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

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

Authors: Katie E. Lane[1], Weili Li[2], Chris Smith[2] and Emma Derbyshire[3]

[1]School of Education, Leisure and Sport Studies, Faculty of Education, Health and Community, IM Marsh Campus, Barkill Road, Aigburth, Liverpool, L17 6BD, United Kingdom. [2]Manchester Metropolitan University, Department of Food and Tourism Management, Hollings Faculty, Cavendish Street, Manchester, M15 6BG, United Kingdom. [3]School of Healthcare Science, Manchester Metropolitan University, John Dalton Building, Chester Street, Manchester, M1 5GD, United Kingdom

Corresponding authors: Katie Lane, k.lane@mmu.ac.uk 0161 24745112 or Emma Derbyshire, e.derbyshire@mmu.ac.uk 0161 24741417

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

Abstract

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

Introduction

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

While the consumption of fish and seafood is one of the easiest ways to improve LC3PUFA intakes, this is not a feasible option for vegetarians or vegans who have been found to have particularly low 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, McClemments *et al*. 2012). Extremely small droplets of

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

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

In summary, while there appears to be some emerging evidence supporting the use of algal DHA as a suitable and bioavailable source of DHA, the potential effects nanoemulsion techniques using vegetarian food vehicles do not appear to have been studied using human populations. To this end, the current study aims to establish whether the bioavailability of an omega-3 rich algal oil is improved by nanoemulsion technology using yogurt as a food vehicle.
Results will be compared against a control bulk oil product created using identical quantities of the same vegetarian LC3PUFA algal oil, but without the application of nanotechnology.

Materials and methods

Yogurt nanoemulsion preparation

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

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

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

Dosage

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

Subjects

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

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

Study design

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

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

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

Blood testing methods

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

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

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

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

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

Statistical analysis

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

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

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

Results

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

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

DHA bioavailability

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

LC3PUFA bioavailability

Figure 4 demonstrates the baseline adjusted LC3PUFA percentage increases for both treatments. LC3PUFA levels peaked at 4 hours for both treatments, indicating a slower rate of absorption to DHA. Analysis of the time intervals using the paired *t*-test and Wilcoxon Signed Rank tests demonstrated that LC3PUFA was significantly more bioavailable for the nanoemulsion from 2 to 4 and 4 to 6 hours following ingestion ($P = 0.020$ and $P = 0.030$ respectively). A comparison of the treatments at all times using mixed models ANOVA demonstrated that the percentage increase of LC3PUFA in blood fatty acids was approaching significance for treatment ($P = 0.067$).

There was also a significant effect for gender, with males having significantly higher AUC for the combined treatments than women ($P = 0.014$) and time intervals ($P = 0.010$). The intercept of treatment, time intervals and gender was a significant factor ($P = 0.001$).

Changes in ARA:EPA ratios

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Acknowledgements

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

References

Acosta, E. (2009). Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Current Opinion in Colloid and Interface Science* **14**(1): 3-15.

Akhtar, M. and E. Dickinson (2003). Emulsifying properties of whey protein-dextran conjugates at low pH and different salt concentrations. *Food Colloids, Biopolymers and Materials Special Issue* **31**(1-4): 125-132.

Akhtar, M., B. S. Murray and E. Dickinson (2006). Perception of creaminess of model oil-in-water dairy emulsions: Influence of the shear-thinning nature of a viscosity-controlling hydrocolloid. *Food Hydrocolloids* **20**(6): 839-847.

Anton, N. and T. Vandamme (2011). Nano-emulsions and Micro-emulsions: Clarifications of the Critical Differences. *Pharmaceutical Research* **28**(5): 978-985.

Armand, M., B. Pasquier, M. André, P. Borel, M. Senft, J. Peyrot, J. Salducci, H. Portugal, V. Jaussan and D. Lairon (1999). Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. *The American Journal of Clinical Nutrition* **70**(6): 1096-1106.

Arterburn, L., H. Oken, J. Hoffman, E. Bailey-Hall, G. Chung, D. Rom, J. Hamersley and D. McCarthy (2007). Bioequivalence of Docosahexaenoic Acid from Different Algal Oils in Capsules and in a DHA-Fortified Food. *Lipids* **42**(11): 1011-1024.

Bates, B., A. Lennox, A. Prentice, C. Bates and G. Swan (2012). National Diet and Nutrition Survey. Headline results from Years 1, 2 and 3 (combined) of the Rolling Programme (2008/2009 – 2010/11). London The Department of Health and the Food Standards Agency

Bell, J. G., E. E. Mackinlay, J. R. Dick, I. Younger, B. Lands and T. Gilhooly (2011). Using a fingertip whole blood sample for rapid fatty acid measurement: method validation and correlation with erythrocyte polar lipid compositions in UK subjects. *British Journal of Nutrition* **106**(09): 1408-1415.

Breivik, H., Ed. (2007). *Long-Chain Omega-3 Speciality Oils*. Bridgwater, The Oily Press.

