

1 **Determinants of DHA incorporation into tumor tissue during dietary DHA**
2 **supplementation**

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23 **Abbreviations:**

24 ARA arachidonic acid

25 ALA alpha-linolenic acid

26 DHA docosahexaenoic acid

27 DPA docosapentaenoic acid

28 EPA eicosapentaenoic acid

29 FAME fatty acid methyl ester

30 LA linoleic acid

31 LCPUFA long-chain PUFA

32 NMU n-methyl-N-nitrosourea

33 PL phospholipids

34 TAG triacylglycerol

35

Abstract

Docosahexaenoic acid (DHA), upon incorporation into tumor tissue, has the potential to sensitize tumors to the effects of chemotherapy or radiation therapy. Although DHA has usually been supplied to tumor tissue in the diet, appropriate dietary conditions required to obtain optimal tumor levels have not been established. Hence, we studied mammary tumor tissue responses in rats fed various durations and doses of DHA. Rats fed a palm-oil enriched diet (diet 0) were switched to diets providing either 0.8 g DHA/d (diet 1) or 1.5 g DHA/d (diet 2). Tumor tissue fatty acid composition was analysed at baseline (diet 0), at weeks 1, 4 and 9 during diet 1 and at week 4 during diet 2. Dietary DHA supplementation differentially increased DHA within phospholipids (PL) and triacylglycerol (TAG) fractions in tumors. DHA level equilibrated between 2 and 4 weeks in PL while DHA increase was more progressive in TAG and did not reach a steady state. A higher dose of DHA further increased DHA content in tumor PL and TAG ($P = 0.018$ and $P < 0.001$ respectively). DHA concentration in plasma PL was positively correlated with DHA in tumor PL ($r = 0.72$; $P = 0.0003$) and TAG ($r = 0.64$; $P = 0.003$). We conclude that dietary DHA supplementation enhances tumor content of DHA in a time- and dose-dependent manner, and that DHA level in plasma PL could be used as a proxy for tumor DHA. These findings have implications for dietary DHA supplementations in cancer patients.

54

Key words: DHA incorporation, dietary DHA supplementation, mammary tumors, tumor phospholipids, tumor triacylglycerol, plasma phospholipids

57

58 **Introduction**

59 Recent studies have indicated that docosahexaenoic acid (DHA), a long chain n-3
60 polyunsaturated fatty acid (LCPUFA) of marine origin, has the potential to increase the
61 efficacy of chemotherapy or radiation therapy in preclinical models and in cancer patients [1].
62 Two pilot studies are now available, demonstrating that dietary intervention with DHA
63 increased the efficacy of chemotherapy in advanced breast or lung cancer patients without
64 additional toxicity to non-tumor tissues [2, 3]. Should these results be confirmed in
65 randomized phase III clinical trials, the consequences could be considerable by opening up
66 the prospect of systematic adjuvant supplementation during cancer treatment, a significant
67 shift in current cancer therapeutic paradigms.

68 DHA has been hypothesized to sensitize tumors to anticancer treatments through a
69 variety of mechanisms involving alteration of cellular functions in cancer cells and
70 modifications of the tumor microenvironment [1, 4, 5]. Enrichment of tumor cell membranes
71 with DHA is crucial because it is assumed to be the initial step of these processes. On the
72 basis of animal experiments, clinical trials were carried out in humans, where DHA was
73 supplied to tumor tissues through a dietary supplementation during chemotherapy. Studies in
74 rodents have shown that DHA-enriched diets administered for several weeks significantly
75 increased tumor tissue DHA content in autochthonous mammary tumors [6, 7]. But beside this
76 observation, little is known about incorporation of preformed DHA in tumor tissue. One pilot
77 study showed that the efficacy of chemotherapy was tightly related to DHA level in plasma
78 phospholipids (PL) during dietary DHA supplementation [2]. In this study, the efficacy of
79 chemotherapy was greater in patients with high plasma levels of DHA compared to patients
80 with low plasma levels of DHA [2]. From these observations, it was hypothesized that blood
81 supply of preformed DHA to tumor tissues varied among patients and that DHA supply to
82 tumors was higher when DHA levels were elevated in plasma PL. It was also hypothesized

83 that plasma could be an indirect indicator of DHA level in tumor tissue. Since all of these
84 hypotheses rely on circumstantial evidences, it appears that the conditions of dietary DHA
85 supplementation needed to obtain optimal DHA accumulation in tumor tissue have to be
86 defined to properly design and interpret studies using dietary DHA intervention aiming at
87 sensitizing tumor tissue to anticancer treatments.

