1 **Is there an advantage in enriching parenteral lipid emulsions containing**
2 **fatty acids from fish oil with medium-chain triglycerides? A study on body**
3 **pool concentrations of omega-3 fatty acids in Lewis rats**

4

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32

33 **CLINICAL RELEVANCY STATEMENT**

34 Omega (n-) 3 polyunsaturated fatty acids (PUFAs) have immunologic properties
35 of clinical interest that highly depend on their availability to leukocytes. Addition
36 of medium-chain triglycerides (MCT) into lipid emulsions rich in n-3 PUFAs from
37 fish oil (FOLE) has been suggested to improve their circulatory clearance and cell
38 delivery. This effect could increase n-3 PUFAs uptake by leukocytes.
39 Furthermore, MCT are preferred energy substrates over long-chain fatty acids
40 and could spare n-3 PUFAs from beta-oxidation, allowing their leukocyte
41 availability for immunological purposes. In our study, n-3 PUFAs provided by the
42 infusion of a FOLE rich in MCT were less incorporated by the liver and remained
43 more available for extra-hepatic cell delivery, in Lewis rats. However, this did not
44 result in a clear benefit in increasing n-3 PUFAs incorporation by peripheral
45 leukocytes. Our data suggest no advantages in adding MCT to FOLEs to increase
46 leukocyte n-3 PUFAs incorporation for immunomodulation.

47

ABSTRACT

Addition of medium-chain triglycerides (MCT) into parenteral lipid emulsions rich in fatty acids from fish oil (FOLE) has been shown to improve their clearance and extrahepatic uptake. We assessed whether this effect could favor the leukocyte uptake of n-3 polyunsaturated fatty acids (PUFAs) for immunomodulatory purposes. **Methods:** Following 5-days adaptation in metabolic cages, 42 male Lewis rats (≈350g) fed with AIN-93M chow were sacrificed (baseline control group [BC]) or submitted to central venous catheterization and distributed into: i) Surgical control group (chow), without parenteral infusion; ii) Test emulsion group (MCT/LCT/FO), with the parenteral infusion of a FOLE containing 40% MCT; and iii) Control emulsion group (LCT/FO), with the parenteral infusion of a FOLE without MCT. The two FOLE had similar n-3 PUFA contents and n-6:n-3 PUFA ratios and were infused during 48 h and 72 h. Concentrations of n-3 and n-6 PUFAs in plasma, liver and blood mononuclear and polymorphonuclear leukocytes were assessed by gas chromatography. **Results:** In both FOLE groups, leukocyte concentrations of n-3 PUFAs peaked after 48 h infusion (*vs.* BC). At this time point, plasma concentrations of n-3 PUFAs were higher in MCT/LCT/FO group than in LCT/FO group and the opposite was found in the liver ($p<0.05$), but no differences in PUFAs concentrations were observed between these groups in leukocytes ($p>0.05$). **Conclusion:** The n-3 PUFAs provided by FOLE rich in MCT were less incorporated by liver and remained more available for extra-hepatic cell delivery, but, this did not result in a clear benefit in increasing their incorporation by peripheral leukocytes.

Descriptors: fatty acids, omega-3; fish oils; parenteral nutrition; leukocytes, mononuclear; neutrophils; rats.

73 **INTRODUCTION**

74 Once incorporated into cells (mainly leukocytes), eicosapentaenoic (EPA)
75 and docosahexaenoic (DHA) acids can attenuate inflammation by serving as
76 precursors of specific lipid-derived immunological mediators or affecting cell
77 signaling and gene transcription. [1,2] The clinical supply of these long-chain omega
78 (n-)3 polyunsaturated fatty acids (PUFAs) has been proposed as an effective
79 adjuvant approach to treat complications associated with deleterious immune
80 responses. [3] Strategies to improve the cellular uptake of n-3 PUFAs may favor
81 better outcomes.

82 Lipid emulsions rich in n-3 PUFAs from fish oils (FOLEs) can be infused to
83 supply EPA and DHA in patients under parenteral nutrition and are commercially
84 available. FOLEs are composed of fish oil (FO) alone or of FO/FO-derived n-3
85 triglycerides mixed with other oils. [4] Due to the low content of essential n-6 PUFAs,
86 FOLE composed only of FO is usually supplied during the infusion of other standard
87 lipid emulsions (LE), composed of soybean oil alone or mixed with olive oil or
88 medium-chain triglycerides (MCT). [5]

89 A recent meta-analysis reported a lower relative risk of infection (40%) and
90 sepsis (56%) as well as reduction in length of intensive care unit (ICU) and
91 hospital stays (by about 2 days) in adult hospitalized patients who received FOLE
92 infusion, in comparison to those patients who received standard lipid emulsions
93 (LEs) without FO. [6] The mortality rate was non-significantly lower in the FOLE
94 group (16%). Improvements in leukocyte uptake of n-3 PUFAs following infusion
95 of FOLEs could contribute to better clinical outcomes, for example significantly
96 lower mortality rates.

