Research Article

# Bioavailability of EPA and DHA delivered by gelled emulsions and soft gel capsules

**Ingvild J. Haug[1], Lise B. Sagmo[2], Daniel Zeiss[2], Inge C. Olsen[3], Kurt I. Draget[1] and Tore Seternes[4]**

[1] Department of Biotechnology/NOBIPOL, Faculty of Natural Sciences and Technology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
[2] Faculty of Health Sciences, Nord-Trøndelag University College, Namsos, Norway
[3] Smerud Medical Research International AS, Oslo, Norway
[4] Ayanda AS, Tromsø, Norway

This study presents data comparing the bioavailability of DHA and EPA delivered by two different formulations: One group received TAG fish oil in traditional soft gel capsules, whereas the other group received the TAG oil as droplets trapped inside a gelatin matrix (gelled emulsions). The incremental area under the curve ($AUC_{0-26\,h}$) of EPA and EPA + DHA in blood plasma from the gelled emulsions was significantly increased by 44.9 and 43.3%, respectively, compared to soft gel capsules. The maximum incremental concentration of EPA and EPA + DHA was significantly increased by 100.4 and 105.6%, respectively, compared to soft gel capsules. These results suggest that improved bioavailability of EPA and DHA may be achieved by incorporating emulsified TAG fish oil in a gel matrix prior to oral ingestion.

**Practical applications:** This study presents a new type of vehicle for the delivery of PUFAs. The vehicles are soft and chewable with the possibility of adding flavours, sweeteners and colour, and this makes the vehicles ideal for delivery of PUFAs to consumers having problems swallowing large capsules or cod liver oil. The vehicles are already applied in products in several countries, including Norway.

**Keywords:** Bioavailability / DHA / Emulsion / EPA / Fatty acid

Received: August 27, 2010 / Revised: November 12, 2010 / Accepted: November 16, 2010

DOI: 10.1002/ejlt.201000450

## 1 Introduction

Intake of omega-3 fatty acids is found to increase the concentration of DHA and EPA in plasma and red blood cells in vivo [1–4]. Ingestion of omega-3 fatty acids has been reported to be advantageous in treatment of disorders such as cardiovascular diseases [5–7], joint pains associated with, for example rheumatoid arthritis, inflammatory bowel disease and dysmenorrhoea [8, 9], and neurocognitive disorders such as attention-deficit hyperactivity disorder (ADHD) [10, 11].

The ingestion of omega-3 oil as incorporated in conventional preparations is, however, not always welcomed by consumers as a result of fishy taste, capsule size and a tendency to produce an unpleasant reflux. Since oils have a lower density than gastric juice the burst of capsules containing omega-3 oils in the stomach may result in concentration of lipids in the upper layers of the gastric juice and may cause such reflux. Development of new oral vehicles with improved acceptability for the consumers would therefore be highly beneficial.

**Correspondence:** Dr. Ingvild J. Haug, Department of Biotechnology/NOBIPOL, Faculty of Natural Sciences and Technology, Norwegian University of Science and Technology (NTNU), Sem Sælandsvei 6/8, N-7491 Trondheim, Norway
**E-mail:** ingvild.haug@biotech.ntnu.no
**Fax:** +47 73 59 12 83

**Abbreviations: AGJ,** artificial gastric juice; **$AUC_{0-26\,h}$,** the incremental area under the plasma concentration curve; **BMI,** body mass index; **$C_{max}$,** the maximal incremental (change from baseline) plasma concentration; **CPMP,** Committee for Proprietary Medicinal Products; **EMEA,** The European Medicines Agency; **GMP,** good manufacturing practice; **GLP,** good laboratory practice; **ICMJE,** The International Committee of Medical Journal Editors; **Non-GMO,** non-gene modified organism; **PBS,** phosphate-buffered saline; **QWP,** quality working party; **SEM,** scanning electron microscope; **$t_{max}$,** time after administration for the maximal incremental plasma concentration to occur

 

Digestion of fat is considered a complex process: The water/lipid interface plays a crucial role in providing a contact area between the lipid digestive enzymes, the lipases, and their substrates. The rate of lipolysis is mainly controlled by the possibility of lipase to bind to the surface of oil droplets in duodenum (and to a lesser extent in the stomach) which is influenced by several chemical, physical and sterical properties. The influence of emulsion structure and stability on lipid digestion and bioavailability has recently been excellently reviewed by Golding and Wooster [12] and McClements et al. [13]. Lipid digestion and bioavailability has been extensively studied during the last decade and the design of nutraceutical delivery vehicles [14], foods [15], formulations of the oil [16], choice of emulsifiers and stabilisers [17] and oil droplet size and emulsion structure [17–21] have been reported to be important factors in controlling the rate of lipolysis and the bioavailability.

The goal of this clinical pre-study was to compare the short-term bioavailability from a single dose administration of EPA and DHA from TAG fish oil released from traditional soft gel capsules and from a gel matrix containing emulsified oil (gelled emulsion).

## 2 Materials and methods

### 2.1 Product technical background

Oral dosage units of gelled emulsions (batch number 161) and soft gel capsules with TAG fish oil (batch number 59605709) were manufactured and supplied by Ayanda AS (Tromsø, Norway). Both formulations contained TAG fish oil (batch number 90421902) supplied by Napro Pharma AS (Brattvåg, Norway). The oil contained mixed tocopherols (1.3 mg/g TAG oil) of non-gene modified organism (non-GMO) quality as an anti-oxidant. The gelled emulsion technology is a new patent pending technology described in WO 2007/085840 [22].