88 To our knowledge, no study has evaluated the influence of the duration of intake or the
89 dose of DHA on tumor tissue content of DHA, or the use of plasma as a surrogate biomarker.
90 Therefore, we used a model of autochthonous mammary tumors induced by a carcinogen in
91 female rats. This model is relevant to address these issues because carcinomas develop
92 autochthonously in the mammary gland, generating a tumor tissue with features mimicking the
93 complexity of common human breast tumors [8, 9]. Phospholipids and triacylglycerol (TAG)
94 are the main tumor lipid fractions in this model. Herein, we report on the response of tumor
95 PL and TAG fatty acid composition to dietary DHA supplementation over time and to
96 increasing dose of DHA. We also investigated whether DHA level in plasma PL can be a
97 proxy for DHA content in tumors.

98

99 **Material and Methods**

100 *Animal procedures*

101 All the work with rats was carried out in accordance with the European guidelines for the care
102 and use of laboratory animals and approved by the local Institutional Animal Care Committee
103 (060NH). Female Sprague Dawley rats received a single dose of NMU (n-methyl-N-
104 nitrosourea) by an inguinal subcutaneous injection (25mg/kg of body weight) at 48 days of
105 age to induce mammary carcinogenesis. Detection of the tumors was performed by palpation
106 twice a week. Tumors developed in the mammary tissue approximately 8 weeks after the
107 induction of carcinogenesis. Diets had the same carbohydrate, protein, mineral, vitamin and
108 total fat content but different fatty acid composition (Table 1). Rats were maintained on a
109 palm oil-based diet (diet 0) until tumor area reached 0.8 cm². Then, rats were fed either diet 1
110 (20 g/d) providing 0.8g DHA/d for 4 weeks (group 1, $n = 4$) or 9 weeks (group 2, $n = 4$) or
111 diet 2 (20 g/d) providing 1.5 g DHA/d for 4 weeks (group 3, $n = 4$). In group 1,
112 transcutaneous biopsies of one tumor per rat and blood collection (0.4 ml) from the tail were
113 performed under general anaesthesia before switching to a DHA enriched diet (basal, diet 0)
114 and at week 1 during diet 1. At the end of the study, rats were killed by cervical dislocation.
115 Tumors were rapidly excised, frozen and stored at -80°C. Blood collected during the study or
116 at sacrifice by cardiac puncture was drawn into EDTA tubes, separated into cells and plasma
117 by centrifugation, and plasma was stored at -80°C. Palm oil was obtained from the Société
118 Industrielle des Oléagineux (Bougival, France) and DHA was obtained from DHASCO oil
119 (Martek Bioscience, Columbia, MD). The daily doses of DHA given to the rats were 4 g/kg
120 body weight for diet 1 and 8 g/kg body weight for diet 2. If conversions used for drugs apply
121 to nutrients, these amounts would correspond to approximately 700 mg/kg and 1300 mg/kg
122 respectively in man [10]. These very high dosages of DHA were aimed at experimentally
123 investigating the extent of changes that takes place in the level of DHA in the tumor and

124 plasma.