97 Previous elegant studies had suggested that combining MCT to omega-3
98 triglycerides in parenteral LE could result in greater n-3 PUFAs delivery to
99 extrahepatic pools and this effect could be important to modulate immune and
100 other responses.[7-10] Here, we aimed to test whether a new commercially available
101 mixed FOLE enriched with MCT could lead to a faster clearance of n-3 PUFAs
102 along with lower liver uptake, leading to a faster and/or higher peripheral
103 leukocytes incorporation. For this purpose, we infused this new FOLE during 72
104 h (a period where a peak of cell n-3 PUFA incorporation was previously observed
105 after infusion of FOLEs) and also during 48 h (as a shorter period) and analyzed
106 the concentrations of n-3 and n-6 PUFAs as well as the n-3:n-6 ratios in plasma,
107 liver and peripheral leukocytes. These analyses were done in comparison to the
108 infusion of a LE emulsion without MCT but with similar n-3 PUFAs amount and
109 n-3:n-6 PUFAs ratio, composed by an experimental mixture of two commercial
110 LEs based only on soybean oil and based only on fish oil .
111
112 **METHODS**
113 **Animals**
114 Forty-two adult male Lewis isogenic rats (350-400 g) were purchased from
115 the Multidisciplinary Center for Research in Biological Science Laboratory Animal
116 Area (Campinas, Sao Paulo, Brazil). Prior to the experimental procedures, the
117 animals were adapted for 5 days in individual metabolic cages at a controlled
118 room temperature (20 – 25°C) with a 12-h light–dark cycle and with free access
119 to AIN-93M diet and water. All the experimental procedures were approved by
120 the Research Ethical Committee, School of Medicine, University of Sao Paulo,
121 Sao Paulo, Brazil (063/15).

122 **Experimental interventions**

123 After the 5-days adaptation, animals were anesthetized by the
124 intraperitoneal injection of ketamine (100 mg/kg body weight; Parke-Davis, Ache,
125 São Paulo, Brazil) and xylazine (8 mg/kg of body weight; Bayer, Leverkusen,
126 Germany) and submitted to humane euthanasia by cardiac puncture (baseline
127 control group - BC group, n = 6) or subjected to central venous system access (n
128 = 36). Intravenous access was achieved by jugular central venous catheterization
129 (CVC) performed according to a standardized technique[11] and by using a specific
130 catheter connected to a gyratory swivel apparatus, which ensured free mobility
131 for the animals. Following surgery, the 36 catheterized animals received a 24 h-
132 infusion (0.25 mL / h) of 6 mL saline solution (0.9%) and were distributed evenly
133 among three different groups: chow group remained only on oral diet AIN-93M
134 (20 g/day); MCT/LCT/FO group remained on oral diet AIN-93M (20 g/day) and
135 also received the parenteral infusion of a MCT-rich FOLE; and LCT/FO group
136 remained on oral diet AIN-93M (20 g/day) and received the parenteral infusion of
137 an experimental FOLE without MCT. The LE supplied in the MCT/LCT/FO group
138 was Lipidem® 20% (BBraun, Melsugen, Germany) containing 40% soybean oil,
139 50% MCT from coconut oil base and 10% fish oil, while the LCT/FO group
140 received a control LE composed by the mixture of 80% soybean oil LE
141 (Lipovenos® 10%, Fresenius-Kabi, Germany) and 20% fish oil LE (Omegaven®
142 10%, Fresenius-Kabi, Germany). Both LE were similar in relation to the amount
143 of n-3 and n-6 PUFAs they contained, and differed only in MCT presence, as
144 shown in Table 1. AIN-93M diet contained 0.05% of total fat as n-3 PUFA as
145 alpha-linolenic acid (ALA), a precursor of EPA and DHA (Table 1). The chow
146 group (n = 12) was followed for 48 h and 72 h after catheterization, while the

147 MCT/LCT/FO (n = 12) and LCT/FO (n = 12) groups received the infusion of their
148 respective FOLEs during these periods, in a concentration of 4.3 g fat / kg body
149 weight[12] and by using a gravity infusion pump (Space® perfusor, BBraun). After
150 both these periods, the humane euthanasia of all the catheterized animals was
151 performed, such that there were 6 animals for each group/ euthanasia period.

152 **Sample collection**

153 All animals were submitted to cardiac puncture for blood collection in a 4
154 mL heparin vacuum tube and laparotomy for liver tissue sampling. Blood samples
155 were processed to yield plasma (10 min, 1800 rpm, 20°C centrifugation). Mono
156 and polymorphonuclear leukocytes were isolated from saline-diluted blood (1:1
157 v/v) by density gradient (30 min, 1800 rpm, room temperature) centrifugation with
158 1:1 v/v Histopaque® 1077 or Histopaque® 1119, respectively (both from Sigma-
159 Aldrich, St. Louis, USA). The plasma and cell samples were frozen at -80 °C and
160 the liver samples were frozen in liquid nitrogen until PUFAs profile analysis.

161 **PUFAs analysis**

162 Concentrations of EPA and DHA, as well as n-3:n-6 ratio, were assessed in
163 plasma, liver and leukocytes by gas chromatography. The n-3:n-6 ratio was
164 calculated by summing the absolute values of EPA, DHA and ALA divided by the
165 sum of the absolute values of linoleic acid (LA) and arachidonic acid (ARA). There
166 was not a sufficient amount of peripheral leukocytes to perform individual sample
167 analyses, so the PUFAs concentrations in these cells were assessed in pools from
168 6 rats, in duplicate, as determined by a previous pilot study (non-published). Plasma
169 and leukocytes lipids were extracted and converted to fatty acid methyl esters as
170 described elsewhere[13]. For liver lipid extraction, 100 mg of hepatic tissue was added
171 to 500 µL of 0.88% KCl solution (Synth, São Paulo, Brazil) and homogenized in a