The excipients of the emulsion and soft gel capsules per unit dose were as follows: Gelled emulsion; gelatin (GELITA GmbH, Mannheim, Germany) 56.7 mg, gum arabic (Harlem foods, Oslo, Norway) 37.0 mg, sorbitol 103.6 mg, xylitol 241.7 mg, citric acid 5.9 mg, and flavour 1.2 mg. Soft gel capsule; gelatin (GELITA) 129.5 mg, glycerol 62 mg, and purified water. Sorbitol and Xylitol were supplied by Food Innovation AS (Oslo, Norway). The gelled emulsions were made by dissolving the water-soluble ingredients at 68°C, before the oil was mixed into the water phase at 50°C by applying a homogeniser. The emulsion was moulded into small discs and sealed in alumina blister trays. The gelled emulsions were produced according to good laboratory practice (GLP) and stored in blister trays under nitrogen atmosphere at 4°C. The soft gel capsules were produced on conventional encapsulation machinery. All capsules were manufactured according to food good manufacturing practice (GMP) and stored at RT, protected from heat, moisture and direct sunlight.

### 2.2 Subjects

Healthy students (19–29 years) from the Nord-Trøndelag University College, Namsos, Norway, were recruited. Exclusion criteria were metabolic syndromes, hypercholesterolemia, high blood pressure, diabetes, fish allergy, obesity (BMI $> 30$), regular intake of non-steroidal anti-inflammatory drugs, anticoagulants or omega-3 supplements.

The final study protocol was approved spring 2009 by the corresponding independent ethics committee in Norway. The study was undertaken in accordance to the Declaration of Helsinki and the laws and regulations for clinical research in the participating countries. All subjects provided written informed consent before any study procedure was undertaken. The study has been registered in www.ClinicalTrials.gov according to the International Committee of Medical Journal Editors initiative (ICMJE) with the registration number NCT01061554.

### 2.3 Study design and diet

The study subjects were randomised to receive two different single-dose treatments of ~5 g omega-3 fatty acids, as summarised in Table 1. The treatments were not blinded, and both the investigator and participants knew the identity of the treatment. The blood sample analyses were performed blinded to treatment.

The participants were instructed to have a zero fish and alcohol diet 1 wk prior to and during the study, and to begin fasting on the morning of the study start. Demographic data (sex and age), measured BMI and baseline blood samples were collected before the study started and blood samples were taken 2, 3, 4, 6, 8 and 26 h post-administration.

All participants were offered standardised meals during day 1 of the study. The participants were allowed to eat at home before the 26 h blood sample but were informed to exclude fish from the meal(s). For safety comparisons adverse events were recorded during the 26 h observation period. No adverse events were observed during this study.

**Table 1.** Total amount of omega-3 fatty acids and number of capsules ingested for the gelled emulsion and soft gel capsule groups

| Fatty acid | Gelled emulsion (mg) | Soft gel capsules (mg) |
|---|---|---|
| EPA | 3089 | 3033 |
| DHA | 2340 | 2298 |
| EPA + DHA | 5429 | 5331 |
| Product weight | 1124 ± 87 | 730 ± 9 |
| Number of capsules ingested | 23 | 17 |

Product weight data: mean ± SD ($n = 40$).

 

### 2.4 Blood specimen collections and plasma analysis

All blood samples (7 mL) were drawn from the antecubital vein, collected in ethylenediaminetetraacetic acid (EDTA) test tubes (BD Vacutainer Plus EDTA 7/6 mL), and centrifuged at 2200 × *g* (3700 rpm) for 11 min at 18°C. Centrifugation was carried out within 30 min after sampling. Two portions of the plasma (1.5 + 0.5 mL samples) were collected in cryo tubes (CryoTubes™, 1.8 mL, Nunc), and immediately frozen before storage at −80°C prior to analyses.

The blood sample analyses were carried out by Vitas AS, Oslo, Norway. EPA and DHA blood plasma concentrations were determined as follows: 40 μL plasma was added 100 μL internal standard (1,2-diheptadecanoyl-*sn*-glycero-3 phosphatidylcholine) and 800 μL methanolic hydrochloric acid, mixed and heated at 80°C for 120 min. The resulting FAME was added 300 μL 3 M potassium hydroxide in water and extracted with 500 μL hexane. GC analyses were performed directly on these solutions using a 6890N GC with a split/splitless injector, a 7683B automatic liquid sampler, and FID (Agilent Technologies, Palo Alto, CA). Separation was accomplished with a SP-2380 (30 m × 0.25 mm i.d. × 0.25 μm film thickness) column from Supelco™.

### 2.5 Statistical analysis

All data analyses were carried out according to recommendations in the Note for Guidance on the Investigation of Bioavailability and Bioequivalence (CPMP/EWP/quality working party (QWP)/1401/98) of The European Medicines Agency's (EMEA) Committee for Proprietary Medicinal Products (CPMP) [23]. The full analysis population, consisting of all eligible randomised subjects was utilised for efficacy analyses. An analysis of covariance (ANCOVA) model was exploited for the incremental area under the plasma concentration curve ($AUC_{0-26\ h}$) variable and the maximal incremental blood plasma concentration ($C_{max}$) variable. All $AUC_{0-26\ h}$ and $C_{max}$ analyses were adjusted for baseline concentration (by including baseline concentration as a covariate in the ANCOVA analyses). Exact Kruskal–Wallis non-parametric test was used to detect the time after administration for the maximal incremental plasma concentration to occur ($t_{max}$). Prior to analysis the $AUC_{0-26\ h}$ and $C_{max}$ variables were transformed using the natural logarithm to adjust for skewness in the data. Pair wise comparisons in the ANCOVA analyses were adjusted for multiple comparisons using the Dunnet–*Hsu* method [24]. All tests were two-sided ($\alpha = 0.05$) and calculations were performed using SAS version 9.2 (SAS institute, Cary, NC, USA).

