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The bioavailability of an omega-3-rich algal oil is improved by nanoemulsion technology using yogurt as a food vehicle

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Lane, KE, Li, W, Smith, C and Derbyshire, E (2014) The bioavailability of an omega-3-rich algal oil is improved by nanoemulsion technology using yogurt as a food vehicle. INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY. 49 (5). pp. 1264-1271. ISSN 0950-5423

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1 **Main title: The bioavailability of an omega-3 rich algal oil is improved by nanoemulsion**
2 **technology using yogurt as a food vehicle.**

3

4 **Running title:** Omega-3 algal oil bioavailability improved by nanoemulsion technology.

5

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16

17 **Key words:** Algae oil, Docosahexaenoic acid, Eicosapentaenoic acid, Nanoemulsion, Omega-3,
18 bioavailability, yogurt.

19

20 **Abstract**

21 Global trends show that habitual omega-3 intakes are short of recommended guidelines, particularly
22 amongst vegetarians. Subsequently, alternative dietary sources of long chain omega-3
23 polyunsaturated fatty acids (LC3PUFA) from vegetarian sources are needed. Food technology
24 methods are advancing and nanoemulsion technologies have improved the bioavailability of certain
25 lipid-based nutrients. This study examined whether ingestion of an omega-3 algal oil nanoemulsion
26 led to improved bioavailability compared to the bulk oil. Eleven subjects completed a single-blind,
27 randomised crossover trial, with a 21-day washout between interventions. Results demonstrated
28 LC3PUFA absorption from the nanoemulsion was significantly higher than the bulk oil. Percentage
29 blood fatty acids were significantly increased for docosahexaenoic acid (DHA) ($P \leq 0.05$) while
30 LC3PUFA: PUFA ratios increased ($P \leq 0.05$) and omega-6:omega-3 ratios were reduced ($P =$
31 0.028). Larger and longer intervention studies are now needed, but these preliminary findings
32 demonstrate that nanoemulsion technology may improve the absorption of omega-3 fatty acids.

33

34 **Introduction**

35 Latest evidence from Western countries indicates that certain population groups may not be
36 consuming enough long chain omega-3 (n-3) polyunsaturated fatty acids (LC3PUFA) (Nelson et al.
37 2007, Elmadfa and Freisling 2009, Bates et al. 2012), with omega-3 intakes in Western regions
38 being found to be 5-fold lower than Japanese intakes (Meyer, 2011). Subsequently, failure to
39 consume enough LC3PUFA for optimal health has led to LC3PUFA deficiency being attributed to
40 at least thirteen morbidity and mortality outcomes, including CHD, CVD, stroke, depression and
41 bipolar disorder (Hibbeln et al. 2006).

42 While the consumption of fish and seafood is one of the easiest ways to improve LC3PUFA intakes,
43 this is not a feasible option for vegetarians or vegans who have been found to have particularly low
44 intakes of docosahexaenoic acid (DHA) (Sanders, 2009). Subsequently, other approaches such as

45 the consumption of foods enriched with LC3PUFA may be an alternative way to improve
46 LC3PUFA intakes and status (Meyer, 2011).

47

48 Nanoemulsions, defined as systems with lipid droplets smaller than 300nm are also gaining interest
49 and are a novel way to improve the absorption of certain nutrients (Gutiérrez et al. 2008, Anton and
50 Vandamme 2011). Nanotechnology methods have already been used and found to improve human
51 absorption of certain hydrophobic macronutrients, including curcumin (Yu and Huang 2012) and
52 lutein (Vishwanathan, Wilson et al. 2009). However, the potential role of nanotechnology methods
53 to improve the absorption and bioavailability of LC3PUFA using vegetarian delivery vehicles
54 appears to be understudied. Previous studies have been confined to animal models and the use of
55 fish oils which are unsuitable for most vegetarians, or vegans (Dey et al. 2012).

56

57 The Food and Drug Administration (2003) defines bioavailability as “the rate and extent to which
58 the active ingredient (in this case omega-3) is absorbed and becomes available at the site of action”
59 (FDA, 2003). Previous studies have shown that the absorption of free fatty acids from EPA and
60 DHA depends largely on the presence of intestinal lipases and is generally highest during the
61 consumption of high-fat meals (Offman et al., 2013). Work by the same authors has also shown
62 that higher absorption rates are reflected by increases in blood EPA and DHA levels (demonstrated
63 using area-under-the curve calculations), with study findings showing that the bioavailability of free
64 fatty acid forms of EPA and DHA were significantly higher than the bioavailability from ethyl ester
65 forms, even under low-fat conditions (Offman et al., 2013).