172 mini Turrax homogenizer. Subsequently, lipids were extracted from the liver
173 homogenate according to the protocol of Bligh and Dyer.[14] An aliquot from each total
174 lipid extract was used in a methylation process with methanol:chloroform:chloridric
175 acid (10:1:1 v/v; chloridric acid from Synth, São Paulo, Brazil) for 2 h at 80 °C and
176 then fatty acid methyl esters (FAME) were extracted three times using hexane. The
177 organic phase was dried and resuspended in 100 µL of hexane. The PUFAs profile
178 in plasma, liver and leukocytes was measured using flame-ionization gas
179 chromatography (SHIMADZU, CG-2010, Kyoto, Japan). Samples (2 µL) were
180 injected via an autosampler into a fused-silica capillary column (DB-FFAP capillary
181 column [15 m× 0.100 mm× 0.10 µm] J&W Scientific from Agilent Technologies,
182 Folsom, CA, USA) in a gas chromatography system fitted with a flame ionization
183 detector and eluted with hydrogen at 3.0 mL/min., with a split ratio of 1:150. The
184 injector and detector were heated to 250°C and 260°C, respectively. The column
185 was temperature programmed from 100°C (hold 0.5 min) to 195°C at 25°C/min, then
186 to 205°C (hold 3 min) at 3.0°C/min. Data on PUFA concentrations (mg/L) were
187 obtained by external standardization using commercial reference standards to
188 construct the calibration curve (Sigma®, Sigma-Aldrich, Missouri, USA).
189

190 **Statistical analysis**

191 For plasma and liver, intragroup analyzes were performed with the
192 nonparametric Kruskal-Wallis test and a post-hoc Mann-Whitney test.
193 Differences between groups were assessed by Wilcoxon test. Evaluations in pool
194 of peripheral leukocytes resulted in the generation of only one representative
195 result for each group studied, so the analyzes of the leukocyte content of PUFA
196 were only descriptive. The analysis and generation of graphs were done using

197 the R program (version 3.2.2; R Core Team, Vienna, Austria), adopting a 5%
198 significance level.

199 **RESULTS**

200 ***Food intake***

201 During the 5 days for adaptation before surgery, the rats ingested, daily,
202 the 20 g of rodent chow AIN-93 M offered. As shown in Table 2, after surgery, all
203 animals decreased their oral intake, including the chow group. At 48h, this
204 variation was not statistically significant in any of the groups studied. At 72h, there
205 was a reduction in food intake among animals in the MCT/LCT/FO ($p = 0.0031$).

206 ***EPA and DHA concentrations***

207 Changes in EPA and DHA concentrations in plasma and liver are shown
208 in Figure 1, while changes in these n-3 PUFAs in both leukocyte populations are
209 shown in Table 3. According to the analysis of BC group, our Lewis rats had
210 undetectable amounts of EPA and DHA in all blood pools studied, except for a
211 low EPA concentration in the mononuclear leukocyte population (1.3%). At the
212 two time points studied (48 and 72hs), the chow group did not exhibit detectable
213 concentrations of EPA in either mononuclear or polymorphonuclear leukocytes,
214 but concentrations of DHA were detectable in both cell types. After 48 h-infusion
215 of both FOLEs (*vs*. BC), the concentrations of EPA and DHA increased in plasma
216 ($p = 0.003$), liver ($p = 0.003$) and leukocytes, but this increase was higher in the
217 MCT/LCT/FO group than in the LCT/FO group in plasma ($p = 0.002$), higher in
218 the LCT/FO group than in the MCT/LCT/FO group in liver ($p = 0.002$), and similar
219 between these groups in both leukocyte populations. After 72 h-infusion of both
220 FOLEs (*vs*. BC), the plasma concentrations of EPA decreased in the
221 MCT/LCT/FO group ($p = 0.003$) and increased in the LCT/FO group ($p = 0.003$),

222 while the concentrations of this n-3 PUFA did not change in leukocytes and the
223 concentrations of DHA were apparently increased in leukocytes and did not
224 change in plasma ($p > 0.05$). Moreover, both EPA and DHA increased in liver (p
225 $= 0.003$ *vs*. BC) with a higher intensity in the LCT/FO group than in the
226 MCT/LCT/FO group ($p = 0.002$) after 72h-infusion of both FOLEs.
227
228 ***n-3:n-6 ratio***
229 As shown in Figure 2, after 48-h infusion of both FOLEs, plasma n-3:n-6
230 ratio was higher in the MCT/LCT/FO group than in the LCT/FO group ($p = 0.030$),
231 while the opposite was observed at the 72-h infusion time point ($p = 0.002$). In
232 the liver, n-3:n-6 ratio was higher in the LCT/FO group than in the MCT/LCT/FO
233 group after 48 ($p = 0.002$) and 72 hours ($p = 0.002$). This effect on n-3:n-6 ratios
234 was accompanied not only by the described increases of EPA and DHA, but also
235 by changes in LA and ARA concentrations, while ALA levels were undetectable
236 in these samples. At the 48-h and 72-h time points (*vs*. BC) the LA concentration
237 in plasma increased in the chow group and decreased to a similar extent in the
238 MCT/LCT/FO and LCT/FO groups ($p = 0.002$), while the concentration of this n-
239 6 PUFA in liver decreased in all groups ($p = 0.002$) to a lower extent in the chow
240 group than in the FOLE groups and to a higher extent in the MCT/LCT/FO group
241 than in the LCT/FO group ($p = 0.002$). In parallel, the ARA concentration in
242 plasma and liver decreased in the chow group and increased in the FOLE groups
243 ($p = 0.002$), but in plasma this increase was similar between the MCT/LCT/FO
244 and LCT/FO groups at 48 h ($p > 0.050$) and higher in the MCT/LCT/FO group
245 than in LCT/FO group at 72 h ($p = 0.002$); while in liver this increase was higher
246 in the MCT/LCT/FO group than in the LCT/FO group at both time points studied