### 2.6 In vitro imaging of the gelled emulsions

The gelled emulsion was cut in thin slices (1 mm thick), primary fixed in McDowell's fixative over night, washed in phosphate-buffered saline (PBS), post-fixated in 1% aqueous $OsO_4$ for 1.5 h, washed in PBS and dehydrated in a graded series of ethanol [7, 25]. The samples were critical point dried in a Balzer Union CPD 020 Critical Point Dryer (Lichtenstein), mounted on aluminium stubs with silver glue and coated with gold/palladium in a Polaron Range Sputter Coater (Ringmer, UK). Micrographs were taken in a Jeol JSM 6300 Scanning Microscope (Tokyo, Japan).

### 2.7 In vitro dissolution testing of the vehicles

One 1 g gelled emulsion was cut into small pieces (mean particle diameter: 3–4 mm) to mimic the fracture during chewing. One soft gel capsule or fractured gelled emulsion was added to 150 mL pre-warmed artificial gastric juice (AGJ) and incubated at 37°C and 50 rpm for 3 h. The AGJ, pH 1.2, without enzymatic activity, was prepared according to European Pharmacopoeia [26]. One dissolved gel matrix was also stored at RT for 48 h. The dissolved gelled emulsion (1 h, 37°C, 50 rpm) was illustrated by the use of a Nikon Eclipse TS 100 microscope, 40× objective, and a Nikon DS-Fi1 camera.

### 2.8 Light scattering—in vitro measurements of oil droplet size

Droplet size measurements were performed using a Coulter LS 230 instrument (Coulter Corporation, Florida, US) to demonstrate that emulsions induced by dissolution of the gelled emulsion were stable at artificial gastric conditions. Dissolved gel matrix (from in vitro dissolution) was applied to the instrument in triplicates at each incubation time (30 min to 48 h). The results are given as mean volume diameters including SDs.

## 3 Results

During spring 2009, the subjects were randomly assigned two formulations containing TAG fish oil: Gelled emulsions ($n = 9$), and soft gel capsules ($n = 9$). One subject was included in the soft gel capsule group although she clearly should not have been due to obesity (BMI = 33.5). This subject has been removed from the results as well as the statistical analyses after the blind was broken.

The baseline values of DHA and EPA in the two groups and the demographics are given in Table 2. The blood plasma concentrations of EPA, DHA and EPA + DHA are presented in Fig. 1 as a function of time post-administration, while Fig. 2 shows the $AUC_{0-26\ h}$-values for EPA, DHA and EPA + DHA. Table 3 presents $C_{max}$ and $t_{max}$, while Table 4 shows the results from the statistical comparison of the bioavailability of EPA, DHA and EPA + DHA for the gelled emulsion versus soft gel capsule treatment.

Statistical analyses showed that the bioavailability as measured by $AUC_{0-26\ h}$ and $C_{max}$ of EPA and

 

**Table 2.** Demographics and baseline characteristics for the bioavailability study

| Parameter | Gelled emulsion ($n = 9$) | Soft gel capsule ($n = 8$) |
|---|---|---|
| Age[a] (years) | 22.4 (1.5) [20–22–25] | 22.3 (2.9) [20–21–28] |
| Female[b] | 6 (66%) | 5 (63%) |
| BMI[a] (kg/m$^2$) | 23.4 (2.3) [19.8–23.3–27.4] | 23.1 (2.5) [19.8–22.6–27.2] |
| EPA[a] (μg/mL) | 11.9 (3.6) [5.6–11.7–17.4] | 19.7 (18.0) [7.7–12.2–62.7] |
| DHA[a] (μg/mL) | 56.6 (12.2) [40.2–54.2–84] | 60.3 (28.3) [25.9–55.4–116.5] |

[a] Mean (SD) [min–median–max].
[b] Number of subjects (%).

EPA + DHA from gelled emulsions was significantly increased compared to the soft gel capsule treatment. The bioavailability of DHA from the gelled emulsion treatment was non-significantly increased by 46.0 and 115.8%, respectively, compared to the soft gel capsule treatment.

Time until maximum concentration, $t_{max}$, of EPA + DHA was determined to 2 and 6 h after administration for the gelled emulsion and the soft gel capsule treatments, respectively. The same trend was also found when EPA was studied alone. When only DHA was investigated $t_{max}$ was determined to 2 h and was equal for both treatments.

A scanning electron microscope (SEM) study was undertaken in order to investigate the size distribution of the emulsified TAG oil droplets in the gel matrix of the gelled emulsion. An image from the SEM is included in Fig. 3 (left) illustrating the oil droplets in the gel matrix. The droplet diameters were determined to be in the range of 1–10 μm with the majority being 2–4 μm.