66

67 While the fat-content of the diet has been found to improve the absorption of free fatty acids,
68 altering the microstructure of foods may also influence this (McClements et al., 2009). For
69 example, lipid emulsions behave differently in the digestive tract according to the droplet size and
70 the interface layer (Armand et al. 1999, McClements et al. 2012). Extremely small droplets of

71 nutrients can easily be transported in the body through cell membranes giving increased blood
72 plasma and erythrocyte concentrations (Huang et al. 2010). It is thought that the incorporation
73 LC3PUFA into foods using nanoemulsions may improve LC3PUFA bioavailability due to the
74 reduced particle sizes and high surface to surface volume ratio (Acosta 2009).

75

76 Given raising concerns over low omega-3 intakes, micro-algal oils, produced in tightly controlled
77 fermentation facilities may also offer an alternative source of omega-3 fatty acids that are suitable
78 for vegetarians, vegans and (Geppert et al. 2006, Derbyshire 2009). Currently, only two studies
79 appear to have investigated the bioavailability of DHA obtained from algal sources but neither have
80 used nanoemulsion techniques. One randomised trial of 109 vegetarians Geppert et al, (2006)
81 found that DHA-rich almost EPA-free macroalgae oil (0.94 g DHA per day over 8 weeks) improved
82 some coronary heart disease risk factors (plasma triglyceride levels and triglyceride: high density
83 lipoprotein ratio) and was regarded as a valuable alternative to fish oil. In another 28-day
84 randomized study Arterburn et al, (2007) also concluded that two varieties of algal oil, in doses of
85 up to 1000 mg DHA per day represented safe and bioequivalent sources of DHA.

86

87 In summary, while there appears to be some emerging evidence supporting the use of algal DHA as
88 a suitable and bioavailable source of DHA, the potential effects nanoemulsion techniques using
89 vegetarian food vehicles do not appear to have been studied using human populations. To this end,
90 the current study aims to establish whether the bioavailability of an omega-3 rich algal oil is
91 improved by nanoemulsion technology using yogurt as a food vehicle.

92 Results will be compared against a control bulk oil product created using identical quantities of the
93 same vegetarian LC3PUFA algal oil, but without the application of nanotechnology.

94

95 **Materials and methods**

96 Yogurt nanoemulsion preparation

97 Currently, there is a trend for the enrichment of yogurt products, which is in response to consumer
98 expectations for functional or wellness foods (Chandan et al. 2008). In the present study, a
99 strawberry yogurt was created using combined levels of natural flavouring (0.4g/100g yogurt) and a
100 sweetening product (13g/100g yogurt). Unbleached natural phospholipid liquid soy lecithin was
101 obtained from Now Foods of Bloomingdale, USA and consisted of 15 per cent phosphatidyl
102 choline, 13 per cent phosphatidyl ethanolamine, 9 per cent phosphatidyl inositol and 19 per cent
103 other phospholipids and lipids. Soy lecithin was used in combination with ultrasound to create a
104 nanoemulsion (patented method (Lane et al. 2012)) containing 50 per cent DHATM-S algae oil and
105 water (see Table 1 for the fatty acid composition of DHATM-S oil). DHATM-S oil is a triglyceride
106 oil produced by the algal species schizochytrium sp. This oil contains around 35 per cent DHA and
107 was obtained from Martek Biosciences through DSM Great Britain Limited.

108 Nanoemulsion droplet sizes were determined using a Malvern Mastersizer 2000 courtesy of
109 Glyndŵr University, Wrexham, UK. Nanoemulsion droplet size distributions were measured in
110 accordance with the methods used by Akhtar et al, (2006) and Akhtar and Dickinson, (2003) using
111 a Malvern Mastersizer MS2000 laser light-scattering analyser with a small sample dispersion unit
112 set to 2000rpm. A drop of each sample amounting to approximately 10µl was pipetted into the
113 dispersion unit. For the emulsion samples an absorption parameter value of 0.001 and the refractive
114 index ratio of 1.472 for flaxseed oil and 1.488 for the algae oil was used (Breivik 2007). Samples
115 were measured in duplicate to ensure accuracy with a 15-second pause between measurements.
116 Droplet size is reported as the d_{32} volume/surface diameter mean or Sauter mean. Droplet size
117 distributions can be found in Figure 1

118 A total of 4.29g nanoemulsion (mean droplet size 258nm) was added per 100g of yogurt and a bulk
119 oil enriched product was created by adding 2.15g bulk oil per 100g yogurt. Participants ingested
120 200g of yogurt during each treatment phase.

