Bioavailability of n-3 fatty acids from enriched meals and from microencapsulated powder

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Supervisor: Alfons Ramel PhD

Thesis for the degree of Master of Science in Human Nutrition Faculty of Food Science and Nutrition, School of Health Sciences University of Iceland
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Lífaðgengi n-3 fitusýra sem viðbætt er í tilbúna rétti og í duftformi

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Leiðbeinandi: Alfons Ramel vísindamaður

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Faculty of Food Science and Nutrition
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ÁGRIP

Bakgrunnur og markmið: Þrátt fyrir hugsaðan jákveiðan ávinnin á neyslu á lóngum omega-3 fjölómëttuðum fitusýrum er hún oft í lágmarki vegna líttillar neyslu á feitu fiskmeti. Órhúðuð fiskioliía í duftformi er hugsaður kostur til þess að auka neysluna. Markmið rannsóknarnarinnar var að kanna lífaðgengi langra omega-3 fjölómëttatöra fitusýra í duftformi og í tilbúnum réttum með viðbættu þorsklýsi.

Aðferðir: Gögnun var safnað í 4 vika tvíblindri fæðu íhlutun, þátttakendur (N = 99, aldur ≥ 50 ára) var skipt í þrjá hópa að handahófi. Hópur 1 (n = 38) fékk 1,75 g/dag af LC n-3 PUFA viðbætt í máltíðir þar sem notast var við fljótandi þorsklýsi og lyfleysu duft, hópur 2 (n = 30) fékk sama magn af LC n-3 PUFA sem órhúðað þorsklýsi í duftformi og hefðbundar máltíðir, hópur 3 (n = 31) var viðmiðunarhópur og fékk lyfleysu duft og hefðbundnar máltíðir. Blóðsýni voru tekin við upphaf og lok rannsóknar.

Niðurstöður: Sjötíu og sjö þátttakendur (77,8%) luku við rannsóknina. EPA magn í blóði tvöfaldaðist í báðum íhlutunanarbópum sem fengu LC n-3 PUFA (P<0.05) en hélst öbreytt hjá viðmiðunarhóp. Breytingar á DHA voru minni en þó var marktækur munur í báðum íhlutunanarbópum. Einnig hækkaði omega-3 index og batnaði n-6/n-3 hlutfallið til muna í íhlutunanarbópum (P<0.05) en hélst öbreytt í viðmiðunarhóp.

Álykun: Eftir fjórar vikur af reglulegri neyslu, jókst LC n-3 PUFA magn í blóði verulega í báðum íhlutunanarbópum en ekki í viðmiðunarhóp. Ífafaðengi órhúðaþra LC n-3 PUFA í duftformi er sambeerilegt við lífaðengi LC n-3 PUFA úr tilbúnum máltíðum sem viðbætt eru með fljótandi þorsklýsi.
ABSTRACT

Background and aims: Despite potential benefits of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), intake is often low due to low consumption of oily seafood. Microencapsulated fish oil powder has been suggested as an alternative way to increase intake. The main aim of the thesis was to examine the bioavailability of LC n-3 PUFA from microencapsulated powder and from meals fortified with liquid cod liver oil.

Methods: Data were collected in a 4-week double-blinded dietary intervention, participants (N = 99, age ≥ 50 years) were randomized into three groups. Group 1 (n = 38) received 1.75 g/d LC n-3 PUFA as ready-to-eat meals enriched with liquid cod liver oil and placebo powder, group 2 (n = 30) received the same amount of LC n-3 PUFA as microencapsulated cod liver oil powder and regular meals; and group 3 (n = 31) was the control group which received placebo powder and regular meals. Blood samples were collected at baseline and endpoint.

Results: Seventy-seven subjects (77.8%) finished the study. The amount of EPA in blood doubled in both groups who received LC n-3 PUFA (P<0.05) but did not change in the control group. The changes in DHA were less but still significant in both intervention groups. The omega-3 index as well as the n-6/n-3 ratio improved dramatically in the two LC n-3 PUFA groups (P < 0.05). No changes were observed in the control group.