247 (p = 0.002). Due to the absence of detectable n-3 or n-6 PUFAs amounts in some
248 samples, the n-3:n-6 ratio was not calculated in leukocytes. However, as shown
249 in Table 3, we observed in leukocytes an apparent decrease of LA concentrations
250 in all groups at 48 h and 72 h (*vs*. BC), where the chow group presented the lower
251 LA concentrations in mononuclear cells and the higher LA concentrations in
252 polymorphonuclear cells. On the other hand, in mononuclear cells the ARA
253 concentrations increased in the chow group but were undetectable in both the
254 FOLE groups, while in polymorphonuclear cells ARA decreased in all the groups.
255
256 ***Dynamics of PUFAs incorporation***
257 As shown in Figure 1 (liver and plasma) and Table 3 (leukocytes),
258 comparing the 48 h infusion to the 72 h infusion time points, the plasma
259 concentrations of EPA at 72 h were higher in the LCT/FO group (p = 0.002) and
260 drastically lower in the MCT/LCT/FO group (p = 0.002), while being lower in both
261 of these groups in liver (p = 0.002) and leukocytes. The same type of comparison
262 for DHA showed, similar lower concentrations of this n-3 PUFA at 72 h (*vs*. 48h)
263 in both the MCT/LCT/FO and LCT/FO groups in plasma (p = 0.002) and
264 mononuclear leukocytes. In addition, at 72h (*vs*. 48h), DHA concentrations were
265 apparently higher in the LCT/FO group and lower in the MCT/LCT/FO group in
266 polymorphonuclear leukocytes (Table 3), and did not change in liver (p > 0.050).
267 No changes were observed in the LA and ARA concentrations in leukocytes
268 among the time points studied, except for apparently higher LA concentrations in
269 polymorphonuclear leukocytes at 72 h than at 48 h in chow and both FOLEs
270 groups (Table 3). As shown in Figure 2, by comparing the time points studied, no
271 changes were observed in the plasma LA concentrations, but higher plasma ARA

272 concentrations were observed at 72 h than at 48 h in both FOLE groups (p =
273 0.002). The same comparison in liver showed that at 72 h time point (*vs*. 48 h)
274 the LA concentrations were lower in the chow (p = 0.002) and MCT/LCT/FO (p =
275 0.015) groups and higher in the LCT/FO group (p = 0.002), while the ARA
276 concentrations were higher in the MCT/LCT/FO group (p = 0.002). In addition,
277 the n-3:n-6 ratio was lower at 72 h than at 48 h time point in plasma (p = 0.002)
278 and liver (p = 0.002) in all the groups studied.

279

280 **DISCUSSION**

281 Some studies have suggested that adding MCTs into FOLEs could
282 improve the circulatory clearance and cell delivery of n-3 PUFAs, by invoking
283 more efficient pathways for the removal of lipid particles from the bloodstream.[15-
284 17] In turn, this effect could favor n-3 PUFAs uptake by leukocytes following FOLEs
285 infusion and contribute to better clinical outcomes. Furthermore, since medium-
286 chain fatty acids are more preferred as energy substrates than long-chain fatty
287 acids,[9,10] n-3 PUFAs infused in the presence of MCTs could be spared from beta-
288 oxidation in leukocytes, remaining available to other cell functions (i.e. synthesis
289 of immunological mediators). Here, in Lewis rats, we did not identify a higher
290 potential in mixing FOLEs with MCTs to improve the leukocyte uptake of n-3
291 PUFAs.

292 Treskova et al. (1999) reported a slower blood clearance of a LE
293 containing only n-3 triglycerides than a LE containing n-3 triglycerides combined
294 to soybean oil (SO) and MCT.[8] Following the same line, Ton et al. (2015)
295 compared the blood clearance of a LE containing only SO and two LEs containing
296 n-3 triglycerides combined to SO and MCT or combined only to MCT. The LE

297 combining n-3 triglycerides only to MCT cleared faster than the other two LEs. In
298 addition, the authors verified that the SO based LE has the greatest liver uptake,
299 while the LE containing n-3 triglycerides combined only to MCT had the greatest
300 lipoprotein lipase-mediated hydrolysis.[7] Accordingly, at 72h time point we
301 observed a higher plasma clearance of EPA along with a lower liver uptake of
302 this n-3 PUFAs in the MCT/LCT/FO group, comparing to the LCT/FO. However,
303 at 48h timepoint we observed only the lower liver uptake of EPA in the
304 MCT/LCT/FO group comparing to the LCT/FO. Our data did not fully confirm a
305 higher potential in mixing FOLEs with MCT to improve the circulatory clearance
306 of n-3 PUFAs, but reinforce that MCT can improve their extra-hepatic uptake.
307 Nevertheless, we could not attribute this effect to an improvement in the n-3
308 PUFAs leukocyte uptake, since EPA and DHA concentrations in leukocytes were
309 similar between MCT/LCT/FO and LCT/FO groups.

310 Hagi and colleagues, found a maximum incorporation peak of n-3 PUFAs
311 in splenocytes after 72 h of intravenous infusion of FOLEs with different n-3:n-6
312 PUFAs ratios.[12] In our study, we infused FOLEs, for 72 h and also for 48 h, to test
313 whether the association with MCT could lead to a faster leukocyte uptake of n-3
314 PUFAs. Surprisingly, we found a maximum incorporation peak of n-3 PUFAs in
315 liver and leukocytes after 48 h infusion, regardless the presence of MCT into
316 FOLE. Differences in experimental protocol may explain this finding: Hagi et al.
317 depleted n-3 and n-6 PUFAs prior to FOLEs infusion, which was done as part of
318 total parenteral nutrition formulations. Before our infusion protocol, we offered an
319 oral diet having both n-3 and n-6 PUFAs and maintained this oral diet during the
320 infusion protocol to avoid nutritional deficits as potential bias, since we only
321 provide fat parenterally. Therefore, the animals of Hagi et al. may have taken an

322 extra time to replace the depleted n-3 PUFAs after infusion. Our data suggest
323 that in physiological conditions the peak of cell uptake of n-3 PUFAs in rats takes
324 48 hours to be achieved after FOLEs infusion and this period did not seem to be
325 shortened by adding MCT to FOLEs. However, we did not test a period shorter
326 than 48 hours of FOLEs infusion to confirm this hypothesis.

