Abstract
Recent years focus on the health benefits of omega-3 has caused foundation for a diverse assortment of omega-3 supplements with regards to quality. Bioavailability of the omega-3 fatty acids from various administration forms has been reported to be highly variable. In this study, a newly developed administration form for omega-3, the omega-3 tablet, has been tested for bioavailability of eicosapentaenoic acid and docosahexaenoic acid as triglycerides. It was found that the bioavailability, measured as relative levels of EPA and DHA in serum, was comparable between tablets and the traditional soft-gel capsules. It was further established that time to maximum concentration of EPA and DHA in serum was significantly shorter when the fatty acid esters were administered in tablets. A proposed explanation is that the uptake from the tablet formulation is less dependent on bile acids.

ABBREVIATIONS
AUC_0-24: Area Under Curve from 0 to 24 hours; β-CD: β-cyclodextrin; Cmax: maximum concentration; DC: Direct Compaction; DHA: Docosahexaenoic acid (22:6 n-3); EE: Ethyl Ester; EPA: Eicosapentaenoic Acid (20:5 n-3); GC: Gas Chromatography; TG: Triglyceride; Tmax: time for maximum concentration

INTRODUCTION
Health-aware individuals often supplement their diet with essential omega-3 fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The diverse selection of omega-3 supplements includes products of various quality. Lately, there has been a greater focus on important product properties, such as oxidative stability and bioavailability. The bioavailability of numerous omega-3 supplements has been reviewed by Schuchardt and Hahn (2013) and Ghasemifard, Turchini and Sinclair (2014). The authors observed pronounced variations in bioavailability depending on chemical binding form and formulation form. The authors further observed great variations in bioavailability caused by parallel nutrient intake and/or other ingredients in the formulation [1,2].

A recent addition to the omega-3 product assortment is the omega-3 tablet. Omega-3, in the form of triglyceride (TG) oil or ethyl ester (EE) oil, has been encapsulated by β-cyclodextrin (β-CD), successfully creating a dry, direct compaction (DC) grade powder. The described powders have made it possible to prepare high-quality tablets comprising up to 30% (w/w) TG oil or 30% (w/w) EE oil [3,4]. It was further observed that the 30% (w/w) TG powder was oxidative stable, with total oxidation values below 30, for 11 months stored at ambient temperatures. The corresponding value for coated tablets prepared from the TG powder was more than 13 months at the same conditions, with expected shelf-life of at least 22 months based on accelerated stability studies [5].

Hard tablets represent an administration form that offer active substances in convenient units. Tablets are solids comprising dry powders and typically exhibit few compatibility issues when more than one active substance is included in the same unit. This is a potential advantage over the traditional administration form for omega-3, the soft-gels, as combining other actives with oil inside the gelatin capsule requires the active to be compatible with the oil.
Most soft-gel capsules comprise of bovine/porcine gelatin and cannot be consumed by those requiring halal diet. Tablets, as an alternative administration form, would eliminate the need for gelatin, whilst the opportunity for an odor-free and tasteless supplement of omega-3 would be maintained.

The primary aim of the research presented was to establish oral bioavailability of omega-3, represented by EPA and DHA in TG form, from the newly developed omega-3 tablets. The oral bioavailability from the tablets was to be compared with soft-gel capsules, an administration form that is also a defined unit intended for oral administration by immediate swallowing. The soft-gels used in this study contained the same TG oil, in comparable doses, as used in the prepared tablets.

**MATERIALS AND METHODS**

**Materials**

Triglyceride concentrate, Vivomega 3322 TG, GC Bieber, Norway (EPA as TG 300 mg/g, DHA as TG 200 mg/g, total omega-3 fatty acid esters as TG 600 mg/g, ethyl esters maximum 10% (w/w)); β-cyclodextrin (Roquette, France); Avicel HFE-102 (IMCD, Sweden); talc (Fluka, Sigma Aldrich); magnesium stearate (Colorcon, UK); tridecanoic (Nu-ChecPrep, Inc., USA); dichloromethane (Ligamed MF-2-V, IMCD, Sweden); Nutraficient (Colorcon, USA); 3Nmethanolic HCl (Sigma Aldrich); dichloromethane, and cannot not be consumed by those requiring halal diet. Tablets, as an alternative administration form, would eliminate the need for gelatin, whilst the opportunity for an odor-free and tasteless supplement of omega-3 would be maintained.