The image in Fig. 3 (right) reveals the fate of the emulsified TAG oil droplets as the gel matrix was dissolved in AGJ at 37°C and 50 rpm. The matrix was fully dissolved within 20 min. The emulsion of TAG oil in AGJ was also studied by light scattering and the results are summarised in Fig. 4. The mean volume diameter of the oil droplets in the dissolved gel matrix was determined to be 1.81 ± 0.02 μm ($n = 12$, 30–2880 min) and the results in Fig. 4 show that the oil droplets released from the gel matrix form an emulsion in AGJ which was stable.

## 4 Discussion

The 5 g dosage in this study was chosen based on the results of another clinical study performed by Marsen et al. [3] to enable detection of increased EPA and DHA concentrations in blood serum within 24 h post-administration. The number of subjects in each group has been selected with reference to similar studies [21, 27, 28].

The results from the present study suggest that the bioavailability of PUFAs may be increased by ingestion of gelled emulsions, even though the results are not unambiguous. Both $AUC_{0-26\ h}$ and $C_{max}$ was significantly higher for EPA and EPA + DHA delivered by the gelled emulsions (Fig. 2 and Table 4). $C_{max}$ of DHA was also significantly higher for the gelled emulsion group. No significant differences were, however, found between the values of $AUC_{0-26\ h}$ for DHA between the two test groups, and we concluded that the overall bioavailability for DHA was not significantly higher for the gelled emulsion group. The results in Figs. 1 and 2, and in Table 4 do, however, indicate that the bioavailability trend may be in favour of the gelled emulsion treatment, but a higher powered study is needed to confirm improved bioavailability of both EPA and DHA from the gelled emulsion treatment.

It has previously been found that pre-emulsification of lipids increases the uptake of fatty acids [18–21, 29] and that especially the uptake of long-chain PUFAs, such as EPA and DHA, was increased by pre-emulsification [21]. The bioequivalence of encapsulated and microencapsulated fish oils has, however, been found to be similar [30]. It has also been reported increased lipase activity in fine versus coarse emulsions [20, 29] and this suggests that emulsification of oils prior to ingestion is likely to have a positive effect on the bioavailability and absorption of PUFAs. Several studies have also provided results supporting increased absorption of EPA and DHA from the ingestion of emulsified oil compared to bulk oil [14, 18, 21, 29].

Results very similar to those presented here were recently reported by another research group [14]: The absorption of omega-3 fatty acids from emulsified (liquid) and encapsulated fish oil after treatment of a single 4 g dosage was studied and a significantly increased absorption of total omega-3 and EPA from the emulsified oil was revealed. These researchers suggest that the differences in absorption could be partly caused by dissimilar delivery forms (liquid emulsion vs. soft gel capsules) [14]. In our study both treatment groups received identical TAG oil using solid vehicles and both vehicles were easily and equivalently dissolved in vitro (within 20 min). This suggests that the observed differences in the bioavailability in our study may be linked to the emulsified oil, but it cannot be ruled out that also the design of the vehicle did influence the bioavailability (chewable, soft vehicle vs. intact, solid vehicle).

The time until maximum concentration of DHA in plasma was not significantly different for the two treatments in this study, while $t_{max}$ for EPA and EPA + DHA was significantly different (Table 3). This may be due to the

 

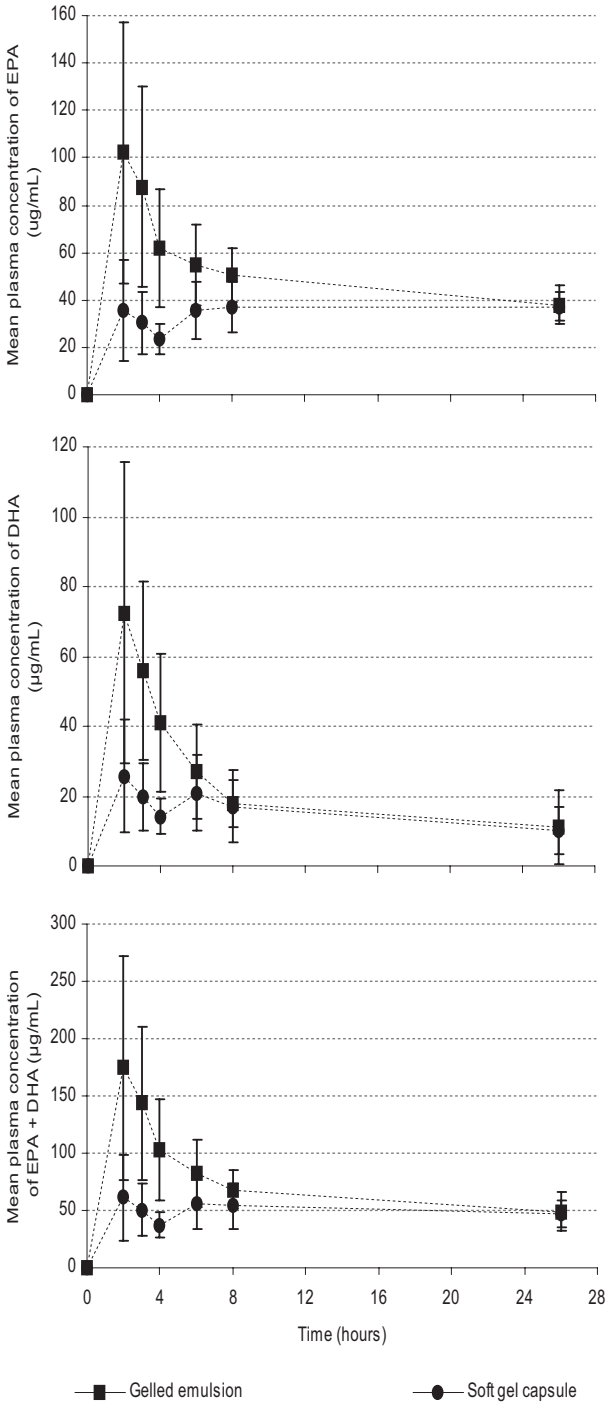


**Figure 1.** Mean blood plasma concentrations of EPA (top), DHA (middle), and EPA + DHA (bottom) from time 0–26 h post-administration of TAG oil via gelled emulsions and soft gel capsules.