Conclusion: After four weeks of regular consumption, the amount of LC n-3 PUFA in blood increased significantly in both intervention groups but not in the control group. Bioavailability of LC n-3 PUFA in encapsulated powder is very similar to bioavailability of LC n-3 PUFA in ready-to-eat meals enriched with liquid cod liver oil.
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The work presented in this thesis was conducted at the Unit for Nutrition Research, University of Iceland & Landspitali National University Hospital, the Faculty of Food Science & Nutrition, University of Iceland and MATÍS.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AA</td>
<td>Arachidonic acid (20:4 n-6)</td>
</tr>
<tr>
<td>ALA</td>
<td>Alfa-linolenic acid (18:3 n-3)</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular diseases</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid (22:6 n-3)</td>
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<tr>
<td>DPA</td>
<td>Docosapentaenoic acid (22:5 n-3)</td>
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<tr>
<td>E %</td>
<td>Percentage of total energy intake</td>
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<tr>
<td>EPA</td>
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<td>Fatty acids</td>
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<tr>
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<td>Icelandic National Nutrition Survey 2002</td>
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<tr>
<td>INNS 2011</td>
<td>Icelandic National Nutrition Survey 2010-2011</td>
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<tr>
<td>LA</td>
<td>Linoleic acid (18:2 n-6)</td>
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<td>LC n-3 PUFA</td>
<td>Long chain omega-3 fatty acids</td>
</tr>
<tr>
<td>LC n-3 PUFA</td>
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</tr>
<tr>
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<td>Monounsaturated fatty acids</td>
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<td>Nordic Nutrition Recommendations</td>
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<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
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<td>SAFA</td>
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1 INTRODUCTION

The n-3 fatty acids EPA and DHA are long chain fatty acids and play an important role in the body such as structure and functions regarding cell membranes.1,2,3 Potential effects from LC n-3 PUFA on various diseases e.g., CHD, have been noticed although studies have not been completely unanimous.4,5,6,7,8,9

According to dietary recommendations,1 energy intake from fat should be 25-40% and thereof should polyunsaturated fatty acids contribute 5-10% of the total energy with an emphasis on oily fish twice a week. Furthermore 1% of energy should come from LC n-3 PUFA which correspond to 2.2 g consumption of n-3 fatty acids in a 2000 kcal diet.1

Despite these recommendations, consumption of oily fish has been decreasing and therefore it is necessary to find other effective ways to meet the requirement of LC n-3 PUFA.10,11 Fortification of foods with LC n-3 PUFA could be a way to increase consumption of these fatty acids. Previous studies have fortified a wide range of products with positive outcomes,12,13 however, it has also been reported to be difficult to fortify food due to the dominant fish flavour which is not desirable in many of the products.14

In general, fortification with microencapsulated nutrients has shown to be successful, this applies also to fat where the microencapsulation additionally has an inhibitory effect on unwanted flavour and odour and is therefore a suitable choice for fish oil supplements.15

However, the absorption of PUFA is a complex process and their bioavailability can vary considerably depending on their chemical form they appear in.16 The bioavailability of omega-3 fatty acid formulations have been investigated at some level for the reason that it depends on numerous factors, such as presence of other components affecting the uptake of the fatty acids, concomitant intake of food and primarily the chemical bonds of the fatty acid.16

Thus, the major objectives and research questions of the thesis were to:

1. Investigate bioavailability of LC n-3 PUFA from meals fortified with liquid fish oil.
2. Investigate bioavailability of LC n-3 PUFA from microencapsulated powder dissolved in water.
3. Compare bioavailability of LC n-3 PUFA from fortified meals to LC n-3 PUFA from microencapsulated powder.
2 REVIEW OF LITERATURE

2.1 Fats

Fat is one of the body's major energy sources beside proteins and carbohydrates. Fat also provides essential fatty acids and fat-soluble vitamins. Most dietary fats consist of triglycerides which are molecules composed of a esterified glycerol and three fatty acid molecules, less than 1% of dietary fatty acids are phospholipids and are found in cell membranes, milk fat globules and eggs. One gram of dietary fat approximately provides 9 kilocalories (kcal) per gram or 37 kilojoules (kJ). In vivo phospholipids and cholesterol are involved in cell membranes, but triglycerides are stored in adipose tissue as energy reserves.

Fatty acids (FA) contain various lengths of a hydrocarbon chain which have a carboxyl group (COOH) at one end and a methyl group (CH3) at the other end. FA are classified according to the number of carbons of the chain and type of bonds that are between the carbons. They are classified as following; saturated fatty acids (SAFA); contain no double bonds, monounsaturated fatty acids (MUFA); contain one double bond and polyunsaturated fatty acids (PUFA) contain two or more double bonds. The position of double bonds can be calculated both from the carboxyl-terminal end of the carbon chain and the methyl end.

SAFA are fully hydrogenated and because of their linear chain due to containing no double bonds between carbon atoms and structural properties, the fatty acids can tightly pack and exist at a solid state at room temperature. The human body can synthesise saturated fatty acids at a low extent.

The most prevailing SAFAs in the diet are lauric, myristic, palmitic, and stearic acid, they originate primarily from animal origin, including meat, eggs and butter or from processed food which contains naturally saturated vegetable oils.

