (C18:4n-3) | - | 0.2 |
| Eicosatetraenoic acid (C20:4n-3) | - | 0.2 |
| Arachidonic acid (C20:4n-6) | 0.3 | 0.4 |
| Eicosapentaenoic acid (C20:5n-3) | - | 3.3 |
| Docosanoic acid (C22:0) | - | 0.2 |
| Docosapentaenoic acid (C22:5n-3) | 0.2 | 0.6 |
| Docosahexaenoic acid (C22:6n-3) | | 2.5 |
| Others | 1.4 | 2.0 |
| Medium-chain triglycerides | 52.1 | 51.9 |
| Omega-9 monounsaturated fatty acids | 13.0 | 11.4 |
| Omega-6 polyunsaturated fatty acids | 27.1 | 22.3 |
| Omega-3 polyunsaturated fatty acids | 3.6 | 9.4 |
| Omega-3:Omega-6 ratio | 1:7.5 | 1:2.4 |

Data were provided by the manufacturer (BBraun, Melsungen, Germany).

Table 2. Concentration of polyunsaturated fatty acids (mg/L) in the plasma and hepatic tissue of Lewis rats sacrificed at different times following the infusion of parenteral lipid emulsion containing fish oil

| Variable | | Plasma (mg/L) | | | | Liver (mg/L) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **EPA** | **DHA** | **ARA** | **n-3:n-6** | **EPA** | **DHA** | **ARA** | **n-3:n-6** |
| Time* | 0 | 1513 (1366-1661) | 0 | 12111 (18459-259) | 0.070 | 1867 (1720-2015) | 3938 (3490-4387) | 22902 (19728-26076) | 0.184 |
| | 2 | 717 (705-775) | 0 | 3015 (4684-276) | 0.129 | 1153 (911-1295) | 1509 (1065-1957) | 14169 (12553-14984) | 0.129 |
| | 6 | 0 | 0 | 2321 (2743-263) | 0 | 988 (908-1270) | 1167 (1058-1825) | 14051 (12974-14973) | 0.108 |
| | 12 | 0 | 0 | 808 (984-250) | 0 | 201 (100-284) | 538 (524-596) | 2466 (2187-2670) | 0.081 |
| P Value[#] | T0 *vs.* T2 | 0.002 | - | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
| | T0 *vs*. T6 | 0.003 | - | 0.002 | 0.003 | 0.005 | 0.002 | 0.002 | 0.002 |
| | T0 *vs*. T12 | 0.003 | - | 0.002 | 0.003 | 0.002 | 0.002 | 0.002 | 0.002 |
| | T2 *vs*. T6 | 0.003 | - | 0.002 | 0.003 | 0.378 | 0.394 | 0.937 | 0.132 |
| | T2 *vs*. T12 | 0.003 | - | 0.002 | 0.003 | 0.002 | 0.002 | 0.002 | 0.002 |
| | T6 *vs*. T12 | - | - | 0.002 | - | 0.005 | 0.002 | 0.002 | 0.002 |

Legend: Data are expressed as the median (minimum-maximum). EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; n-3:n-6, omega-3:omega-6 ratio. *Hours after the end of the lipid emulsion infusion; [#]Wilcoxon Test

**FIGURE CAPTIONS**

Figure 1. Concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) and omega-3:omega-6 (n-3:n-6) ratio in plasma samples of Lewis rats sacrificed at different times following the ending of parenteral infusion of lipid emulsion with or without fish oil

Legend. Data are expressed as mg/L (Y axis) at different times (X axis), representing the number of hours between the end of the parenteral infusion and sacrifice (T). BC, baseline values in Lewis rats for each polyunsaturated fatty acid, defined by analyses of animals without any surgical or nutritional intervention; SC, animals submitted only to catheterization of the central venous system; EC, animals submitted to catheterization of the central venous system and receiving parenteral infusion of lipid emulsion control, without fish oil; FO, animals submitted to catheterization of the central venous system and receiving parenteral infusion of lipid emulsion containing fish oil. Data on BC group were obtained at a single time point and applied for comparison between groups across all the time points studied.

Figure 2. Concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) and omega-3:omega-6 (n-3:n-6) ratio in liver samples of Lewis rats sacrificed at different times following the ending of parenteral infusion of lipid emulsion with or without fish oil

Legend. Data are expressed as mg/L (Y axis) at different times (X axis), representing the number of hours between the end of the parenteral infusion and sacrifice (T). BC, baseline values in Lewis rats for each polyunsaturated fatty acid, defined by analyses of animals without any surgical or nutritional

intervention; SC, animals submitted only to catheterization of the central venous system; EC, animals submitted to catheterization of the central venous system and receiving parenteral infusion of lipid emulsion control, without fish oil; FO, animals submitted to catheterization of the central venous system and receiving parenteral infusion of lipid emulsion containing fish oil. Data on BC group were obtained at a single time point and applied for comparison between groups across all the time points studied.

Figure 3. Concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) acids omega-3:omega-6 (n-3:n-6) ratio in mononuclear leukocytes from Lewis rats sacrificed at different times following the ending of parenteral infusion of lipid emulsion with or without fish oil

Legend. Data are expressed as mg/L (Y axis) at different times (X axis), representing the number of hours between the end of the parenteral infusion and sacrifice (T). BC, baseline values in Lewis rats for each polyunsaturated fatty acid, defined by analyses of animals without any surgical or nutritional intervention; SC, animals submitted only to catheterization of the central venous system; EC, animals submitted to catheterization of the central venous system and receiving parenteral infusion of lipid emulsion control, without fish oil; FO, animals submitted to catheterization of the central venous system and receiving parenteral infusion of lipid emulsion containing fish oil. Data on BC group were obtained at a single time point and applied for comparison between groups across all the time points studied.

Figure 4. Concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) and omega-3:omega-6 (n-3:n-6) ratio in polymorphonuclear leukocytes from Lewis rats sacrificed at different times following the ending of parenteral infusion of lipid emulsion with or without fish oil

Legend. Data are expressed as mg/L (Y axis) at different times (X axis), representing the number of hours between the end of the parenteral infusion and sacrifice (T). BC, baseline values in Lewis rats for each polyunsaturated fatty acid, defined by analyses of animals without any surgical or nutritional intervention; SC, animals submitted only to catheterization of the central venous system; EC, animals submitted to catheterization of the central venous system and receiving parenteral infusion of lipid emulsion control, without fish oil; FO, animals submitted to catheterization of the central venous system and receiving parenteral infusion of lipid emulsion containing fish oil. Data on BC group were obtained at a single time point and applied for comparison between groups across all the time points studied.