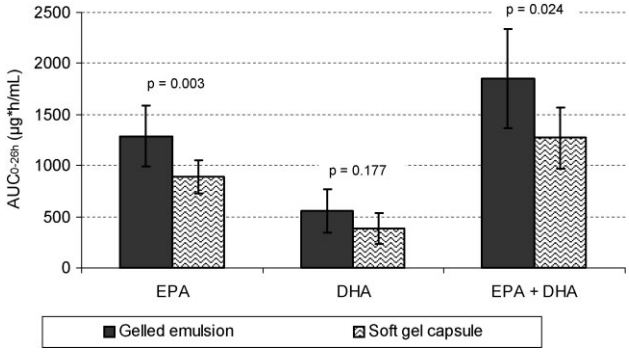


**Figure 2.** Incremental area under the curve from time 0–26 h ($AUC_{0–26\ h}$) post-administration of TAG oil delivered by gelled emulsions and soft gel capsules. Plotted values are means ± SD from the baseline adjusted ANCOVA analyses, adjusted for multiple comparisons using the Dunnet–Hsu method [24].

low number of subjects in each treatment group, but could also be due to the relatively long gap between administration and the first blood sampling. It is difficult to visually detect the maximum concentrations of EPA and DHA from the mean blood plasma concentrations (Fig. 1). The $t_{max}$-values were determined from statistical analyses, providing different values for the two test groups. The lack of distinct maximum peaks for the soft gel capsule group may indicate that the uptake of EPA and DHA from this treatment could be more sustained due to larger oil droplets in the duodenum. Larger droplets provide a smaller surface area for the lipases to attack resulting in a slower lipolysis, as illustrated by Armand et al. [20]. The initial slopes of the plasma concentration curves in Fig. 1 clearly show that there is a difference in the rate of absorption for the two test groups which may be caused by different rates of lipolysis for the two test groups. The rate of lipolysis is probably one of the most important factors for the rate of uptake of PUFAs and in vivo studies of the lipolysis must be performed to gain a full understanding of the bioavailability of fatty acids. Garaiova et al. [21] reported the

**Table 3.** Descriptive statistics of the results from the two treatment groups

| Outcome measure | Parameter | Treatment group: Gelled emulsion ($n = 9$) | Treatment group: Soft gel capsule ($n = 8$) |
|---|---|---|---|
| $C_{max}$ [a] (µg/mL) | EPA | 106.1 ± 52.5 | 49.4 ± 10.3 |
| | DHA | 74.4 ± 41.6 | 31.6 ± 12.4 |
| | EPA + DHA | 179.5 ± 93.8 | 79.1 ± 24.2 |
| $t_{max}$ [b] (h) | EPA | 2 [2–3] | 6 [2–17] |
| | DHA | 2 [2–2] | 2 [2–6] |
| | EPA + DHA | 2 [2–2] | 6 [2–17] |

$C_{max}$, the maximal incremental (change from baseline) plasma concentration; $t_{max}$, time after administration for the maximal incremental plasma concentration to occur.
[a] Mean ± SD.
[b] Median [25–75 percentile].

 

**Table 4.** Summary of relative bioavailability of DHA and EPA administered by the gelled emulsions or soft gel capsules

| | | Comparisons[a)] |
|---|---|---|
| Outcome | Parameter | Gelled emulsion versus soft gel capsule |
| $AUC_{0-26\ h}$-ratio | EPA | 1.45 (1.13–1.86); $p = 0.003$[b)] |
| | DHA | 1.46 (0.87–2.46); $p = 0.177$ |
| | EPA + DHA | 1.43 (1.05–1.96); $p = 0.024$[b)] |
| $C_{max}$-ratio | EPA | 2.00 (1.27–3.16); $p = 0.003$[b)] |
| | DHA | 2.16 (1.14–4.09); $p = 0.017$[b)] |
| | EPA + DHA | 2.06 (1.21–3.49); $p = 0.008$[b)] |

$C_{max}$, the maximal incremental (change from baseline) plasma concentration; $AUC_{0-26\ h}$, the incremental area under the plasma concentration curve.

[a)] Data are ratios of least square means (95% CI) from the baseline adjusted ANCOVA analyses, adjusted for multiple comparisons using the Dunnet–Hsu method [24].

[b)] Gelled emulsion group significantly increased compared to soft gel capsule group ($p < 0.05$).

total fatty acids incorporated into the phospholipid fraction of plasma and found $t_{max}$-values for EPA and DHA similar to data presented here and concluded that the emulsified oil had a shorter $t_{max}$ compared to bulk oil. The $t_{max}$-values of EPA and DHA have also been reported to be 8 and 24 h, respectively, in another similar study [14]. The reason for the differences in the $t_{max}$-values between different studies may be due to the fact that different compartments of the blood samples were analysed and due to different test products.