327 It is worth to note that our oral diet ALA as the only n-3 PUFA source and
328 a low n-3:n-6 PUFA ratio, similar to that usually found in human consuming
329 western diets.[18] The gas chromatography analysis did not identify ALA in any of
330 the body pool here studied, suggesting that it was used for energy generation or
331 endogenously converted to other n-3 PUFA. In humans, most of ingested ALA is
332 used as energy source and only 8.0-20.0% and 0.5-9.0% are converted to EPA
333 and DHA, respectively.[19] No study has directly compared desaturase activities or
334 EPA and DHA synthesis rates between humans and rats, but Domenichiello et
335 al. (2015) found percentages of ALA conversion to DHA in rats similar to those
336 previous estimated in humans.[20.] Although significantly higher, the total amount of
337 n-6 PUFAs provided per kilogram of oral diet was 10.20 g as LA – approximately
338 4 times lower than the amount of LA provided per liter of the FOLEs studied. LA
339 also needs to be endogenously converted to generate ARA. Therefore, the
340 amount and quality of n-3 and n-6 PUFAs available in the oral diet are unlikely to
341 interfere in our results, even under the oral intake differing across studied groups.

342 Cell uptake of PUFAs may depend on their half-life in the circulation for
343 delivery. In the human body, lipids can be used for the synthesis of energy,
344 membranes, hormones and inflammatory mediators, as well as for the transport
345 of some vitamins.[5] So, the half-life of circulating lipids infused as parenteral
346 formulations may change according to low or high body needs. In our study,

347 animals from both FOLEs groups were not depleted of PUFA and did not receive
348 an additional stress stimulus, except for the same surgical catheterization.
349 Therefore, similar circulating half-life of PUFAs between the LCT/FO and
350 MCT/LCT/FO groups is expected.

351 According to the analysis of the BC group, blood mononuclear and
352 polymorphonuclear leukocytes from Lewis rats exhibited almost undetectable
353 levels of EPA and DHA and high levels of LA at baseline. The almost absence of
354 EPA and DHA in our samples was unexpected, as other studies report detectable
355 amounts of these PUFAs in plasma and liver phospholipids from animals fed the
356 same standard diet.[21-23] However, we have repeated the chromatographic
357 analyzes and confirmed our findings in BC group.

358 After infusion of both lipid emulsions (MCT/LCT/FO and LCT/FO) for 48
359 and 72 h, the n-3 PUFA (EPA and DHA) concentrations increased and the n-6
360 PUFA concentrations exhibited distinct behavior on each biological sample
361 evaluated: liver and plasma showed decreased LA and increased AA
362 concentrations, while in the leukocytes the opposite happened, increased LA
363 concentrations and decreased AA concentrations, which may indicate a lower
364 conversion between these fatty acids or differences in utilization. Thus, the effect
365 of administering a LE containing fish oil is in line with other studies that
366 investigated the incorporation of oral n-3 PUFAs into serum phospholipids and
367 cell membranes.[24-28]

368 In the present study, we observed an inverse proportional relationship
369 between n-3 and n-6 PUFAs after parenteral infusion of different LEs: AA and LA
370 concentrations in polymorphonuclear leukocytes and plasma decreased at the
371 same time as EPA and DHA concentrations increased in these body pools. High

372 levels of AA-derived eicosanoids may be found in patients with sepsis and
373 hypercatabolism. Part of the proinflammatory and immunosuppressive effects of
374 these mediators may be the result of their influence on cytokine production. For
375 example, 4-series leukotrienes increase IL-1, IL-2, and IL-6 production and
376 lymphocyte proliferation. On the other hand, prostaglandin E2, in spite of inducing
377 fever and increasing vascular permeability and vasodilation, inhibits the
378 production of proinflammatory cytokines IL-1, IL-2, IL-6 and TNF-α which activate
379 leukocyte functions. With increased supply of n-3 PUFAs, the synthesis of
380 prostaglandins and leukotrienes from AA is reduced and thus favourable
381 modulation of inflammatory cytokines occurs, particularly in humans. [29-31]

382 The n-6 and n-3 fatty acid families compete for the enzymes involved in
383 fatty acyl chain desaturation and elongation reactions. Although these enzymes
384 have higher affinity for n-3 PUFAs, the conversion of alpha-linolenic acid to EPA
385 and DHA is strongly influenced by dietary linoleic acid levels. Thus, the increase
386 in the n-3:n-6 ratio is associated with less intense inflammatory responses and
387 also with a reduction in proinflammatory cytokine production. The recommended
388 n-3:n-6 ratio for promoting immunomodulation is about 1:2.5 as demonstrated
389 previously by Masters and colleagues.[32] On the other hand, ratios of less than
390 1:1 are not recommended. In our study, we observed that the ratios between n-
391 3:n-6 PUFAs and between EPA:AA were higher in the LE-infused groups than in
392 the BC and chow groups.