British National Formulary (BNF). (2012). British National Formulary. [Internet document] Accessed 17/10/2012, URL http://www.bnf.org/bnf/index.htm.

Brouns, F., I. Bjorck, K. N. Frayn, A. L. Gibbs, V. Lang, G. Slama and T. M. S. Wolever (2005). Glycaemic index methodology *Nutrition Research Reviews* **18**: 145-171.

Burdge, G. C. and P. C. Calder (2005). α-Linolenic acid metabolism in adult humans: the effects of gender and age on conversion to longer-chain polyunsaturated fatty acids. *European Journal of Lipid Science and Technology* **107**(6): 426-439.

Burdge, G. C., A. E. Jones and S. A. Wootton (2002). Eicosapentaenoic and docosapentaenoic acids are the principal products of a-linolenic acid metabolism in young men*. *British Journal of Nutrition* **88**: 355-363.

Burdge, G. C. and S. A. Wootton (2006). Dietary a-linolenic acid and health-related outcomes: a metabolic perspective. *Nutrition Research Reviews* **19**: 26-52.

Calzada, C., R. Colas, N. Guillot, M. Guichardant, M. Laville, E. Véricel and M. Lagarde (2010). Subgram daily supplementation with docosahexaenoic acid protects low-density lipoproteins from oxidation in healthy men. *Atherosclerosis* **208**(2): 467-472.

Chandan, R. C., A. Kilara and N. P. Shah (2008). Dairy Processing and Quality Assurance. Iowa, John Wiley & Sons.

Davidson, M. H., J. Johnson, M. W. Rooney, M. L. Kyle and D. F. Kling (2012). A novel omega-3 free fatty acid formulation has dramatically improved bioavailability during a low-fat diet compared with omega-3-acid ethyl esters: The ECLIPSE (Epanova® compared to Lovaza® in a pharmacokinetic single-dose evaluation) study. *Journal of Clinical Lipidology* **6**(6): 573-584.

Derbyshire, E. (2009). Functional LC ω-3 PUFAs in pregnancy: a short review of literature. *Journal of Foodservice* **20**: 224-229.

Dey, T. K., S. Ghosh, M. Ghosh, H. Koley and P. Dhar (2012). Comparative study of gastrointestinal absorption of EPA and DHA rich fish oil from nano and conventional emulsion formulation in rats. *Food Research International* **49**(1): 72-79.

Elmadfa, I. and H. Freisling (2009). Nutritional status in Europe: methods and results. *Nutrition Reviews* **67**: S130-S134.

European Food Safety Authority (2006). REGULATION (EC) No 1924/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 December 2006 on nutrition and health claims made on foods. 1924/2006. E. Parliament. Brussels**:** 1-26.

Food and Drug Administration (2003) Guidance for Industry. Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations. Available at: http://www.fda.gov/downloads/Drugs/.../Guidances/ucm070124.pdf

Galli, C., F. M. Maggi, P. Risé and C. R. Sirtori (2012). Bioequivalence of two omega-3 fatty acid ethyl ester formulations: a case of clinical pharmacology of dietary supplements. *British Journal of Clinical Pharmacology* **74**(1): 60-65.

Garaiova, I., I. A. Guschina, S. F. Plummer, J. Tang, D. Wang and N. T. Plummer (2007). A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification. *Nutrition Journal* **6**: 1-9.

Geppert, J., V. Kraft, H. Demmelmair and B. Koletzko (2006). Microalgal sources of docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: a randomised trial. *British Journal of Nutrition* **95**: 779-786.

Glasgow Health Solutions and R. McLellan (2012). The Omega Blood Count™. Poulton Le Fylde,.

Godfray, H. C. J., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir, J. Pretty, S. Robinson, S. M. Thomas and C. Toulmin (2010). Food Security: The Challenge of Feeding 9 Billion People. *Science* **327**(5967): 812-818.

Gutiérrez, J. M., C. González, A. Maestro, I. Solè, C. M. Pey and J. Nolla (2008). Nano-emulsions: New applications and optimization of their preparation. *Current Opinion in Colloid & Interface Science* **13**(4): 245-251.

Hahn, A., H. Meyer, J. Neubronner, I. Schneider, J. P. Schuchardt and C. von Schacky (2011). Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations - a comparative bioavailability study of fish oil vs. krill oil. *Lipids in Health and Disease* **10**: 145.

Hibbeln, J. R., L. R. Nieminen, T. L. Blasbalg, J. A. Riggs and W. E. Lands (2006). Healthy intakes of n–3 and n–6 fatty acids: estimations considering worldwide diversity. The American *Journal of Clinical Nutrition* **83**(6): S1483-1493S.

Huang, Q., H. Yu and Q. Ru (2010). Bioavailability and delivery of nutraceuticals using nanotechnology. *Journal of Food Science* **75**(1): R50-R57.

Lane, K. E., E. Derbyshire, W. Li and C. J. Smith (2012). Nanoemulsions, methods of forming the same and uses thereof (UK Patent) Manchester Metropolitan University. United Kingdom.