The single-dose treatment in this study is higher than normal daily omega-3 ingestions, but the results still reveal valuable information regarding the bioavailability and pharmacokinetics of DHA and EPA using the gelled emulsion technology. The pharmacokinetics of fatty acids from fish oil preparations have been studied by others and these studies revealed that the increase in plasma concentrations of EPA and DHA is dose dependent [3, 4, 31–33]. Even though the short-time absorption of EPA and DHA is dose dependent, different doses are found to result in comparable blood plasma concentrations over time [3].

In vitro experiments were performed to check that the gelled emulsions dissolved comparable to the conversional soft gel capsules. The distribution of the oil in the gel matrix and the emulsion formed after dissolution in vitro is illustrated in Fig. 3. It was found that the oil trapped inside the gel matrix was released and formed stable emulsions in vitro (Figs. 3 and 4). The release of oil from the soft gel capsules in vitro was not emulsified but merged on top of the AGJ (not measurable due to lack of small oil droplets and data are thus not included). During the in vitro dissolution testing only gentle stirring was applied (50 rpm) and this was most likely not enough to imitate the mechanical forces present in the stomach. In addition, the dissolution tests were performed without addition of enzymes (proteases and gastric lipase). It is well known that mechanical forces in the stomach, presence of proteases and gastric lipases and emptying of the stomach content through the antrum/pylorus contribute to emulsify oil in the stomach [12, 18]. This would probably influence both the release of bulk oil from capsules and the dissolved gelled emulsions to a greater extent than what was observed during the in vitro experiments. The experiments did, however, show that the biopolymers stabilising the emulsion could tolerate low pH and ionic strengths comparable to gastric conditions.

The droplet diameters found from the dissolved gelled emulsion during in vitro release are in the range of oil droplet diameters measured by other researchers [18–20]. From the experiments of the emulsion stability in this study it is tempting to assume that the oil released from the gelled emulsions

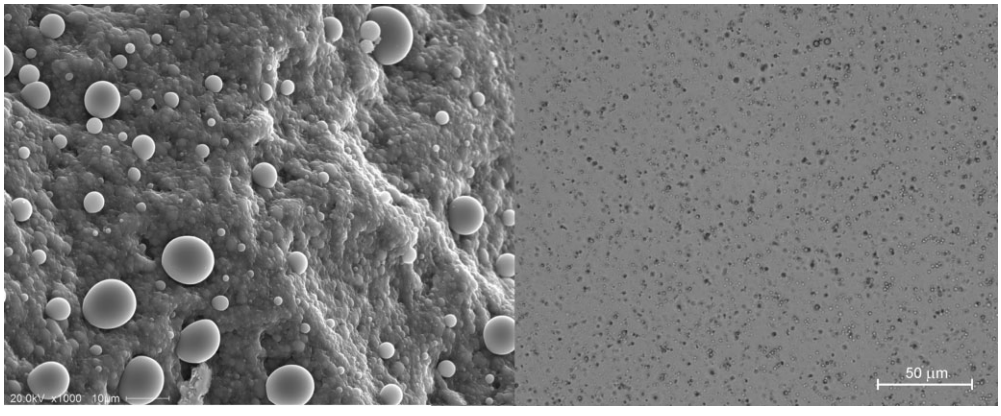


**Figure 3.** (left) Scanning electron microscope image (magnification 1000×) showing the emulsified oil (scale bar: 10 μm) inside the gel matrix. The average droplet diameter seems to be in the range of 2–5 μm and most droplets have diameters <10 μm. (right) Oil droplets distributed in AGJ after release from the gelled emulsion (light microscope, magnification 400×, scale bar: 50 μm).

 

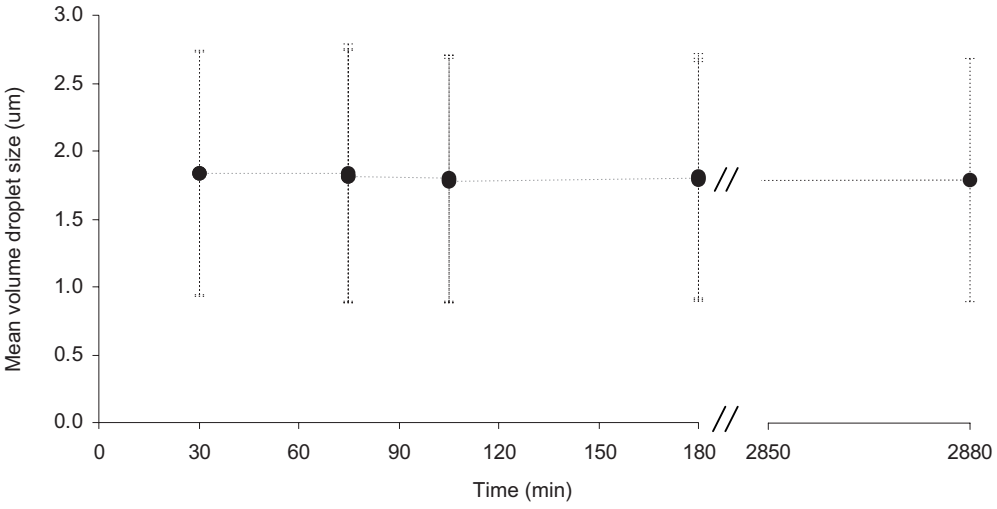


**Figure 4.** Mean volume droplet diameters in AGJ containing dissolved gel matrix (incubation at 37°C and 50 rpm). The SDs illustrate the distribution of droplet sizes in each replicate.