125

126 *Fatty acid composition analysis*

127 The fatty acid composition of the experimental diets, plasma and tumors was determined by
128 gas chromatography as previously described [2, 11]. Diet, plasma and tumor tissue lipids were
129 extracted using chloroform/methanol (2:1, by vol; containing 50 mg/L BHT) [12]. PL and
130 TAG were separated by one-dimensional silicagel thin layer chromatography. Fatty acids
131 were transmethylated with 14% boron trifluoride in methanol at 100°C for 90 min and 30 min
132 respectively for PL and TAG. FAME were extracted with hexane. Fatty acid methyl esters
133 were resolved on an AS 2000 gas chromatograph (ThermoFinnigan, France) equipped with a
134 cold on-column injector and a 60 meters BPX 70 capillary column, with the aid of an
135 automatic injector. The temperature protocol was adapted from reference [13]. FAME were
136 identified by comparison with authentic standards (Supelco, USA). Peak area was determined
137 using ChromQuest software and results are presented as percent of total peak area.

138

139 *Statistics*

140 GraphPad Prism (version 4, GraphPad Software, Inc., San Diego, USA) was used for
141 statistical analysis. The effects of time on fatty acid composition were assessed by one-way
142 ANOVA completed with Bonferroni test. The effects of dose on fatty acid composition were
143 assessed by unpaired t-test. Spearman test was used to analyse the relationship between DHA
144 concentration in plasma and in tumor tissue. Significance was defined as $P < 0.05$ (two-
145 sided).

146

147 **Results**

148 *DHA accumulation in tumor PL*

149 The fatty acid composition of tumor PL during dietary DHA supplementation is presented in
150 Table 2. DHA level in tumor PL rose to equilibrate between 2 and 4 weeks. DHA level
151 increased by 2 fold after 4 weeks of intake. No further increase was noted after 4 weeks.
152 When the dose of DHA was doubled, the amount of DHA in tumor PL increased by 1.3 fold.
153 There was a time- and dose-dependent increase in tumor PL EPA content and a time-
154 dependent increase in DPA n-3. No significant change was noted in the amount of ALA.
155 Tumor LA content increased in a time-dependent manner with diet 1 and maintained while on
156 diet 2. There was a reciprocal decrease in tumor PL ARA over time that was enhanced by a
157 higher dose of DHA. Since oleic acid was present in large amounts in the diets, an increase in
158 tumor PL oleic acid content was also noted.

159

160 *DHA accumulation in tumor TAG*

161 The fatty acid composition of tumor TAG during dietary DHA supplementation is presented
162 in Table 3. DHA increase in tumor TAG was more progressive and did not equilibrate during
163 the time course of the study compared to tumor PL. The level of 5.5 % DHA was reached in 9
164 weeks in the TAG fraction compared to 4 weeks in the PL fraction. The proportion of DHA in
165 tumor TAG increased 2.6 fold when the dose of DHA was doubled. EPA increased after one
166 week of dietary DHA supplementation to reach a steady state. EPA further increased in
167 response to a higher dose of DHA. DPA n-3 increased in a time- and dose-dependent manner.
168 There was a time-dependent increase in the amount of ALA and LA. Tumor TAG ARA
169 content decreased in a time- and dose-dependent manner. There was also an increase in tumor
170 TAG oleic acid and a decrease in stearic acid.

171

172 *Relationship between DHA concentration in plasma PL and in tumor tissue*

173 DHA increase in plasma PL was rapid and reached a steady state after one week of intake
174 (Table 4). A higher dose of DHA induced a 1.7 fold increase in DHA concentration in plasma
175 PL. DHA concentration in plasma PL followed a pattern close to that of tumor PL. Changes in
176 EPA and ARA followed a pattern similar to that of tumor PL. An increase of ALA was noted
177 at week 9 during diet 1. The proportion of LA increased during diet 1 but decreased during
178 diet 2 when the amount of LA in the diet reduced. No significant change in DPA n-3 level
179 was noticed.

180 Plasma PL DHA showed a correlation with tumor PL DHA ($r = 0.72$; $P < 0.001$; Figure 1a)
181 and tumor TAG DHA ($r = 0.64$; $P = 0.003$; Figure 1b).