MUFA's have one double bond which often is located at carbon nine (n-9) when counted from the methyl end. MUFA's are present in a great variety of foods such as nuts, vegetables, seed oils, meats and dairy products. Oleic acid is one of the abundant fatty acids found in foods and is present in high amounts in olive oil, canola oil, avocados and almonds. Several other MUFA's exist but are present in low amounts in the diet.
2.1.1 Polyunsaturated fatty acids – classification and structure

Fatty acids that contain two or more double bonds are called polyunsaturated fatty acids (PUFA). The most common PUFA are omega-3 (n-3) and omega-6 (n-6) thereof are α-linolenic acid and linoleic acid considered essential fatty acids because humans cannot synthesise these fatty acids. The reason is that human organisms lack the enzymes Δ 12-and Δ 15-desaturase that insert double bonds in the n-6 and n-3 positions.¹

![Diagram of polyunsaturated fatty acid metabolism]

**Figure 1:** Metabolism of polyunsaturated fatty acids¹
The n-6 and n-3 fatty acids compete for the same enzymes (e.g., desaturases, elongases and cyclo-oxygenases), however n-6 FA cannot convert into n-3 FA and vice versa. The n-3 FA have a higher affinity for the enzymes but if the diet is imbalanced, between n-6 and n-3 FA it might influence in further metabolism on n-6 FA, this is referred as the n-6/n-3 ratio.1 PUFA are further metabolised into a group of biologically active substances including prostaglandins, prostacyclins, leukotrienes and thromboxanes which are classified as eicosanoids.1

Naturally occurring PUFA found in plants and wild fish are mainly cis- fatty acids. PUFA serve important functions in the organism, they are important structural components of cell membranes, are essential for various membrane characteristics and functions including fluidity, permeability, activity of membrane-bound enzymes and receptors, and signal transduction.1

2.1.1.1 Omega-3 fatty acids
Omega-3 polyunsaturated fatty acids are classified based on the position of the first double bond, which is located at the third carbon atom from the methyl end of the fatty acid. The main omega-3 fatty acids obtained from the diet are α-linolenic acid (ALA; 18:3) which is considered a shorter n-3 chain, eicosapentaenoic acid (EPA; 20:5), docosahexaenoic acid (DHA; 22:6) and docosapentaenoic acid (DPA; 22:5). Omega-3 fatty acids that have 20 carbons or more are called long-chain omega-3 fatty acids (LC n-3 PUFA) which refer to the latter three fatty acids.

LC n-3 PUFA play an important role in the body, for example as structural components of the cell membrane and in various membrane functions. E.g., DHA is required in the nervous system for optimal neuronal and retinal function, growth of the brain and other membrane-rich tissues in foetal and early postnatal life.1

Oily seafood is the main source of LC n-3 PUFA such as salmon, herring and trout, fish oil supplements are also a relevant source.3

2.1.1.2 Omega-6 fatty acids
Omega-6 fatty acids have their first double bond on the sixth carbon atom counted from the methyl end of the fatty acid. Linoleic acid (LA) is considered the most common dietary PUFA in Western diet, the quantity of Arachidonic acid (AA) is relatively smaller.3 The body can
synthesise AA from LA and therefore AA is strictly not classified as an essential FA. N-6 PUFA are mostly originated from plant oils rich in LA such as corn oil, sunflower oil and soybean oil.\textsuperscript{3}

![Figure 2: Structures of ALA, EPA, DHA and LA](image)

### 2.2 Recommendations

Fat should provide 25-40% of total energy intake (E\%) according to recommendations from the Nordic Nutrition Recommendations (NNR).\textsuperscript{1} MUFAs and PUFA should make up at least two thirds of the total FA, divided as 10-20 E\% from cis-MUFA and cis-PUFA should contribute 5-10 E\%. SAFA should not exceed 10 E\% calculated as triglycerides. LC n-3 PUFA should at least contribute 1 E\% for adults and children from 2 years of age.\textsuperscript{1} That corresponds to 2.2 g of n-3 FA in a diet containing 2000 kcal.