Matthews, J. N. S., D. G. Altman, M. J. Campbell and P. Royston (1990). Analysis of serial measurements in medical research *British Medical Journal* **300**: 230-235.

McClements, D. J. and H. Xiao (2012). Potential biological fate of ingested nanoemulsions: influence of particle characteristics. *Food & Function* **3**(3): 202-220.

McClements, D.J., E.A. Decker EA and Y. Park (2009) Controlling lipid bioavailability through physicochemical and structural approaches. Crit Rev Food Sci Nutr. 2009 Jan;**49**(1):48-67.

Mercer, P. and R. E. Armenta (2011). Developments in oil extraction from microalgae. *European Journal of Lipid Science and Technology* **113**(5): 539-547.

Meyer, B.J. (2011) Are we consuming enough long chain omega-3 polyunsaturated fatty acids for optimal health. *Prostaglangins, Leukotrienes & Essential Fatty Acids* **85**(5): 275-80.

Mintel (2011). Functional Food and Drink. A. Beckett. London Mintel

Mun, S., E. Decker and D. McClements (2007). Influence of emulsifier type on in vitro digestibility of lipid droplets by pancreatic lipase. *Food Research International* **40**: 770 - 781.

Nelson, M., B. Erens, B. Bates, S. Church and T. Bashier (2007). Low income diet and nutrition survey. F. s. agency. London, The Stationery Office

Offman, E., T. Marenco, S. Ferber, J. Johnson, D. Kling, D. Curcio and M. Davidson (2013) Steady-state bioavailability of prescription omega-3 on a low-fat diet is significantly improved with a free fatty acid formulation compared with an ethyl ester formulation: the ECLIPSE II study. *Vascular Health Risk Management* **9:**563-573.

Pallant, J. (2010). *SPSS survival manual: a step by step guide to data analysis using SPSS*. Maidenhead, Open University Press/McGraw-Hill.

Raatz, S. K., J. B. Redmon, N. Wimmergren, J. V. Donadio and D. M. Bibus (2009). Enhanced Absorption of n-3 Fatty Acids from Emulsified Compared with Encapsulated Fish Oil. *Journal of the American Dietetic Association* **109**(6): 1076-1081.

Ritter-Gooder, P. K., N. M. Lewis, K. Barber-Heidal and M. Waltz-Hill (2008). Development and pilot testing of an omega-3 fatty acid food frequency questionnaire. *Journal of Food Composition and Analysis* **21**(Supplement 1): S43-S49.

Sabeena Farvin, K. H., C. P. Baron, N. S. Nielsen and C. Jacobsen (2010). Antioxidant activity of yoghurt peptides: Part 1-in vitro assays and evaluation in ω-3 enriched milk. *Food Chemistry* **123**(4): 1081-1089.

Sanders, T.A. (2009) DHA status of vegetarians. *Prostaglandins Leukot Essent Fatty Acids* **81**(2-3):137-41.

Schram, L. B., C. J. Nielsen, T. Porsgaard, N. S. Nielsen, R. Holm and H. Mu (2007). Food matrices affect the bioavailability of (n - 3) polyunsaturated fatty acids in a single meal study in humans. *Food Research International* **40**(8): 1062-1068.

Schuchardt, J. P., I. Schneider, H. Meyer, J. Neubronner, C. Von Schacky and A. Hans (2011). Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations - a comparative bioavailability study of fish oil vs. krill oil *Lipids in Health and Disease* **10**(145): 1-7.

Schulz, K. F., D. G. Altman and D. Moher (2011). CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. *International Journal of Surgery* **9**(8): 672-677.

Sealed envelope. (2012). Randomisation and online databases for clinical trials. Accessed 04/08/2012, 2012, URL http://www.sealedenvelope.com/.

Simopoulos, A. (2011). Evolutionary Aspects of Diet: The Omega-6/Omega-3 Ratio and the Brain. *Molecular Neurobiology* **44**(2): 203-215.

Vishwanathan, R., T. A. Wilson and R. J. Nicolson (2009). Bioavailability of a nanoemulsion of lutein is greater than a lutein supplement. *Nano Biomedicine And Engineering* **1**(1): 38-49.

Wakil, A., G. Mackenzie, A. Diego-Taboada, J. G. Bell and S. L. Atkin (2010). Enhanced bioavailability of eicosapentaenoic acid from fish oil after encapsulation with plant spore exines as microcapsules. *Lipids* **45**: 645-649.

World Medical Association (2008). World Medical Association Declaration of Helsinki, *Ethical Principles for Medical Research Involving Human Subjects*. Department of Health 2008. Helsinki.

Yu, H. and Q. Huang (2012). Improving the Oral Bioavailability of Curcumin Using Novel Organogel-Based Nanoemulsions. *Journal of Agricultural and Food Chemistry* **60**(21): 5373-5379.

Conflict of interests, sources of funding and authorship

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

541
542
543