393 We do not observe visual differences in the incorporation of PUFAs
394 between the two types of leukocyte studied. We found that polymorphonuclear
395 leukocytes had higher n-3 PUFA concentrations than mononuclear cells. This
396 difference in the cellular incorporation of fatty acids could be explained by the

397 distinct differences in the turnover and metabolism of these cells. While
398 monocytes have an average half-life of 10 to 20 h in the circulation, neutrophils
399 have a half-life range of 6 to 7 h in circulation. Thus, polymorphonuclear
400 leukocytes would need more lipids incorporated into their membrane as they are
401 in a constant process of cell replication. [33,34]

402 This study has methodological limitations that deserve to be highlighted:
403 The number of leukocytes in the blood of an adult rat weighing about 350 grams
404 is insufficient to allow individual fatty acid analysis by gas chromatography. To
405 circumvent this difficulty, we chose to pool leukocytes from different animals.
406 Studies in this area generally choose to analyze the cellular incorporation of these
407 FAs in platelets or red blood cells because they are more numerous, however,
408 they do not reflect the incorporation in mono and polymorphonuclear leukocytes
409 - considered important agents in the inflammatory state. It is noteworthy here that
410 the use of isogenic animals enables a more uniform response, reducing the bias
411 of pooled sample analysis. We observed that other experimental studies in this
412 area use a similar number of animals as the present study, but the animals used
413 are not isogenic. For example, Barros et al. compared parenteral and enteral
414 administration routes of lipid emulsion on n-3 PUFA incorporation in white and
415 red cells, adipose tissue, liver, lung and hearts of Wistar rats (n = 6 animals per
416 group).[35] In this sense, we are comfortable with determining the size of our
417 sample studied, as genetic similarity leads to phenotypic uniformity, thus fewer
418 animals are needed in an experiment to achieve a given level of statistical power.
419 LCT/FO group received re-esterified n-3 fatty acids from fish oils, in contrast to
420 the MCT/LCT/FO that received purified body fish oils. Benefits in improving the
421 clearance and extra-hepatic uptake of FOLEs enriched with MCT were observed

422 across groups receiving n-3 triglycerides.[7,8] We did not identity studies in this field
423 using FOLEs containing pure fish oil. One study reported that increases in the
424 absolute serum amounts of EPA and DHA after two-weeks oral supplementation
425 as re-esterified triglycerides were superior compared to the supplementation as
426 natural fish body oil (non-significant level) and cod liver oil (significant level), while
427 this was not influenced by the stereochemistry of fatty acid in acylglycerols.[36]
428 Although the authors attributed their findings to facilitated conditions for normal
429 acylglyceride absorption in the intestine, we cannot exclude the possibility that
430 circulating availability and cell uptake of n-3 PUFAs will not be influenced by the
431 biochemical properties in which they are provided parentally by FOLEs.
432 Nevertheless, our data in leukocytes do not suggest any advantage in infusing n-
433 3 PUFAs as triglycerides, regardless the presence of MCT in the parenteral
434 formulation.

435 Taken together, and under the conditions of the present investigation, our
436 data suggest that there was no benefit in adding MCTs into FOLEs to achieve
437 early and/or higher leukocyte incorporation of n-3 PUFAs. Studies that consider
438 the induction of infectious/inflammatory stress could help to elucidate if this
439 dynamic alters with the presence of inflammation. Therefore, combining MCT to
440 n-3 triglycerides in parenteral lipid emulsions may not represent a clinical
441 advantage in terms of immunomodulation by FOLEs, at least in the absence of
442 infectious/inflammatory stimuli.

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562

563 Table 1 – Fatty acid composition of the control (LCT/FO – Lipovenus® 10%+
564 Omegaven® 10% - 8:2 v/v), medium-chain triglycerides-containing (Lipidem®
565 20%) parenteral lipid emulsions studied and the oral diet offered.

566

Fatty acid	LCT/FO[1] (g/L)	MCT/LCT/FO[2] (g/L)	AIN-93M (g/kg)
Caprylic acid (C8:0)	-	46.8	0.40
Capric acid (C10:0)	-	35.3	0.10
Myristic acid (C14:0)	1.8	-	0.10
Palmitic acid (C16:0)	9.6	10.9	5.90
Palmitoleic acid (C16:1n-7)	1.5	-	0.10
Stearic acid (C18:0)	0.4	4.6	2.10
Oleic acid (C18:1n-9)	21.5	20.4	11.40
Linoleic acid (C18:2n-6)	43.0	39.2	10.20
Stearidonic acid (C18:4n-3)	-	0.3	-
Arachidonic acid (C20:4n-6)	0.4	-	-
Alpha-linolenic acid (C18:3n-3)	6.8	5.1	0.50
Eicosatetraenoic acid (C:20:4n-3)	-	0.3	-
Dihomo-gamma-linolenic acid (20:3n-6)	-	0.8	-
Eicosapentaenoic acid (C20:5n-3)	4.2	5.9	-
Docosanoic acid (22:0)	-	0.3	-
Docosapentaenoic acid (C22:5n-3)	0.6	1.0	-
Docosahexaenoic acid (C22:6n-3)	4.3	4.4	-
Medium-chain fatty acids	0	82.1	0.50
Omega-9 monounsaturated fatty acids	21.5	20.4	11.50
Omega-6 polyunsaturated fatty acids	44.0	41.0	10.20
Omega-3 polyunsaturated fatty acids	15.3	15.7	0.50
Omega-6:Omega-3 ratio	2.9:1	2.6:1	20.4:1

567 Data of lipid emulsions were provided by the manufacturer [1](Fresenius Kabi, Bad
568 Homberg, Germany;); [2](B. Braun, Melsungen, Germany); data of oral diet was
569 assessed by gas chromatography (MA-006 methodology).CG- ASSOCIATIONS OF
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571 2007.