182

183 **Discussion**

184 This study describes the evolution of mammary tumor tissue fatty acids during dietary DHA
185 supplementation over time and with increasing dose of DHA in rats. We found that DHA
186 accumulation in tumor tissue increased in a time- and dose-dependent manner in response to
187 supplementary dietary DHA and that DHA concentration in plasma PL can serve as a proxy
188 biomarker for tumor DHA.

189 Enrichment of cancer cell membranes and tumors with DHA has been associated with
190 enhanced efficacy of anticancer treatments and is therefore assumed to be a critical step
191 leading to this effect. Although increased *de novo* lipogenesis is a property of cancer cells
192 [14], these cells cannot synthesize LCPUFA *de novo* because mammalian cells cannot
193 introduce double bonds beyond the $\Delta 9$ position. Diet is a simple and efficient way to supply
194 DHA to tumors as previously documented in this model [6, 7]. In the present study, we
195 showed that dietary DHA supplementation induced a rapid accumulation of DHA in tumor PL
196 over time. DHA level equilibrated between two and four weeks of intake in the PL fraction
197 while no steady state was reached within the 9 weeks of intake in the TAG fraction. This
198 suggests that a dietary DHA supplementation for two to four weeks would be necessary to
199 significantly increase tumor PL content of DHA. As a practical consequence for future
200 clinical trials, it would be necessary to initiate dietary DHA supplementation at least two
201 weeks prior to chemotherapy to ensure optimal DHA accumulation into tumor tissue. When
202 this is not possible, assessment of chemotherapy efficacy should not be performed before one
203 month of dietary DHA supplementation has been achieved.

204 We provided 0.8 g DHA/d in diet 1 based on previous studies indicating that this dose
205 significantly increased tumor DHA content and sensitized tumors to chemotherapy and
206 radiation therapy in the same model [6, 7]. Despite the saturation of DHA level in tumor PL
207 with this dose of DHA, tumor tissue continued to incorporate DHA in response to the

208 considerable amount of DHA provided by diet 2 (1.5 g/d). DHA accumulation in the TAG
209 fraction was greater than in the PL fraction in response to a higher dose of DHA. These
210 results suggest that tumors have a high propensity to incorporate preformed DHA, even in
211 large amounts. A possible mechanism could be an up-regulation in tumor lipid transporters to
212 meet their high requirement for structural lipids. Whether the rate of growth or proliferation in
213 the tumors could interfere with DHA accumulation in tumor tissue should be examined.

214 Diet has been considered to be the main source of DHA supplied to tissues including
215 tumors either as preformed DHA or as its precursor, the essential fatty acid α -linolenic acid
216 (18:3n-3, ALA). DHA synthesis from ALA requires elongation, desaturation by delta 6 (EC
217 1.14.19.3) and delta 5 desaturases (EC 1.14.19.4), and β -oxidation [15]. The activity of the
218 desaturation/elongation pathway in the liver is the most important in terms of supply of ALA
219 metabolites to other tissues. Tumor cells might also contribute to this pathway since several
220 cancer cell lines are capable of processing exogenous essential fatty acids [16, 17]. During
221 diet 0, endogenous synthesis of DHA may be regarded as the likely supply to tumor tissue,
222 since no dietary source of preformed DHA was available. However, this pathway could not
223 have markedly contributed to the increase in DHA level during dietary DHA supplementation
224 because the amount of ALA and the ratio of LA:ALA were approximately similar in diet 1
225 and diet 0, and diet 2 contained only traces of ALA. The formation of EPA during dietary
226 DHA supplementation, while diets were free from EPA, could be a result of DHA
227 retroconversion [18]. This process mainly takes place in the liver and requires saturation of
228 the Δ 4 double bond by Δ 4 enoyl CoA reductase (EC 1.3.1.34) and rearrangement of the
229 double bond structure by Δ 3, Δ 2 enoyl CoA isomerase (EC 5.3.3.8) [19, 20]. The formation
230 of DPA n-3 could be a result of DHA retroconversion or a chain-elongation of the
231 retroconverted EPA. ARA decrease concomitant with increase in LA during dietary DHA
232 supplementation may result from a competition with EPA and a lower conversion from LA.