also could have been more extensively emulsified in the stomach and in the duodenum in vivo compared to oil released from soft gel capsules. Further analysis must, however, be performed to determine if the dissolution of the gelled emulsion results in a stable emulsion in vivo and to investigate how enzymes influence the oil droplets, their stability and the rate of lipolysis. Results from Armand et al. have shown that fine emulsions (from 0.7 μm to 6–8 μm) seems to coalescence into larger droplets to a greater extent than coarser emulsions (from 10 μm to 20–40 μm), and this may also be the case in our study. Still, they found that the emulsions containing the smallest droplets prior to ingestion also gave the smallest droplets in vivo and that these emulsions exhibited increased rate of lipolysis compared to the coarser emulsion, as previously mentioned [20]. Golding and Wooster [12], however, state that 'whilst considerable differences in emulsion structure and surface area can be achieved in the stomach, the rate of lipid uptake in the small intestine can be very similar' and refer to unpublished data [34]. This reflects the complexity of lipid digestion and shows that interpreting results from clinical studies is not straight forward based strictly on in vivo data.

Klinkesorn and McClements tested the emulsion stability and lipase digestibility of emulsions containing tuna oil (5% w/w tuna oil, 1% w/w lecithin, 100 mM acetate buffer and pH 3) in an in vitro human digestion model. They found that an oily layer was observed on top of the emulsion after passing through the model containing pancreatic lipases [17, 35]. The destabilisation did, however, also occur in the absence of lipases indicating that the coalescence probably was caused by other factors than the lipases. The authors indicate that the reason may be that lecithin did not stabilise the emulsions properly under the harsh condition in the in vitro digestion model. Testing emulsions in vitro is a complex process but may give indications whether an emulsion may pass through the stomach and intestines without total destabilisation and should also be performed for the gelled emulsions and the oil release from the soft gel capsules in this study.

It should be kept in mind that the results from this study only reveal the short-term absorption of EPA and DHA from a single dose. Other studies have, however, reported coherence between the short-time absorption of EPA and DHA, and the effect of long-term consumption [3, 36]. To be able to conclude regarding the long-term absorption and incorporation of EPA and DHA from the gelled emulsions a more thorough study should be performed.

There are some shortcomings in this study which should be mentioned and taken into consideration: The specifications of primary and secondary endpoints and their analyses were only vaguely described in the protocol, and most analyses were defined after blind-breaking. The analyses of bioavailability studies are, however, quite standardised and the analyses of this study follow the recommendations from EMEA [23]. Therefore, the late determination of analyses is not regarded as imposing any biases in the results.

In conclusion, this study suggests that increased bioavailability of EPA and DHA can be obtained when TAG fish oils are delivered by gelled emulsions compared to soft gel capsules. The increased bioavailability may be caused by the pre-emulsification of the oil, as also suggested by other researchers, but different designs of the vehicles may also contribute.

*The authors would like to thank Anne-Ma H. Carlsen, Department of Biotechnology, NTNU, Thomas E. Gundersen, Vitas AS, Randi Olsen, Faculty of Health Sciences, Department of Electron Microscopy, University of Tromsø, and Bjørn Flatnes, SINTEF,*

 

*Trondheim, for technical assistance. The research has been financed by Ayanda AS, The Research Council of Norway (SkatteFUNN) and Innovation Norway. MD Njål Flem Mæland, Ayanda AS, is thanked for input and help with the study protocol.*

*The authors have declared no conflict of interest.*

## References

[1] Cao, J., Schwichtenberg, K. A., Hanson, N. Q., Tsai, M. Y., Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin. Chem.* 2006, *52*, 2265–2272.

[2] Marangoni, F., Angeli, M. T., Colli, S., Eligini, S., et al., Changes of N-3 and N-6 fatty-acids in plasma and circulating cells of normal subjects, after prolonged administration of 20/5 (EPA) and 22/6 (DHA) ethyl-esters and prolonged washout. *Biochim. Biophys. Acta* 1993, *1210*, 55–62.

[3] Marsen, T. A., Pollok, M., Oette, K., Baldamus, C. A., Pharmacokinetics of omega-3-fatty-acids during ingestion of fish oil preparations. *Prostaglandins, Leukotrienes Essent. Fatty Acids* 1992, *46*, 191–196.

[4] Vidgren, H. M., Agren, J. J., Schwab, U., Rissanen, T., et al., Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. *Lipids* 1997, *32*, 697–705.

[5] Bucher, H. C., Hengstler, P., Schindler, C., Meier, G., N-3 polyunsaturated fatty acids in coronary heart disease: A meta-analysis of randomized controlled trials. *Am. J. Med.* 2002, *112*, 298–304.

[6] Tavazzi, L., Maggioni, A. P., Marchioli, R., Barlera, S., et al., Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): A randomised, double-blind, placebo-controlled trial. *Lancet* 2008, *372*, 1223–1230.

[7] Wang, C. C., Harris, W. S., Chung, M., Lichtenstein, A. H., et al., n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: A systematic review. *Am. J. Clin. Nutr.* 2006, *84*, 5–17.

[8] Brunborg, L. A., Madland, T. M., Lind, R. A., Arslan, G., et al., Effects of short-term oral administration of dietary marine oils in patients with inflammatory bowel disease and joint pain: A pilot study comparing seal oil and cod liver oil. *Clin. Nutr.* 2008, *27*, 614–622.

[9] Goldberg, R. J., Katz, J., A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. *Pain* 2007, *129*, 210–223.