Recommendations on LC n-3 PUFA intake published by various scientific entities are in good agreement. The American Dietetic Association recommends consumption of two or more servings of oily fish per week to provide at least 500 mg EPA and DHA per day with the aim of 0.5\%-2.0\% energy as n-3 FA.\textsuperscript{17} This is comparable to recommendations published by WHO.\textsuperscript{2} EFSA indicates an adequate intake of 250 mg/d of EPA + DHA which corresponds to 1-2 fatty fish meals per week for adults based on cardiovascular considerations.\textsuperscript{3} Long-term supplemental intake of EPA + DHA up to 5 g/d and supplemental intake for EPA alone up to 1.8 g/d do not raise safety concerns for adults.\textsuperscript{18}
2.3 Consumption
Despite recommendations and awareness of the benefits from fish consumption, food habits have changed over the years and currently only few people meet the recommendations of consumption; two fish meals per week with a focus on oily fish.\textsuperscript{1,10,11} This is possibly the leading reason for such a low intake of LC n-3 PUFA which is also the case in Iceland.\textsuperscript{19}

According to the Icelandic National Nutrition Survey 2010-2011 (INNS 2011) approximately half the population reached the recommendations on fish consumption (300g per week) which is comparable to the findings of the Icelandic National Nutrition Survey from 2002 (INNS 2002).\textsuperscript{10,11} According to these studies older people usually consume more fish than younger people. Furthermore INNS 2011 showed that only 13\% of the fish consumption comes from oily fish, which has a considerably higher amount of LC n-3 PUFA in contrast to lean fish.\textsuperscript{10} The Icelandic population gets LC n-3 PUFA mostly from cod liver oil (51\%) and from fish and fish products (34\%). The rest divides to meat and meat products (9\%), eggs (2\%), cheese (1\%), cereals (1\%), soups and sauce (1\%) and other fats (1\%).\textsuperscript{10}

2.4 Omega-3 supplements
Omega-3 FA can be ingested in various forms as supplements. There are many types of capsules and pills available that contain n-3 and there is also the option of supplements in oil form. The origins of the FA are diverse and can for example come from fish, algae, nuts, oils and seeds. The quality of n-3 FA can be different both between its origin, brands and manufacturers. This topic has to be examined further to get an overall perspective.

2.4.1 Fish liver oil
There is a long tradition in Iceland for the use of fish liver oil. Fish liver oil has been produced in Iceland since the 18\textsuperscript{th} century and has been an important source of LC n-3 PUFA for Icelandic people for decades. From 1931-1970 cod liver oil was routinely poured into the mouths of children attending elementary schools to prevent and reduce malnutrition and vitamin-D deficiency among children in Iceland.\textsuperscript{20}

Cod liver oil is rich in EPA and DHA fatty acids and also contains A, D and E vitamins. In recent years cod liver oil has also been produced in capsules which makes the ingestion easier for people who do otherwise not tolerate the strong fish flavour of cod liver oil.\textsuperscript{21}
Still today there is a large group of people in Iceland that consume fish liver oil as a dietary supplement for n-3 fatty acids and the INNS 2011 showed that 21% of participants reported that they consumed fish liver oil on a daily basis and another 13% consume fish liver oil 1-6 times per week. This group also meets the recommendations for consumption of vitamin D, nonetheless poor vitamin D status is a concern in Iceland.\textsuperscript{10}

Nutritional status of the Icelandic population can be connected to consumption of fish liver oil, furthermore higher vitamin D and vitamin E ingestion can be connected to changes that have been made in the production of fish liver oil through the years.\textsuperscript{10}

2.5 LC n-3 PUFA and disease

Studies have shown potential benefits of LC n-3 PUFA against various diseases.\textsuperscript{22} Studies for example indicate that EPA and DHA can reduce cardiovascular disease (CVD) risk in persons with high risk factors.\textsuperscript{22} LC n-3 PUFA have also been suggested to reduce risk of death from coronary heart diseases in healthy adults with a reasonable intake target with a range of 400-500 mg/d.\textsuperscript{19,23,24} EFSA has given dietary recommendations for EPA + DHA consumption based on CVD risk considerations for adults between 250 – 500 mg/d.\textsuperscript{18} Large intakes of supplemental EPA + DHA up to 5 g/d do not raise safety concerns for adults.\textsuperscript{18}

A systematic review focusing on clinical trials and randomized control trials of LC n-3 PUFA observed a fall of approximately 10% in CVD events, lower frequency of cardiac death and coronary events within intervention groups that ingested LC n-3 PUFA.\textsuperscript{22} A meta-analysis in epidemiological studies conducted in the United States focusing on dose-response of omega-3 intake and CHD death also observed positive outcomes.\textsuperscript{23} An overall risk reduction around 40% was noticed in groups with high intake of EPA+DHA where the average intake of 560 mg/d showed the greatest overall reduction in risk from CHD.\textsuperscript{23} A comparison of two trials between fish and purified EPA+DHA showed simular reductions in coronary mortality in post-myocardial infraction patients which strongly suggests that it is the LC n-3 PUFA present in oily fish that are the cardioprotective factors.\textsuperscript{23}

