

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

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

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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 DHA<sup>TM</sup>-S algae oil and  
105 water (see Table 1 for the fatty acid composition of DHA<sup>TM</sup>-S oil). DHA<sup>TM</sup>-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 10 $\mu$ l 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  $1264.69 \pm 44.91$ 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 ( $\pm 10.3$  years), BMI was  
235 23.89 ( $\pm 2.11$ ), 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



385 **References**

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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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