The primary aim of the research presented was to establish oral bioavailability of omega-3, represented by EPA and DHA in TG form, from the newly developed omega-3 tablets. The oral bioavailability from the tablets was to be compared with soft-gel capsules, an administration form that is also a defined unit intended for oral administration by immediate swallowing. The soft-gels used in this study contained the same TG oil, in comparable doses, as used in the prepared tablets.

**Materials**

![Tablets and soft-gel capsules used in the study](image)

The study was organized as a randomized crossover study where ten (10) participants were given a single dose triglyceride oil (3 g) formulated as tablets followed by soft-gel capsules, or the reverse sequence. A washout period of 14 days was applied between administrations of the individual dosage forms. The baseline serum concentrations of EPA and DHA were measured prior to administration at the respective test-days and the relative increase in individual levels of EPA and DHA was followed for 24 hours. Blood samples were taken before administration of omega-3 (baseline) and 2, 3, 5, 8 and 24 hours after administration.

The participants were asked to avoid certain types of food (fish, linseed, linseed oil, soy oil, walnuts, products containing grapefruit, vitamin supplements and supplements in general) for 1 week prior to the first test-day and through the entire duration of the study. In addition, they were asked to avoid caffeine and alcohol 72 hours prior to, and during, each test-day.

The diet on each 24 h test-day was standardized and the participants were asked to avoid eating or drinking from 8 p.m. the day before testing. The standardized breakfast was a fat-rich meal (mammalian fat) served immediately after administration of omega-3.

**Participants:** Ten (10) participants were included in the study. All participants signed a written informed consent prior to entering the study.

The following inclusion/exclusion criteria were applied; sex (only males were included), age (only men >18 years) and body mass index (restricted to 20-28 kg/m²). The participants were non-smokers, non-abusers of alcohol/narcotics, were not taking any regular medication, had not donated blood the last 2 weeks and had not participated in other clinical studies the last 30 days prior to the study.

All included participants finalized the entire bioavailability study.

**Handling of serum samples:** Blood samples were drawn into BD Vacutainer SST II Plus Advance 7 (5 ml) and left standing in room temperature for 45 min. Samples were centrifuged at room temperature (2780 rpm) for 10 min, serum decanted and stored refrigerated (4-6°C) for 24 to 48 hours until analysis.

**Quantitative measurement of fatty acids in serum by GC:** A full fatty acid profile from each participant's serum was obtained using gas chromatography (GC). Serum samples were vortexed, centrifuged and pipetted into vials. Internal standard (tridecanoin) was added and samples were methylated with 3N MeOH/HCl. Methylated fatty acids were extracted with hexane, and then neutralized with 3N KOH in water. After mixing and centrifuging the hexane phase was injected into GC-FID. Analysis was performed on a 7890A GC with a split/splitless injector, a 7683B automatic liquid sampler, and flame ionization detection (Agilent Technologies, Palo Alto, CA). Separation was performed on a SP-2380 (30 m × 0.25 mm i.d. × 0.25 μm film thickness) column (Supelco, USA). Temperature program as follows; initially 90°C for 0.5 min, then 50°C/min to 150°C, 10°C/min to 225°C and finally 120°C/min to 245°C for 3 min. Total run time was 12.37 minutes. Fatty acids were quantified against internal standard tridecanoin. For EPA and DHA, calculated response factors obtained from a mixture of commercial FAMEs (NuCheck, Inc) were used. For all other fatty acids, theoretical responses were used.

**Tablets and soft-gel capsules used in the study:** The tablets used in the bioavailability study were prepared from 30% (w/w) TG DC grade powder as described in "Characterization of Omega-3 Tablets" by Vestland, Jacobsen, Sande, Myrset and Klaveness (2015). The content of EPA and DHA in each tablet was 86 mg and 71 mg, respectively. A total of 19 tablets were administered as one single administration (equals to 1634 mg EPA and 1349 mg DHA).