121

122 Dosage

123 A dosage of 1264.69 ± 44.91 mg DHA was used for each treatment. This was based on the key
124 findings from a preliminary bioavailability trial (unpublished results) and previously published
125 LC3PUFA randomised crossover trials (RCT). For example, Raatz et al, (2009) used a crossover
126 trial to ascertain whether pre-emulsification improved the bioavailability of LC3PUFA and a dose
127 of 712mg DHA and 440mg EPA was given. A further trial by Schuchardt et al, (2011) compared
128 the bioavailability of fish oil and krill oil using a crossover trial with a formulated dose of 1680mg
129 EPA and DHA. In terms of tolerable upper intakes the European Food Safety Authority (EFSA)
130 state that of 1g/day DHA should be suitable for the general population and that while doses of 2 to
131 4g per day have been associated with increases in LDL-cholesterol concentrations by 3 per cent,
132 this should not have an adverse effect on CVD risk (European Food Safety Authority 2006).
133 Although Calzada et al, (2010) found that high levels of up to 3 or 4g/d DHA could have pro-
134 inflammatory consequences.

135

136

137 Subjects

138 Trial subjects were recruited from Manchester Metropolitan University (MMU) Hollings Faculty.
139 Invited participants were healthy adult men and women (aged 18 to 60 years) who did not consume
140 fish oil or vegetarian LC3PUFA dietary supplements, regularly eat oily fish or be willing to
141 complete a one month washout period prior to the trial (see Table 2 for study inclusion criteria).
142 Potential volunteers were interviewed and date of birth, sex, height, weight, medical history,
143 pregnancy status, smoking history, dietary supplement use, alcohol consumption, exercise activity
144 levels, and concomitant medications obtained. Participants with relevant food allergies, obesity
145 (BMI >30) any significant health problems or underlying conditions that may affect fat absorption
146 (determined using the British National Formulary (2012)) were excluded from the trial. All data
147 was fully anonymized.

148

149 The LC3PUFA consumption status of the subjects was assessed using a validated food frequency
150 questionnaire from Ritter-Gooder et al, (2008), which gathered daily dietary intakes of LC3PUFA,
151 ALA, EPA and DHA. Participants whose dietary intake of EPA and DHA exceeded 200 mg DHA
152 per day were excluded or asked to complete a one-month washout period before taking part in the
153 trial, using similar approaches to Schuchardt et al, (2011).

154

155 Study design

156 This intervention study was conducted according to the guidelines laid down in the Declaration of
157 Helsinki (World Medical Association 2008) and all procedures involving human subjects were
158 approved by the Manchester Metropolitan University Ethics Committee on 30th July 2012. Written
159 informed consent was obtained from all subjects. The trial was designed in accordance with the
160 CONSORT statement for randomized controlled trials (Schulz, Altman et al. 2011).

161

162 A randomised single blinded crossover trial was utilised to determine the bioequivalence of DHA
163 from bulk and nanoemulsified oil enriched yogurt. Subjects were randomised and allocated to
164 treatments in an A/B or B/A pattern using the ‘Sealed Envelope’ randomisation program (Sealed
165 envelope 2012). A total of six blood samples were taken for each treatment phase with the aim of
166 capturing optimum levels to make use of the area under the curve calculation. As described by
167 Wakil et al, (2010), participants completed a 21-day minimum washout period between each
168 treatment phase before the trial was repeated using the other treatment (see Figure 2).

169

170 The minimum washout period of 21 days between treatments was based on previously published
171 LC3PUFA crossover studies and it was anticipated that this would be sufficient to allow baseline
172 values to return to normal levels (Garaiova et al. 2007, Schuchardt et al. 2011). Applicants were
173 also asked to avoid the restricted foods during the washout period in accordance with a recent trial
174 by Davidson et al, (2012). This was assessed using three-day dietary recalls, which were analysed
175 using Netwisp (Version 3) to include a full fatty acid analysis.