233 DHA and EPA were shown to downregulate the expression of delta 5- and delta 6-desaturases
234 [21]. As a result of ARA changes, there was a dramatic decrease in n-6/n-3 ratio in tumor
235 lipid fractions. Thus, we were able to profoundly modify the fatty acid composition of tumor
236 tissue with a DHA-enriched diet. It is likely that this would have consequences on tumor
237 metabolic pathways, particularly the production of inflammatory eicosanoids.

238 Blood is the route of delivery of dietary lipids to tissues. Plasma DHA in total lipids
239 has been shown to correlate with DHA accumulation in brain, retina or liver in experimental
240 animals [22, 23]. We found that DHA accumulation in tumor PL followed a pattern close to
241 that of plasma PL and that DHA in tumor PL and TAG correlated with DHA level in plasma
242 PL in this model. Studies exploring this issue in cancer patients are difficult to perform
243 because of technical problems and ethical concerns. One study reported that the DHA
244 increase in gastro-intestinal tumors was concomitant with its increase in plasma PL after 5
245 days of dietary supplementation with fish oil [24]. These results suggest that plasma, beside
246 its use as a biomarker of intake, could be a relevant surrogate biomarker of tumor tissue DHA
247 content.

248 In conclusion, this study establishes the evolution of tumor tissue fatty acid
249 composition in response to different durations or doses of dietary DHA supplementation and
250 demonstrates that accumulation of preformed DHA into tumor tissue is time- and dose-
251 dependent. These results could provide a basis for a rational design of dietary DHA
252 supplementation in cancer patients.

253

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258

259 **Conflict of interest**

260 The authors report no conflict of interest.

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Tables

Table 1: Composition of diets

	Diet 0	Diet 1	Diet 2
Peanut oil, <i>g/kg diet</i>	46.7	46.7	0
Rapeseed oil, <i>g/kg diet</i>	23.3	23.3	0
Palm oil, <i>g/kg diet</i>	80	0	0
DHASCO, <i>g/kg diet</i>	0	80	150
Total SFA, <i>g/kg diet</i>	62.77 ± 0.58	40.65 ± 0.18	54.61 ± 1.88
Palmitic acid	31.12 ± 1.84	11.16 ± 0.06	11.88 ± 0.39
Stearic acid	24.64 ± 0.17	1.75 ± 0.08	1.04 ± 0.08
Total monounsaturated fatty acids, <i>g/kg diet</i>	59.77 ± 2.01	60.63 ± 0.67	36.29 ± 0.99
Oleic acid	57.20 ± 2.30	55.21 ± 0.30	30.60 ± 0.59
Total (n-6) fatty acids, <i>g/kg diet</i>	19.41 ± 0.86	16.34 ± 1.06	1.79 ± 0.07
Linoleic acid	18.86 ± 1.15	15.77 ± 0.64	1.66 ± 0.06
Total (n-3) fatty acids, <i>g/kg diet</i>	2.94 ± 0.03	30.10 ± 0.33	55.22 ± 1.16
ALA	2.71 ± 0.15	2.07 ± 0.20	0.06 ± 0.00
EPA	ND*	0.05 ± 0.01	0.13 ± 0.01
DHA	0.12 ± 0.04	27.66 ± 0.45	54.34 ± 1.05
LA:ALA ratio	6.96	7.62	27.67

Carbohydrates (578 g/kg): corn starch, 372 g/kg; cellulose, 20 g/kg; sugar, 186 g/kg.

Proteins (221.6 g/kg): casein, 220 g/kg; methionine, 1.6 g/kg.

Mineral mix (40 g/kg, per kg mix): calcium, 8.1 g; phosphorus, 17.1 g; sodium, 3.16 g; chloride, 3.16 g; potassium, 10.8 g; magnesium, 0.9 g; iron, 390 mg; copper, 46 mg; manganese, 230 mg; zinc, 230 mg; cobalt, 1 mg; iodine, 2 mg; selenium, 1 mg; fluorine, 2 mg.