The soft-gel capsules used in the study contained the exact same oil as the tablets and was kindly donated by the supplier of the oil (section 2.1). Each soft-gel capsule contained 270 mg EPA and 219 mg DHA. Six soft-gel capsules were administered as a single dose (equals to 1620 mg EPA and 1314 mg DHA). The quantitative content of EPA and DHA in each administration form was analysed specifically for this study, see section 2.2.5.

**Quantitative fatty acid analysis of tablets and soft-gel capsules:**

**Tablets:** Five tablets were grounded into powder, extracted...
with hexane:dichloromethane:methanol with added internal standard tridecanoin and then methylated with 3N MeOH.HCl.

**Soft-gel capsules:** Ten oil capsules were cut in two and the content dissolved in hexane:dichloromethane:methanol. Internal standard tridecanoin was added and samples were methylated with methanolic HCl.

The fatty acid methyl esters from both administration forms were analyzed by GC-FID as detailed in section 2.2.3.

**Statistical analyses:** The results from the quantitative analyses of EPA and DHA in serum were analysed with Wilcoxon signed-rank test and p-values were recorded for the differences observed in data from the two administration forms (SPSS, version 18). Relative bioavailability was assessed by AUC, ratios of soft-gel:tablet for EPA and DHA, respectively. AUC was calculated using the linear trapezoidal rule.

H0: there is no difference in bioavailability of EPA and DHA from the two administration forms, omega-3 tablets and soft-gel capsules.

H1: there is difference in bioavailability of EPA and DHA from the two administration forms, omega-3 tablets and soft-gel capsules.

Significance level was 5% (p-value = 0.05).

**RESULTS AND DISCUSSION**

Bioavailability is defined as the fraction of an administrated dose of a molecule that reaches systemic circulation [6]. However, the ability of a molecule to reach systemic circulation does not necessarily describe its ability to reach intended in vivo targets. The presence of a molecule in systemic circulation is nevertheless accepted to be a relevant indicator, as the systemic circulation is the link between different compartments of the body [1,2].

In the current study, presence of EPA and DHA in systemic circulation of the participants after ingestion of fatty acid esters in TG form in tablets or soft-gels were used to compare the bioavailability from the respective administration forms. The results showed that both EPA and DHA had a non-significant numerical higher systemic exposure following administration of omega-3 tablets, compared to soft-gels (Figure 1, Table 1, 2).

It must be noted, however, that the absorption of EPA and DHA from the omega-3 tablets was faster than expected and the study design was unfortunately not successful in registering the true concentration peak for tablets. The first sample obtained (T=2 h) was at the apparent Cmax for the tablet formulation, hence, it cannot be excluded that the true concentration peak came earlier and was higher (Figure 1).

While the differences in systemic exposure of EPA and DHA observed between the two administration forms were not statistically significant, the differences in time for maximum concentration (Tmax) were (Table 1).

The significant differences in Tmax clearly show that the administration form had influence on the rates of absorption of the fatty acid esters. Upon oral administration of the two administration forms, they will appear closely similar, no odour or taste, comparable size, indented for immediate swallowing.

The gelatin layer of the soft-gel capsules will after ingestion be punctured and the oil will, unless the soft-gel is enteric coated, be released in the stomach. TG oil is lighter than water and in cases where portions of the oil initially floats on top of the liquid stomach content, there will be a certain degree of retention of oil from the soft-gels in the ventricle. This lag time will vary between individuals, as can be observed also in this study by the relatively high standard deviations (SD) observed in Tmax after administration of EPA and DHA as soft-gels (Table 2).

The tablet will upon disintegration in the stomach release the fatty acid esters in complex with the β-CD. β-CD form complexes with lipophilic compounds through non-covalent interactions between the hydrophobic cavity of β-CD and the hydrophobic part of the guest molecule. One of the most common reasons for use of β-CD or other cyclodextrins is their ability to increase the bioavailability of lipophilic substances, among other by increasing their solubility in the hydrophilic environment in vivo through complexing [7-9]. The surface of β-CD is hydrophilic and this will facilitate mixture of the β-CD:fatty acid ester complex with the hydrophilic stomach content. The fatty acid esters then avoid the lag time normally caused by their relative insolubility in water.