572 Table 2 – Amount of rodent chow intake by Lewis rats before and after 48 and 72
573 hours infusion of fish oil-containing lipid emulsions with or without medium-chain
574 triglycerides

Group	Before	48h	72h
Chow	20.00 ± 0.00	16.83 ± 1.84	17.83 ± 3.68
LCT / FO	20.00 ± 0.00	16.44 ± 1.99	18.21 ± 1.10
MCT / LCT / FO	20.00 ± 0.00	16.39 ± 1.42	9.92 ± 5.56*

575 Data expressed as grams. FO: lipid emulsion control group, with central venous
576 catheterization and infusion of a fish oil-containing lipid emulsion without medium-
577 chain triglycerides; LA: linoleic acid; LCT/FO: test group, with central venous
578 catheterization and infusion of a fish oil-containing lipid emulsion rich in medium-
579 chain triglycerides; chow: surgical control group, with central venous
580 catheterization and without lipid emulsion infusion.

581 * $p<0.05$ in relation to the time before and 48h

582

583

Table 3. Concentrations of polyunsaturated fatty acids in peripheral leukocytes from Lewis rats after 48 and 72 hours infusion of fish oil-containing lipid emulsions with or without medium-chain triglycerides

	Mononuclear leukocytes										**Polymorphonuclear leukocytes**									
Group	**EPA**		**DHA**		**ALA**		**LA**		**ARA**		**EPA**		**DHA**		**ALA**		**LA**		**ARA**	
	48h	**72h**	**48h**	**72h**	**48h**	**72h**	**48h**	**72h**	**48h**	**72h**	**48h**	**72h**	**48h**	**72h**	**48h**	**72h**	**48h**	**72h**	**48h**	**72h**
Chow	0.0	0.0	0.0	3.7	0.0	0.0	7.8	7.0	24.2	22.1	0.0	0.0	1.5	1.7	0.0	0.0	8.4	9.1	3.1	0.0
LCT / FO	1.8	0.0	5.5	4.2	0.0	0.0	9.9	9.0	0.0	0.0	4.4	0.0	5.5	6.1	0.0	0.0	5.3	7.7	1.8	0.0
MCT / LCT / FO	1.9	0.0	5.0	3.8	0.0	0.0	9.3	9.4	0.0	0.0	4.1	0.0	5.7	5.2	0.0	0.0	4.8	7.4	2.0	0.0
BC	**1.3**		**0.0**		**0.0**		**13.4**		**21.8**		**0.0**		**0.0**		**0.0**		**10.1**		**20.5**	

Data expressed as mean percentage of total fatty acids. ALA: alpha-linoleic acid; ARA, arachidonic acid; BC: baseline control group, sacrificed at a single time point without any surgical or nutritional intervention; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; LCT/FO: lipid emulsion control group, with central venous catheterization and infusion of a fish oil-containing lipid emulsion

without medium-chain triglycerides; LA: linoleic acid; MCT/LCT/FO: test group, with central venous catheterization and infusion of a fish oil-containing lipid emulsion rich in medium-chain triglycerides; chow: surgical control group, with central venous catheterization and without lipid emulsion infusion.

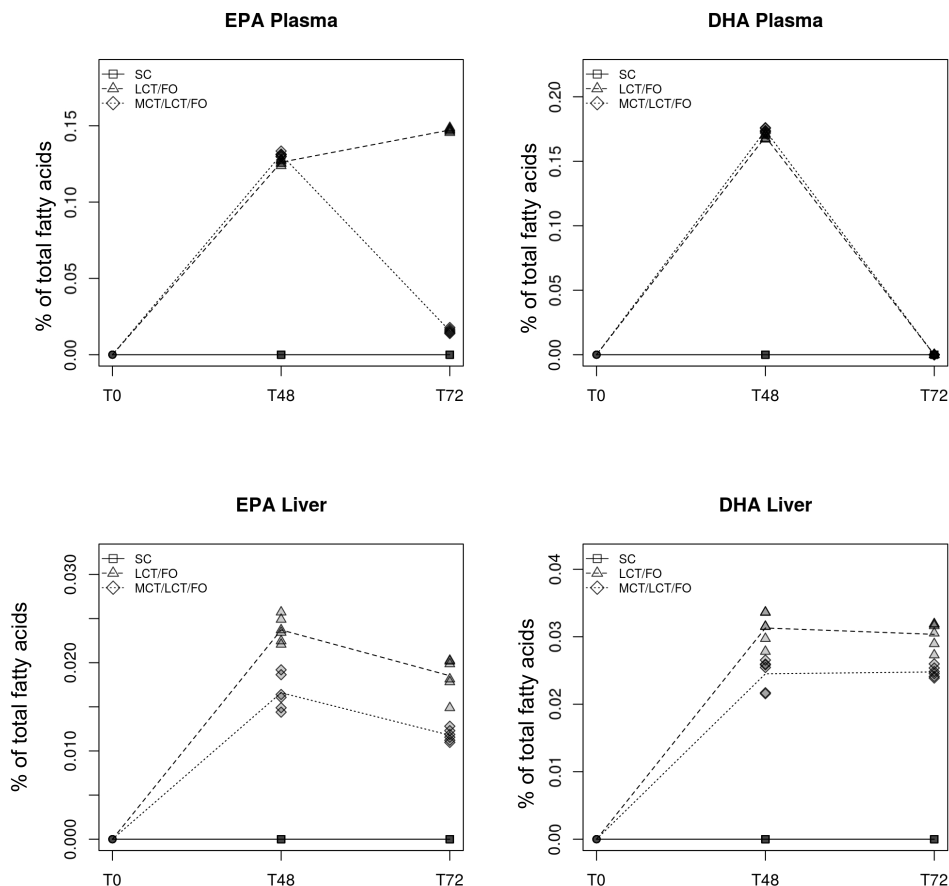

Figure 1. Concentrations of eicosapentaenoic and docosahexaenoic acids in plasma and liver samples from Lewis rats after 48 and 72 hours infusion of fish oil-containing lipid emulsions with or without medium-chain triglycerides

Legend. The figure shows the concentrations of eicosapentaenoic and docosahexaenoic acids in plasma (A and B, respectively) and liver (C and D, respectively). EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid; FO: lipid emulsion control group, with central venous catheterization and infusion of a fish oil-containing lipid emulsion without medium-chain triglycerides; LCT/FO: test group, with central venous catheterization and infusion of a fish oil-containing lipid emulsion rich in medium-chain triglycerides; SC: surgical control group, with central venous catheterization and without lipid emulsion infusion (chow).