The therapeutic value of LC n-3 PUFA in the treatment of inflammatory diseases has also been noticed with a particular focus on rheumatoid arthritis.\textsuperscript{25,26} The effect of LC n-3 PUFA on inflammatory is due to the decrease in production of inflammatory eicosanoids and cytokine.\textsuperscript{25} Studies have also suggested that supplementation of LC n-3 PUFA in parenteral
nutrition is safe and may for instance improve immune- and hyper-inflammatory response in surgical intensive care unit patients after major surgery.²⁷

However, not all studies are in good agreement and several meta-analyses and systematic reviews did not find convincing proof on the protective effects of LC n-3 PUFA.⁵,⁶,²⁸,²⁹

Recent findings have suggested that LC n-3 PUFA play a role in enhancing prostate tumorigenesis.³⁰ A nested case-cohort study indicates that a high content of LC n-3 PUFA in plasma phospholipids was associated with increased risk of total-, low-, and high-grade prostate cancer.³⁰ However, this study has been criticized pointing out flaws such as inappropriate measures of dietary exposure to LC n-3 PUFA and insufficient data to conclude that LC n-3 PUFA are conclusively linked to increased risk of prostate cancer.³¹,³² Other studies drawing similar conclusions can also be criticised for inappropriate measures.³³ Findings regarding LC n-3 PUFA and prostate cancer are inconsistent and other studies have even noticed association between LC n-3 PUFA and decreased risk of prostate cancer.³⁴,³⁵ This subject has to be further investigated in order to draw a more accurate conclusion in this matter.

2.6 Omega-3 index

The omega-3 index is a biomarker which reflects the amount of LC n-3 PUFA in red blood cells expressed as a percentage of total fatty acids.³⁶ The index was developed in course of a 6-month dose response study in which subjects ingested LC n-3 PUFA in 3 different amounts and was then applied to various data from epidemiological and intervention studies to predict heart disease mortality. It was concluded that average omega-3 index associated with the lowest risk for death of CHD was about 8% and the highest risk was below 4%. The omega-3 index may be useful assessing baseline risk and changes in risk as a function of intake.³⁷

2.7 Omega-6 / Omega-3 ratio

It has been suggested that the human kind evolved on a diet with a very low ratio between n-6 and n-3 FA, as low as 1:1.³⁸ Due to the large consumption of corn, safflower and sunflower oil and other food products that contain only small sources of LC n-3 PUFA the typical western diet has developed an n-6/n-3 ratio of approximately 10-15:1.³⁹
There have not been established recommendations for the n-6/n-3 ratio as there are insufficient data on clinical and biochemical endpoints in humans. The total intake of each n-6 and n-3 FA should be of more importance than the ratio, as long as the basic dietary requirements are met. However, some studies imply that a low ratio is important for human health and protective against degenerative pathologies. According to them it is preferable to keep the ratio at low-levels which is possible for instance on a Mediterranean diet.

2.8 Fortification

Considering the low consumption of fish and seafood in large parts of the population, attempts have been made to fortify food with LC n-3 PUFA. It has been shown that enriched foods with LC n-3 PUFA result in higher plasma phospholipid DHA and EPA content indicating acceptable bioavailability. However, it can be problematic to fortify foods with cod fish oil, because it has a strong odour and taste that can be hard to hide. Therefore it is a challenge to find the right amount of LC n-3 PUFA for fortification in order to get both the health benefits as well as still a satisfactory flavour. A study on sensory quality of certain instant foods such as milk and jelly fortified with fish powder showed that instant foods containing milk powder or fat might be better suited for high levels of fortification than other types of food. Microencapsulation could also be preferable alternative because such coating can reduce side effects such as reflux or heartburn and therefore increase patient compliance.

2.8.1 Microencapsulation

When nutrients are incorporated into foods there can slowly degrade and lose their activity or even become hazardous because of oxidative reactions. They can also react with components present in the food leading to changes in colour and taste of the product and limit the bioavailability of certain nutrients. Microencapsulation reduces these reactions.

Fortification with microencapsulated nutrients has been successfully used, e.g., with vitamins, minerals, fatty acids and antioxidants. The technology in microencapsulation can be divided into two groups; using liquid as suspending medium or using gas as a suspending medium into which a liquid phase is sprayed (spray-drying). Spray-drying is the industrial method of choice when fatty acids and other oil-based compounds are
encapsulated. Using celluloses, especially methylcellulose, has shown improvements in stability when fish oil is spray dried. Microencapsulated fish oil seems to have little or no effect on the flavour of food products and good acceptance has been shown in taste panel evaluations.