[10] Raz, R., Gabis, L., Essential fatty acids and attention-deficit-hyperactivity disorder: A systematic review. *Dev. Med. Child Neurol.* 2009, *51*, 580–592.

[11] Schuchardt, J. P., Huss, M., Stauss-Grabo, M., Hahn, A., Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *Eur. J. Pediatr.* 2010, *169*, 149–164.

[12] Golding, M., Wooster, T. J., The influence of emulsion structure and stability on lipid digestion. *Curr. Opin. Colloid Interface Sci.* 2010, *15*, 90–101.

[13] Mcclements, D. J., Decker, E. A., Park, Y., Controlling lipid bioavailability through physicochemical and structural approaches. *Crit. Rev. Food Sci. Nutr.* 2009, *49*, 48–67.

[14] Raatz, S. K., Redmon, J. B., Wimmergren, N., Donadio, J. V., Bibus, D. M., Enhanced absorption of n-3 fatty acids from emulsified compared with encapsulated fish oil. *J. Am. Diet. Assoc.* 2009, *109*, 1076–1081.

[15] Lundin, L., Golding, M., Structure design for healthy food. *Aust. J. Dairy Technol.* 2009, *64*, 68–74.

[16] Dyerberg, J., Madsen, P., Møller, J. M., Aardestrup, I., Schmidt, E. B., Bioavailability of marine n-3 fatty acid formulations. *Prostaglandins, Leukotrienes Essent. Fatty Acids* 2010, *83*, 137–141.

[17] Klinkesorn, U., McClements, D. J., Influence of chitosan on stability and lipase digestibility of lecithin-stabilized tuna oil-in-water emulsions. *Food Chem.* 2009, *114*, 1308–1315.

[18] Armand, M., Borel, P., Dubois, C., Senft, M., et al., Characterization of emulsions and lipolysis of dietary lipids in the human stomach. *Am. J. Physiol.* 1994, *266*, G372–G381.

[19] Armand, M., Borel, P., Pasquier, B., Dubois, C., et al., Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol.* 1996, *34*, G172–G183.

[20] Armand, M., Pasquier, B., Andre, M., Borel, P., et al., Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. *Am. J. Clin. Nutr.* 1999, *70*, 1096–1106.

[21] Garaiova, I., Guschina, I. A., Plummer, S. F., Tang, J., et al., A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification. *Nutr. J.* 2007, *6*, 4.

[22] Haug, I. J., Draget, K. I., WO2007/085840, 2007.

[23] EMEA., Note for Guidance on the investigation of bioavailability and bioequivalence. *CPMP/EWP/QWP/1401/98* London: The European Agency for the Evaluation of Medicinal Products, 2001.

[24] Hsu, J. C., The factor analytic approach to simultaneous inference in the general linear model. *J. Comput. Graph. Stat.* 1992, *1*, 151–168.

[25] McDowell, E. M., Trump, B. F., Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch. Pathol. Lab. Med.* 1976, *100*, 405–414.

[26] European Directorate for the Quality of Medicines & HealthCare: European Pharmacopoeia Strasbourg Cedex, France, 2008.

[27] Elvevoll, E. O., Barstad, H., Breimo, E. S., Brox, J., et al., Enhanced incorporation of n-3 fatty acids from fish compared with fish oils. *Lipids* 2006, *41*, 1109–1114.

[28] Schram, L. B., Nielsen, C. J., Porsgaard, T., Nielsen, N. S., et al., Food matrices affect the bioavailability of (n-3) polyunsaturated fatty acids in a single meal study in humans. *Food Res. Int.* 2007, *40*, 1062–1068.

[29] Borel, P., Pasquier, B., Armand, M., Tyssandier, V., et al., Processing of vitamin A and E in the human gastrointestinal tract. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2001, *280*, G95–G103.

[30] Barrow, C. J., Nolan, C., Holub, B. J., Bioequivalence of encapsulated and microencapsulated fish-oil supplementation. *J. Funct. Foods* 2009, *1*, 38–43.

 

[31] Arterburn, L. M., Hall, E. B., Oken, H., Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J. Clin. Nutr.* 2006, *83*, 1467S–1476S.

[32] Arterburn, L. M., Oken, H. A., Hoffman, J. P., Bailey-Hall, E., et al., Bioequivalence of docosahexaenoic acid from different algal oils in capsules and in a DHA-fortified food. *Lipids* 2007, *42*, 1011–1024.

[33] Lund, E. K., Harvey, L. J., Ladha, S., Clark, D. C., Johnson, I. T., Effects of dietary fish oil supplementation on the phospholipid composition and fluidity of cell membranes from human volunteers. *Ann. Nutr. Metab.* 1999, *43*, 290–300.

[34] Golding, M., Wooster, T. J., Day, L., Lundin, L., et al., Impact of gastric structuring on the lipolysis of emulsified lipids. in preparation.

[35] Klinkesorn, U., McClements, D. J., Impact of lipase, bile salts, and polysaccharides on properties and digestibility of tuna oil multilayer emulsions stabilized by lecithin–chitosan. *Food Biophys.* 2010, *5*, 73–81.

[36] Rusca, A., Di Stefano, A. F. D., Doig, M. V., Scarsi, C., Perucca, E., Relative bioavailability and pharmacokinetics of two oral formulations of docosahexaenoic acid/eicosapentaenoic acid after multiple-dose administration in healthy volunteers. *Eur. J. Clin. Pharmacol.* 2009, *65*, 503–510.

 