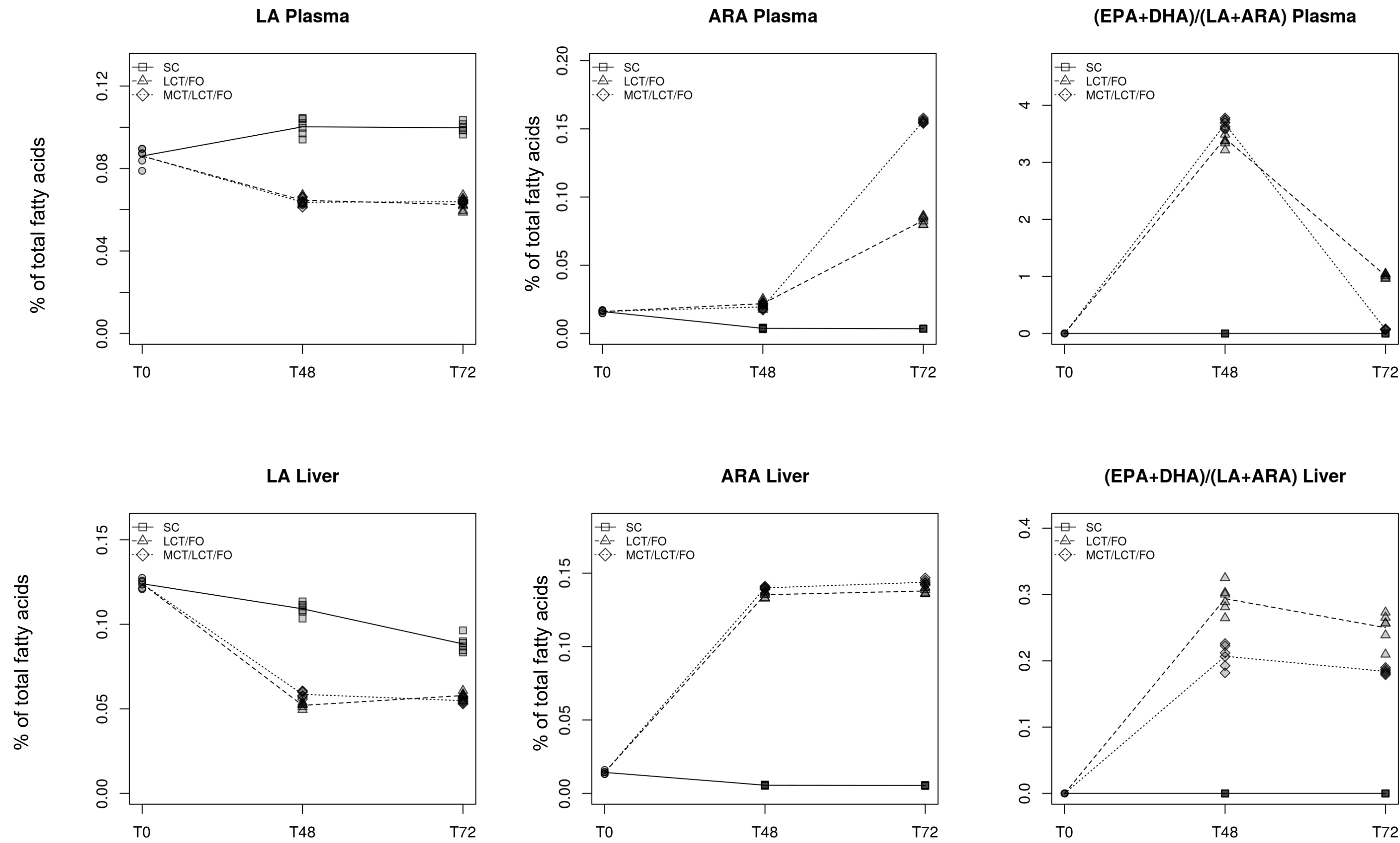

Figure 2. Concentrations of linoleic and arachidonic acids and ratios of omega-3 to omega-6 polyunsaturated fatty acids in plasma

and liver samples from Lewis rats after 48 and 72 hours infusion of fish oil-.containing lipid emulsions with or without medium-chain triglycerides

Legend. The figure shows the concentrations of linoleic and arachidonic acids and the ratios of omega-3 to omega-6 polyunsaturated fatty acids (n-3:n-6) in plasma (A, B and C, respectively) and liver (C, D and E, respectively). ARA, arachidonic acid; FO: lipid emulsion control group, with central venous catheterization and infusion of a fish oil-containing lipid emulsion without medium-chain triglycerides; LA: linoleic acid; LCT/FO: test group, with central venous catheterization and infusion of a fish oil-containing lipid emulsion rich in medium-chain triglycerides; SC: surgical control group, with central venous catheterization and without lipid emulsion infusion (chow).