2.9 Bioavailability

Bioavailability of nutrients is every bit as important as the amount of the respective substance in the food. Bioavailability can be described as the magnitude by which a nutrient can be absorbed and moved to systematic circulation or a site of physiological activity. The absorption of PUFA is a complex process and can differ after the chemical form they appear in. The bioavailability of n-3 FA formulations have been investigated at some level because it depends on numerous factors, such as presence of other components affecting the uptake of the fatty acids, concomitant intake of food, but primarily the chemical bonds of the fatty acid. LC n-3 PUFA found in fish are mostly triglycerides and free fatty acids in a minor extent, as found in unrefined raw fish oil.

Capsule coating which is resistant to gastric acid can increase acceptability and decrease gastric side effects of fish liver oil. Furthermore, encapsulation leads to finely dispersed oil droplets which might result into a higher bioavailability because of an easier breakdown by lipases compared with liquid fish oil.

2.9.1 Bioavailability - previous studies

A study from 2000 compared the bioavailability of fortified foods with microencapsulated fish oil and fish oil capsules. Both intervention groups got the same amount of n-3 PUFA or 0.9 g/d for 4 weeks. The result showed no significant difference between the intervention groups which indicates that foods enriched with microencapsulated fish oil display the same bioavailability as fish oil capsules and can be considered as an alternative. Another study shows similar results.

A randomized parallel study investigating potential difference of bioavailability between fish meals and fish oil supplements found out that consumption of similar amounts of LC n-3 PUFA either from two weekly servings of salmon or a daily dosage of salmon oil capsules (in this case 6 capsules) were equally effective in increasing LC n-3 PUFA status in healthy individuals.
Similar results came from a 16 week randomized controlled trial comparing bioavailability between fish consumption and fish oil capsules. No significant differences were found between groups, however during the first month of the intervention EPA concentrations increased faster in the fish group compared to the capsule group, nevertheless, this difference disappeared during the course of the intervention. These finding suggest that over a short term, EPA may be more bioavailable when consumed from fish meals than from capsules.

Visioli et al. did not come to the same conclusion, their findings indicated that fish is more efficient than capsules in providing LC n-3 PUFA. Elvevoll et al. support Visioli’s findings and conclude that consumption of 2 meals per week of fat fish such as salmon (containing 1.2 g EPA + DHA on average per day) is almost as effective as 15 g daily intake of cod liver oil (containing 3.0 g EPA + DHA on average per day) when serum concentrations of EPA + DHA are examined.
3 METHODS

Methods regarding the intervention and analysis related to my thesis are described detailed in the manuscript, chapter 4. Data collected from the intervention were also used in the thesis “Ready to eat meals enriched with omega-3. Product development and consumer study” written by Valgerður Lilja Jónsdóttir.

3.1 Author’s contribution

The work process regarding my Masters project can be divided into three milestones.

1. Preparation and applications regarding the intervention

My work for the study started January 2013. The first part was to prepare and turn in an application to the National Bioethics Committee getting the approval to perform the intervention study and an application to The Data Protection Authority. Permissions were received in March 2013.

2. Recruitment of subjects and intervention

The second part of the study was the recruitment of subjects and the intervention. My part was to prepare and distribute the advertisement for recruitment, receive submissions, inform subjects what involved in participating in the study and distribute appointments. My part in the intervention was to receive and inform the participants when arriving at the research facility and keep records and attendances in order. Data collection which I participated in started in May 2013 and was finished in July 2013.

3. Data processing, writing of the manuscript and thesis

The third part was data processing where everything was entered into excel and prepared to import to SPSS for analysis. Statistical analysis presented where made with supervision from Alfons Ramel. In cooperation with my supervisor and Valgerður, we wrote a report for Matís to present the latest results for organizations related to the research. A draft of the manuscript “Bioavailability of long chain n-3 fatty acids from enriched meals and from microencapsulated powder” was written and has been submitted to the European Journal of Clinical Nutrition. In February 2014 I participated in the annual educational review day from the Faculty of Food Science and Nutrition, University of Iceland, with a short overview and poster on the project, and at last the thesis was written.
4 MANUSCRIPT

Title: Bioavailability of long chain n-3 fatty acids from enriched meals and from microencapsulated powder.

Running title: Bioavailability of long chain n-3 fatty acids.

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Abstract

**Background:** Despite potential benefits of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), intake is often low due to low consumption of oily seafood. Microencapsulated fish oil powder has been suggested as an alternative way to increase intake. We investigated the bioavailability of LC n-3 PUFA from microencapsulated powder.

**Methods:** Participants (N=99, age ≥ 50 years) of this 4-week double-blinded dietary intervention were randomized into three groups. Group 1 (n=38) received 1.75g/d LC n-3 PUFA as ready-to-eat meals enriched with liquid cod liver oil, group 2 (n=30) received the same amount of LC n-3 PUFA as microencapsulated cod liver oil powder and regular meals; and group 3 (n=31) was the control group which received placebo powder and regular meals. Blood samples were collected at baseline and endpoint.