176

177 Blood testing methods

178 Blood samples were taken for each tranche of the trial at baseline 2, 4, 6, 24 and 48-hour intervals.
179 Samples were obtained after overnight fasting for baseline, 24 and 48-hour measurements. ACCU-
180 CHEK Safe-T-Pro Plus lancets with three depth settings of 1.3, 1.8, and 2.3mm were used to obtain
181 finger prick blood samples by puncturing the fingertip. Depth settings of 1.8mm were used for
182 females and 2.3mm for males. Blood samples were collected using ‘The Omega Blood Count’ test
183 provided by Glasgow Health Solutions (2012). The finger prick test provided blood measures for
184 total LC3PUFA:PUFA and omega-6 arachidonic acid (ARA):EPA ratios with ALA, EPA and DHA
185 percentages. After collection, fingertip samples were placed in the test kit box with a desiccation
186 pouch and allowed to dry for a minimum of three hours.

187 Once dry, the collection cards were placed in a sealed plastic storage bag with a desiccant pouch
188 and sent directly by post to Stirling University.

189

190 Blood analysis was carried out at The Institute of Aquaculture, Nutrition Analytical Service,
191 Stirling University, using purpose built analytical equipment. Samples were tested in accordance
192 with the methods stated by Bell et al, (2011). The dried whole blood sample was detached from the
193 collection devise using scissors and forceps and placed in a screw top vial containing 1ml of
194 methylating solution (1.25 M in methanol-HCl). The temperature of the vials was then increased to
195 70°C using a hot block, where the sample remained for one hour. The vials were then allowed to
196 cool to room temperature, then 2ml of distilled water and 2ml saturated potassium chloride solution
197 were added. Fatty acid methyl esters (FAME) were extracted using 2x2ml isohexane.

198

199 To extract the remaining haem and cholesterol, 500mg/6ml of solid phase extraction silica gel was
200 used after which the samples were dried down in preparation for analysis using gas-liquid
201 chromatography (GLC). FAME were separated then quantified by GLC. (ThermoFisher Trace,
202 Hemel Hempstead, Herts, UK) using a 60m x 0.32mm x 0.25µm film thickness capillary column
203 (ZB Wax; Phenomenex, Macclesfield, Cheshire, UK). Hydrogen was used as the carrier gas at a
204 flow rate of 4.0ml/min and the temperature programme was from 50 to 150°C at 40°C/min then to
205 195°C at 2°C/min and finally to 215°C at 0.5°C/min.

206 Individual FAME were identified by comparison to well-characterised in-house standards as well as
207 commercial FAME mixtures.

208

209 Statistical analysis

210 Data are presented as mean \pm standard error for continuous variables, number of subjects (n) and
211 percentage (%) for categorical variables. All whole blood levels were corrected to baseline levels.
212 The area under the curve (AUC) was calculated for DHA and LC3PUFA:PUFA.

213 Values were determined for each person during the intervention period according to the trapezoid
214 rule (Matthews et al. 1990, Brouns et al. 2005). ARA:EPA ratios were analysed using baseline
215 adjusted values due to decreases in these levels. Compatibility checks using Kolmogorov-Smirnov
216 and Shapiro-Wilk tests established that the baseline adjusted means and AUC results had normal
217 and non-normal distributions. Statistical analysis was completed using mixed repeated measures
218 two-way analysis of variance (MMANOVA) as described by Pallant (2010).

219 To further identify specific differences paired t-tests and Wilcoxon Signed Rank tests were
220 completed to evaluate differences between treatments and time intervals in comparison to baseline.
221 Values of $P \leq 0.05$ were considered to be statistically significant. All statistical analyses were
222 carried out using SPSS software (version 19.0).

223

224 **Results**

225 A total of thirteen volunteers were recruited, with two potential subjects being excluded. One was
226 taking regular medication known to affect the metabolism of fatty acids; the other was unable to
227 travel to the faculty before having breakfast. Previous literature demonstrates that a number of
228 studies have utilised a crossover design to assess absorption of LC3PUFA using a similar number of
229 participants to this study (Garaiova et al. 2007, Raatz, et al. 2009, Wakil et al. 2010, Schuchard et
230 al. 2011). Comparative randomised crossover studies by Raatz et al, (2009) examined the
231 absorption of LC3PUFA from emulsified fish oil compared to bulk oil supplements using 10
232 participants and Garaiova et al, (2007) compared absorption of pre-emulsified fish oil to bulk oil
233 with 13 volunteers.