Vitamin mix (10 g/kg, per kg mix): retinol, 5000 UI; cholecalciferol, 2500 UI; vitamin E (α-tocopherol), 50 UI; thiamine, 10 mg; riboflavin, 10 mg; pyridoxine, 10 mg; cyanocobalamin, 0.0135 mg; ascorbic acid, 100 mg; menadione, 1 mg; folic acid, 2 mg; nicotinic acid, 45 mg; pantothenic acid, 30 mg; choline, 0.75 mg; inositol, 50 mg; biotin, 0.2 mg.

Values are means ± SD, n = 3; *ND: not detected.

Table 2: Tumor PL fatty acid composition in response to different durations and doses of DHA supplementation

Fatty acid	Diet 0	Diet 1			Diet 2	ANOVA <i>P</i>	T-test <i>P</i>
	Basal	Week 1	Week 4	Week 9	Week 4	Time	Dose
16:0	22.91 ± 1.04	24.68 ± 0.63	23.29 ± 1.65	22.66 ± 0.81	23.90 ± 1.09	0.189	0.350
18:0	16.07 ± 1.87	15.13 ± 1.92	14.50 ± 2.20	14.34 ± 1.42	14.03 ± 0.69	0.579	0.555
18:1n-9	11.80 ± 1.04	12.48 ± 0.95	13.19 ± 1.55	15.64 ± 1.01 ^{abc}	15.88 ± 1.99	0.001	0.003
18:2n-6	2.41 ± 0.59	3.12 ± 0.63	5.10 ± 1.32 ^a	5.49 ± 0.91 ^{ab}	4.64 ± 0.93	0.001	0.386
20:4n-6	19.44 ± 0.64	15.44 ± 2.59	12.37 ± 2.69 ^a	9.50 ± 2.29 ^{ab}	6.47 ± 2.45	<0.001	<0.001
18:3n-3	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.074	0.180
20:5n-3	0.04 ± 0.02	0.43 ± 0.13	1.26 ± 0.34 ^{ab}	1.56 ± 0.41 ^{ab}	3.39 ± 0.34	<0.001	<0.001
22:5n-3	0.29 ± 0.06	0.46 ± 0.11	0.75 ± 0.23 ^a	0.80 ± 0.18 ^a	0.93 ± 0.19	<0.001	0.079
22:6n-3	2.45 ± 0.29	3.47 ± 0.07	5.23 ± 1.23 ^{ab}	5.11 ± 0.52 ^a	6.85 ± 1.04	<0.001	0.018
n-6/n-3 ratio	9.58	4.83	2.66	2.40	1.18		

Values are mean ± SD, n=4. ^a Different from basal, ^b different from week 1, ^c different from week 4, *P* < 0.05

Table 3: Tumor TAG fatty acid composition in response to different durations and doses of DHA supplementation

Fatty acid	Diet 0		Diet 1		Diet 2	ANOVA <i>P</i>	T-test <i>P</i>
	Basal	Week 1	Week 4	Week 9	Week 4	Time	Dose
16:0	23.54 ± 0.79	26.70 ± 4.38	20.14 ± 1.88 ^b	21.41 ± 0.91 ^b	18.56 ± 2.53	<0.001	0.153
18:0	11.48 ± 1.65	12.42 ± 3.74	7.70 ± 0.94 ^{ab}	6.02 ± 1.76 ^{ab}	4.30 ± 1.82	<0.001	<0.001
18:1n-9	29.71 ± 8.74	23.18 ± 8.14	42.73 ± 5.90 ^{ab}	37.81 ± 4.18 ^b	42.51 ± 3.93	<0.001	0.928
18:2n-6	5.82 ± 2.92	4.48 ± 2.16	10.02 ± 1.41 ^{ab}	12.18 ± 1.66 ^{ab}	9.05 ± 1.85	<0.001	0.243
20:4n-6	4.28 ± 1.96	5.32 ± 2.53	0.67 ± 0.38 ^{ab}	0.70 ± 0.37 ^{ab}	0.25 ± 0.17	<0.001	0.008
18:3n-3	0.27 ± 0.19	0.11 ± 0.05	0.56 ± 0.13 ^{ab}	0.55 ± 0.18 ^b	0.45 ± 0.13	<0.001	0.098
20:5n-3	0.03 ± 0.02	0.18 ± 0.10 ^a	0.18 ± 0.09 ^a	0.19 ± 0.10 ^a	0.32 ± 0.14	0.045	0.027
22:5n-3	0.24 ± 0.15	0.38 ± 0.12	0.23 ± 0.10	0.77 ± 0.32 ^{ac}	0.55 ± 0.22	0.001	0.005
22:6n-3	1.64 ± 1.01	3.26 ± 1.33	3.40 ± 1.09	5.50 ± 1.95 ^a	8.99 ± 1.18	0.004	<0.001
n-6/n-3 ratio	6.65	3.42	2.54	1.81	0.93		