The time limiting step in the absorption of omega-3 from the tablets was probably the time the tablets needed to disintegrate and release the β-CD:fatty acid ester complex. Disintegrants were not added to the tablets used in this study, an excipient that would
Table 1: It could be observed from the statistical analysis (section 2.2.6) that the differences between T_max were significant for the uptake of both EPA and DHA in serum; hence, the uptake of EPA and DHA from omega-3 tablets was significantly faster than from soft-gel capsules. Statistical significance can be observed as p-values < 0.05.

<table>
<thead>
<tr>
<th>p-values (Wilcoxon)</th>
<th>AUC_0-24</th>
<th>C_max</th>
<th>T_max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values for EPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.88</td>
<td>0.51</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Values for DHA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.72</td>
<td>0.58</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean values and standard deviations (SD) for the overall relative level of EPA and DHA in serum (relative area under curve, 0-24 hours) for all ten participants in the bioavailability study.

<table>
<thead>
<tr>
<th>Values for EPA</th>
<th>AUC_0-24 (µg*h/ml)</th>
<th>C_max (µg*h/ml)</th>
<th>T_max (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG tablet</td>
<td>Mean 686</td>
<td>58</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>SD 210</td>
<td>19</td>
<td>0.5</td>
</tr>
<tr>
<td>TG soft-gel</td>
<td>Mean 660</td>
<td>49</td>
<td>7.3</td>
</tr>
<tr>
<td>capsule</td>
<td>SD 241</td>
<td>18</td>
<td>6.7</td>
</tr>
<tr>
<td>Values for DHA</td>
<td>AUC_0-24 (µg*h/ml)</td>
<td>C_max (µg*h/ml)</td>
<td>T_max (h)</td>
</tr>
<tr>
<td>TG tablet</td>
<td>Mean 425</td>
<td>47</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>SD 250</td>
<td>17</td>
<td>1.0</td>
</tr>
<tr>
<td>TG soft-gel</td>
<td>Mean 415</td>
<td>37</td>
<td>4.6</td>
</tr>
<tr>
<td>capsule</td>
<td>SD 246</td>
<td>18</td>
<td>2.3</td>
</tr>
</tbody>
</table>

reduce the time needed for this step. Addition of disintegrants in the tablets would, hence, presumably further reduce observed Tmax of EPA and DHA.

The absorption of fat initiates in the small intestine. Normally, as will be the case for oil from soft-gels, fat in the small intestine is emulsified by bile acids and lipases will cut off fatty acids from the triglycerides, creating monoglycerides and free fatty acids. Lipases will only be able to work in the water-fat interface due to their solubility, and hence, the enzymes will only have access to the surface of fat droplets [10].

The triglycerides from the omega-3 tablets will be released from the complex due to the highly diluted and dynamic environment in the gastrointestinal system. The triglycerides will then be present as individual molecules, this secures immediate access for lipase and may eliminate or reduce the need for bile acids.

The β-CD is mainly metabolised by bacteria in caecum and colon; only a limited amount (1-2%) is absorbed after oral administration [8].

Schuchardt and Hahn (2013) conclude in their review that future guidelines on dietary supplementation for omega-3 should include taking the supplement with a sufficient amount of fat [1]. The ingestion of the extra fat triggers additional bile activity, hence enhancing the bioavailability of omega-3. Omega-3 tablets may prove to be far less dependent of such simultaneous additional fat intake.

CONCLUSIONS

It was established in this study that EPA and DHA was readily bioavailable from omega-3 tablets prepared from β-CD:fatty acid ester complexes. It was further observed that the bioavailability from tablets was comparable to soft-gels whenomega-3oil type and dose was the same for both administration forms. C_max was reached significantly faster when the administration form was the newly developed tablet. A proposed reason for the faster uptake was the possibility for more rapid passage through the gastrointestinal system due to altered solubility of fatty acid esters in complex with β-CD and less dependence of emulsifying bile during absorption.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

Tina Lien Vestland is a shareholder and employee in Omegatri AS. Jo Klaveness is shareholder in the company.

REFERENCES


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