234 The average age of the 11 participating subjects was 33 years 6 months (± 10.3 years), BMI was
235 23.89 (± 2.11), six volunteers were male and five female. There was no evidence of significant
236 differences between baseline blood values or correlations between oily fish or supplement intake,
237 which confirms that the washout periods were effective and that there was no evidence of
238 LC3PUFA carryover between treatments. The paired t-test showed there were no significant
239 differences between dietary intakes at baseline for both treatments for energy (Kcal) ($P = 0.655$),
240 total fat ($P = 0.908$), monounsaturated fatty acids (MUFA) ($P = 0.697$), polyunsaturated fatty acids
241 (PUFA) ($P = 0.403$), saturated fats ($P = 0.863$), protein ($P = 0.346$) and carbohydrates. ($P = 0.817$).
242 This indicated that the self-reported dietary intakes were consistent among participants for each
243 treatment.

244

245 DHA bioavailability

246 The mean baseline adjusted percentage DHA increases for the bulk and nanoemulsion treatments
247 can be found in Figure 3. The nanoemulsion enriched yogurt gave rapid increases in DHA levels,
248 which peaked 2 hours after ingestion. Paired t-tests and Wilcoxon Signed Rank tests were
249 completed to assess the time intervals (see Table 4), which demonstrated significant differences
250 between nano and bulk treatments for the 0 to 2 and 2 to 4 hour values ($P = 0.001$ and $P = 0.040$
251 respectively). DHA from the nanoemulsion enriched yogurt was statistically significantly more
252 bioavailable than the bulk oil for up to four hours after ingestion. A comparison of the treatments at
253 all times using mixed models ANOVA demonstrated that the percentage increase of DHA in blood
254 fatty acids was not statistically significant for the two treatments ($P = 0.803$) and that there was also
255 no significant effect for gender ($P = 0.311$). Overall time intervals following digestion were found
256 to be a statistically significant factor ($P = 0.010$). The intercept of treatment, time intervals and
257 gender was also a significant factor ($P = 0.006$).

258

259 LC3PUFA bioavailability

260 Figure 4 demonstrates the baseline adjusted LC3PUFA percentage increases for both treatments.
261 LC3PUFA levels peaked at 4 hours for both treatments, indicating a slower rate of absorption to
262 DHA. Analysis of the time intervals using the paired t-test and Wilcoxon Signed Rank tests
263 demonstrated that LC3PUFA was significantly more bioavailable for the nanoemulsion from 2 to 4
264 and 4 to 6 hours following ingestion ($P = 0.020$ and $P = 0.030$ respectively). A comparison of the
265 treatments at all times using mixed models ANOVA demonstrated that the percentage increase of
266 LC3PUFA in blood fatty acids was approaching significance for treatment ($P = 0.067$).
267 There was also a significant effect for gender, with males having significantly higher AUC for the
268 combined treatments than women ($P = 0.014$) and time intervals ($P = 0.010$). The intercept of
269 treatment, time intervals and gender was a significant factor ($P = 0.001$).

270

271 Changes in ARA:EPA ratios

272 The ratio of omega-6 (n-6) to n-3 is important for long-term health and the arachidonic
273 acid/eicosapentaenoic acid (ARA/EPA) ratio may be used as an indicator of fatty acid balance and
274 long-term health (Simopoulos 2011). Baseline adjusted blood ARA:EPA percentage values are
275 shown in Figure 5. Statistical analysis was completed using the baseline adjusted ARA:EPA
276 values. A comparison of the treatments at all times using mixed models ANOVA demonstrated that
277 the percentage decrease in ARA:EPA blood fatty acids ratios was significantly higher for the
278 nanoemulsion treatment ($P = 0.028$) and that time and gender were approaching significance ($P =$
279 0.063 and $P = 0.058$ respectively). The intercept of treatment, time and gender was a significant
280 factor ($P = 0.001$). Women had larger but non-significant reductions in ARA:EPA ratios for both
281 treatments than men (nano treatment males -2.58 , females -6.84 per cent, bulk treatment males $-$
282 0.98 , females -2.07 per cent) although these were only approaching significance ($P = 0.063$).