**Results:** Seventy-seven subjects (77.8%) finished the study. The amount of EPA in blood doubled in both groups who received LC n-3 PUFA (P<0.05) but did not change in the control group. The changes in DHA were less but still significant in both intervention groups. The omega-3 index as well as the n-6/n-3 ratio improved dramatically in the two LC n-3 PUFA groups (P < 0.05). No changes were observed in the control group.

**Conclusion:** After 4 weeks of regular consumption, the amount of LC n-3 PUFA in blood increased significantly in both intervention groups but not in the control group. Bioavailability of LC n-3 PUFA in encapsulated powder is very similar to bioavailability of LC n-3 PUFA in ready-to-eat meals enriched with liquid cod liver oil.

**Keywords:** Bioavailability, double-blinded intervention study, long chain fatty acids, enrichment, powder.
Introduction

Studies have shown potential benefits of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) against various diseases, e.g., cardiovascular disease, where eicosapentsenoic acid (EPA) and docosahexaenoic acid (DHA) can reduce cardiovascular risk in persons with high risk factors. LC n-3 PUFA have even been suggested to reduce risk of death from coronary heart diseases in healthy adults with a reasonable intake target with a range of 400-500 mg/d. The therapeutic value of LC n-3 PUFA in treatment in inflammatory diseases has also been noticed with a particular focus on rheumatoid arthritis. Studies have also suggested that supplementation of LC n-3 PUFA in parenteral nutrition is safe and may for instance improve immune and hyper-inflammatory response in surgical intensive care unit patients after surgery.

Oily seafood is the main source of LC n-3 PUFA. The Nordic Nutrition Recommendations recommend that LC n-3 PUFA should at least contribute 1% of total energy intake (E%) for adults and children from 2 years of age. Despite recommendations and awareness of the benefits from fish consumption, food habits have changed over the years and few people meet the recommendations of consumption which is two fish meals per week with a focus on oily fish.

Considering the low consumption of fish and seafood in large parts of the population, attempts have been made to fortify food with LC n-3 PUFA from fish liver oil. It has been shown that enriched foods with LC n-3 PUFA result in higher plasma phospholipid DHA and EPA content indicating bioavailability. However, it can be problematic to fortify foods with fish liver oil,
because it has a strong odour and taste that can be hard to hide. Therefore it is a challenge to find the right amount of LC n-3 PUFA for fortification in order to get both the health benefits as well as still a satisfactory flavour.

As an alternative, flavour neutral microencapsulated fish liver oil in powder form has been suggested for fortification of foods. In this context it is important to consider bioavailability, because bioavailability of LC n-3 PUFA has been reported to depend on their chemical form.\textsuperscript{14} Few studies that have been conducted in this area have though indicated mostly comparable bioavailability of microencapsulated fish oil compared to fish oil gelatine.\textsuperscript{15,16} However, according to our best knowledge there have not been any human studies comparing bioavailability of LC n-3 PUFA in powder form (microencapsulated fish oil) in comparison to meals enriched with liquid fish oil.

Given these considerations, the current randomized, doubly blinded, dietary intervention study investigated the bioavailability of LC n-3 PUFA; 1) either consumed as microencapsulated cod liver oil powder or 2) consumed in meals enriched with liquid cod liver oil over a four weeks period.

\textbf{Methods and materials}

\textbf{Subjects}

All participants (N = 99) from the capital area of Iceland were recruited through advertisements on the internet, through e-mail lists at the University of Iceland and through advertisements published in regional health care facilities. The study was conducted from May until October 2013. Inclusion criteria were age 50 years or over and regular consumption of fish or fish meals.
The only exclusion criterion was a previous record of digestive disease which could interfere with the digestion or absorption of dietary fat. The study was approved by the National Bioethics Committee (VSNb201302008/03.07) and was notified by the Data Protection Authority (S6241/2013). All persons gave their informed consent prior to their inclusion in the study.

**Study design**

This was a 4-week randomized, placebo controlled, doubly-blinded dietary intervention study. Subjects were randomized into three groups. Group 1 (n = 38) received 6 meals/week fortified with a liquid oil blend (see below) providing 1.75 g EPA and DHA daily and 6 sachets of placebo powder. Group 2 (n = 30) received 6 conventional meals/week and 6 sachets of microencapsulated powder providing 1.75 g EPA and DHA daily (see below) and group 3 (n = 31) was the control group which received conventional meals and placebo powder.