Values are mean ± SD, n=4. ^a Different from basal, ^b different from week 1, ^c different from week 4, *P* < 0.05

Table 4: Plasma PL fatty acid composition in response to different durations and doses of DHA supplementation

Fatty acid	Diet 0		Diet 1			Diet 2	ANOVA <i>P</i>	T-test <i>P</i>
	Basal	Week 1	Week 4	Week 9	Week 4	Time	Dose	
16:0	16.65 ± 1.28	21.34 ± 1.11 ^a	23.11 ± 1.05 ^a	26.12 ± 1.35 ^{ab}	27.79 ± 1.91	<0.001	0.015	
18:0	42.30 ± 2.96	35.85 ± 1.92 ^a	34.14 ± 0.85 ^a	36.22 ± 1.75 ^a	30.28 ± 2.17	0.003	0.049	
18:1n-9	3.71 ± 0.24	4.67 ± 0.43	5.33 ± 0.46 ^a	4.51 ± 0.61	5.99 ± 0.41	0.015	0.088	
18:2n-6	5.85 ± 1.68	9.08 ± 1.26 ^a	11.04 ± 0.38 ^a	7.89 ± 0.71	4.74 ± 0.55	0.003	<0.001	
20:4n-6	16.59 ± 2.86	6.30 ± 0.83 ^a	3.33 ± 0.15 ^a	2.15 ± 0.59 ^{ab}	1.23 ± 0.79	<0.001	0.009	
18:3n-3	0.04 ± 0.02	0.04 ± 0.03	0.03 ± 0.03	0.24 ± 0.11 ^{abc}	0.03 ± 0.01	0.004	0.575	
20:5n-3	0.10 ± 0.04	2.27 ± 0.95 ^a	1.90 ± 0.41 ^a	1.82 ± 0.40 ^a	4.24 ± 1.17	<0.001	0.032	
22:5n-3	0.13 ± 0.02	0.24 ± 0.09	0.25 ± 0.12	0.26 ± 0.03	0.27 ± 0.05	0.250	0.745	
22:6n-3	3.70 ± 0.86	6.83 ± 1.67 ^a	6.74 ± 2.27 ^a	6.04 ± 1.21 ^a	11.64 ± 1.47	0.011	0.007	
n-6/n-3 ratio	5.20	1.50	1.63	1.19	0.40			

Values are mean ± SD, n=4. ^a Different from basal, ^b different from week 1, ^c different from week 4, *P* < 0.05

1 **Figures legend**

2

3 Figure 1: **Positive correlation between tumor PL (a) or TAG (b) DHA levels and DHA**
4 **concentration in plasma PL.** Spearman test results are $r = 0.72$, 95% CI = 0.39 to 0.88, $p =$
5 0.0003 , $n = 20$ (a) and $r = 0.64$, 95% CI = 0.26 to 0.85, $p = 0.003$, $n = 20$ (b). Lines represent
6 the least-squares regression analysis of best linear fit (GraphPad Prism Software, version 4).
7 Linear regression results are $r^2 = 0.43$, $P = 0.0018$ (a) and $r^2 = 0.52$, $P = 0.0003$ (b).