283

284 **Discussion**

285 To the knowledge of the authors, this study was the first to demonstrate that an algal based
286 vegetarian LC3PUFA oil nanoemulsion was absorbed more effectively than the same bulk oil
287 control. Nanoemulsion enriched foods may offer an effective vehicle to increase habitual
288 LC3PUFA intakes which currently fall below recommended guidelines for the general population
289 (Bates et al. 2012).

290

291 This study demonstrated that, compared with a bulk oil enriched yogurt, the consumption of a
292 yogurt enriched with a nanoemulsion of algae oil (mean droplet size 258nm) offers an enhanced
293 rate and extent of absorption for LC3PUFA. The increased levels of DHA and LC3PUFA coupled
294 with decreased ARA:EPA markers may be beneficial to health (Simopoulos 2011). Blood marker
295 levels peaked between two and four hours after ingestion for both treatments (Table 4). The levels
296 of DHA percentage fatty acids for the nanoemulsion were 1.81 and 1.61 times the bulk oil levels,
297 two and four hours following ingestion ($P = 0.001$ and $P = 0.04$ respectively).

298

299 Blood LC3PUFA levels were significantly elevated to 1.78 and 1.62 times the bulk oil respectively,
300 four and six hours after ingestion ($P = 0.020$ and $P = 0.030$). The mean ratio of ARA/EPA in blood
301 fatty acids was also significantly lowered by the nanoemulsion in comparison to the bulk oil
302 product for the duration of the trial ($P = 0.011$).

303

304 The findings from this RCT are in line with those reported by Garaiova et al, (2007), who found
305 that blood DHA levels peaked three hours after ingestion, although in this instance a larger dosage
306 of pre-emulsified fish oil was used. When compared to bulk oil capsules, EPA and DHA blood
307 plasma triacylglycerol levels were found to be significantly higher in the pre-emulsion group (P
308 <0.001 and $P = 0.0355$ respectively). For the pre-emulsification study, LC3PUFA from the pre-
309 emulsion treatment which had a median droplet size of $1.3\mu\text{m}$ was significantly more bioavailable

310 than bulk oil using AUC calculations ($P = 0.018$). Participants were given 30ml of the fish oil
311 based formula with fatty acids comprising of 16.8 per cent EPA and 11 per cent DHA in bulk or
312 pre-emulsified form with a crossover between treatments.

313

314 Raatz et al, (2009) made further findings, which validate the results of this trial in a bulk
315 oil/emulsion crossover study. Statistically significant increases were found in the bioavailability of
316 LC3PUFA from a 4g dose of bulk or emulsified fish oil (droplet size not stated). As with the
317 current trial ARA/EPA ratios were significantly more reduced with the emulsion treatment ($P =$
318 0.01) and enhanced absorption was noted for EPA ($P < 0.01$) and total LC3PUFA ($P = 0.05$).

319

320 In terms of the possible mechanisms, the observed rises in the bioavailability of nanoemulsions may
321 have occurred due to increased lipase activity at the surface area and interfacial layer of oil droplets
322 as droplet surface areas increase as droplet sizes decrease (Garaiova et al. 2007, Raatz et al. 2009,
323 Yu and Huang 2012). Very fine droplets also have high specific surface areas and high curvatures
324 giving different surface reactivity than bulk oils, which can lead to amended bile salt accumulation
325 and activity (McClements and Xiao 2012). In addition, small droplets of nutrients can easily be
326 transported in the body through cell membranes, giving increased blood plasma and erythrocyte
327 concentrations (Huang et al. 2010). Once in circulation, blood lipids are in constant exchange with
328 major tissues containing LC3PUFA, namely the intestine, liver and peripheral organs. This may
329 explain decreases in blood levels that were particularly noticeable in some participants 24 hours
330 after ingestion.

331

332 This study used a validated fingertip blood sampling method, which has previously been
333 demonstrated to give good correlations to erythrocyte plasma and whole blood fatty acid levels
334 (Bell et al. 2011). Due to the nature of fingertip blood sampling, it was possible to obtain
335 percentage fatty acid levels for the blood markers but not precise levels.

336 It was not therefore possible to calculate exactly how much of the 1264 mg dose of DHA entered
337 the bloodstream. However, for this initial trial, fingertip blood sampling was a far less invasive
338 sampling method in comparison to cannulation blood test methods, which may have been
339 unsuitable for several of the participants in this trial.

340

341 With regard to study limitations, in the present study, participants underwent a four-week washout
342 period, which may have left some internal organs and tissues LC3PUFA depleted and further
343 increased tissue uptakes from blood. The dosages used in this trial were based on previous research
344 and ethical considerations.

345 The use of lecithin as the emulsifier in the nanoemulsion samples and not the bulk oil control may
346 act as a potential confounder in this trial. Lecithin may have improved or decreased the
347 bioavailability of the fatty acids contained in the algae oil. A study by Mun et al, (2007) found that
348 lecithin decreased the access of pancreatic lipase to emulsified fats in comparison to protein based
349 emulsifiers such as caseinate and whey protein isolates.

350 It was necessary to use lecithin to create stable nanoemulsion systems. The bulk oil product was
351 stable to separation when formulated, so additional emulsifying agents were not considered
352 necessary. The aim of the trial was to compare nanoemulsions to bulk oil. It was decided not to
353 give a sole dose of bulk oil only to participants as it may have caused unpleasant taste and
354 mouthfeel sensations. Yogurt was chosen as the food vehicle for this trial as it facilitated the
355 provision of a relatively high dose of nanoemulsion and potentially offered improved oxidation
356 stability (Sabeena Farvin et al. 2010). The use of yogurt as an enrichment vehicle may have
357 affected fatty acid absorption. Schram et al, (2007) found that yogurt provided the best matrix for
358 fast of absorption of lipids in general including n-3 when comparing supplements, fitness bars
359 yogurt and bread and butter.

360 Therefore to ensure consistency, the bulk oil was incorporated into the same yogurt product, which
361 also ensured that volunteers remained blinded to the treatment pattern. Lecithin was not added to
362 the bulk oil product to ensure that the oil remained as much in bulk form as possible and did not
363 instantaneously form fine emulsion droplets as this might act as a further confounder.

364 Larger doses (>4g LC3PUFA) used by Davidson et al, (2012) and Galli et al, (2012) may have
365 achieved more detectable changes in blood concentrations after a single one off treatment.
366 However, a dose of 1264 mg DHA did demonstrate significant differences in blood percentage
367 levels soon after ingestion when the nanoemulsion was compared to the bulk product. In future,
368 long term intervention trials are needed to investigate the sustained effects of consumption of
369 vegetarian LC3PUFA nanoemulsion fortified foods on LC3PUFA bioavailability using a RCT
370 study design.

371

372 To conclude, the present study demonstrated that when compared to a bulk oil enriched yogurt, the
373 consumption of a yogurt with an added nanoemulsion of algae oil offers an enhanced rate and
374 extent of absorption of DHA and total LC3PUFA, with a decline in ARA:EPA levels, which may
375 broaden health benefits. It is believed that this study is the first to investigate the bioavailability of
376 an algal based vegetarian LC3PUFA nanoemulsion, using fingertip blood sampling and yogurt as a
377 food vehicle. While larger and extended studies are needed, these findings indicate that
378 nanoemulsion enriched foods may improve LC3PUFA intake and uptake, helping to bridge the gaps
379 with dietary recommendations that currently exist.

380

381 **Acknowledgements**

382 We would like to give thanks to John Dobson at DSM, Martek for the provision of the oil and
383 Omega Health Solutions based at Stirling University for their support with the blood analyses.

384

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531 **Conflict of interests, sources of funding and authorship**

532 None of the authors had a personal or financial conflict of interest. KL developed the bulk oil and
533 nanoemulsion enriched strawberry yogurt drinks and was responsible for the recruitment of
534 participants, study data collection and analysis, as well as the preparation and writing of the
535 research paper. ED supervised the crossover study, contributed to the development of the study
536 materials, the receipt of ethical approval and also supported the writing and editing of the paper.
537 WL provided support with nanoemulsion and bulk oil enriched strawberry yogurt product
538 development and the editing of the paper. CS supported the editing of the paper. This research
539 received no specific grant from any funding agency in the public, commercial or not-for-profit
540 sectors.

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