The meals were fortified with cold liver and olive oil blend provided by BioActive Foods AS, Trondheim, Norway (www.1life63.com). The microencapsulated LC n-3 powder was also from BioActive Foods in Norway and is based on the same oil blend (see Table 1). The participants received 6 powder sachets each week and were given written instructions on how to use the powder. The meals were produced by Grimur Kokkur ehf, Vestmanneyjar, Iceland (www.grimurkokkur.is/en). All the dishes were kept frozen until cooking or heating. The conventional meals did not provide any noteworthy amount of LC n-3 PUFA. Protein powder with light vanilla flavor was used as placebo powder in groups 1 and 3; unfortified meals were used in groups 2 and 3. Subjects were told to exclude all LC n-3 PUFA from their diet at least for 2 weeks before the intervention and also while the intervention lasted.
All measurements were conducted at baseline and at endpoint of the study.

**Anthropometric measurements**

Body weight was measured in light clothing on a calibrated scale (model no. 708, Seca, Hamburg, Germany). Height was measured and body mass index (BMI) was calculated from the recorded height and weight (kg/m²). For the measurement of waist circumference subject stood erect with the abdomen relaxed, arms at the sides, feet together and with their weight equally divided over both legs. The lowest rib margin was first located. Then the iliac crest was palpated in the midaxillary line. A flexible tape was then applied horizontally midway between the lowest rib margin and the iliac crest and tied firmly so that it stayed in position around the abdomen about the level of the umbilicus. Body fat percentage was estimated using a hand held bioimpedance measurement device (Body Fat Monitor BF 306, Omron Healthcare UK Ltd, Milton Keynes, United Kingdom).

**Blood parameters**

Blood samples from fingertip where collected using a home test kit and sent to Norway for analysis on fatty acids, based on the methods published by Saga et al. The content of the home test kit was as follows: user manual (Grako AS, Norway), an absorption paper for the blood spot samples (Grako AS), a disposable automatic lancing device (Med-Kjemi AS, Norway), aluminum bag (Whatman, Denmark) for preservation and storage of the test paper and an enclosed envelope (Grako AS, Norway) addressed to St. Olav’s Hospital, Trondheim University Hospital, Norway, where the analyses were conducted. Omega-3 index was calculated as follows:

\[
\text{Omega-3 Index} \% = \text{Whole blood EPA + DPA + DHA (}) \% \times 0.95 + 0.35
\]
Blood glucose was measured using a finger prick test (Accu-Check, Roche Diagnostics GmbH, Mannheim, Germany).

**Questionnaires**

Each participant was asked questions about socioeconomic status (i.e., relationship status, children, education, children and living area), health-related behavior, e.g., smoking habits, alcohol consumption, health status (food allergy or intolerance), usage of medication and then which medication, questions about physical activity and there were also questions about general purchases and consumption of fish and fish meals.

**Statistical analyses**

The data was entered into the SPSS statistical package, version 21.0 (SPSS, Chicago, IL, USA), and checked for normality using the Kolmogorov-Smirnov test. Data are presented as means ± standard deviations (SD). The paired-samples T-test was used to assess whether the content of fatty acids measured in fingertip blood changed during the course of the intervention. The significance level was set at P ≤ 0.05.

**Results**

Seventy seven subjects finished the study (77.8%). Dropout rates were 28.9%, 16.7% and 19.4% for group 1, group 2 and group 3, respectively (not significantly different). The most common reason for dropout was lack of time or lack of interest.
Baseline characteristics of the participants are shown in Table 2. More than two thirds of the participants were women. Results from the fingertip blood measurements are shown in Table 3. An increase in n-3 fatty acids and related changes in the n6/n3 ratio as well as in the omega-3 index were observed in group 1 and 2, but not in group 3. The significant changes in both LC n-3 groups and no changes in the control group indicate good compliance to the study protocol. No significant difference was between women and men (not shown in table). According to the questionnaires more than 97% of the meals were eaten during the intervention.

**Discussion**

The present study investigated the bioavailability of LC n-3 PUFA in microencapsulated cod liver oil powder and in ready-to-eat meals enriched with liquid cod liver oil. The results show that after 4 weeks of regular consumption, the amount of EPA in blood approximately doubled in both groups who received LC n-3 PUFA but did not change in the control group. The changes in DHA were less pronounced, however, still significant and in the same direction as changes in EPA. The n-3 index as well as the n-6/n-3 ratio improved dramatically in the two LC n-3 PUFA groups but did not change in the control group. The study also shows that the bioavailability of LC n-3 PUFA in encapsulated powder is very similar to bioavailability of LC n-3 PUFA in ready-to-eat meals enriched with liquid cod liver oil. Therefore it can be assumed that both ways can be equally used if increased intake of LC n-3 PUFA is indicated.

**Bioavailability**

Bioavailability of LC n-3 PUFA depends on several factors. Bioavailability of fatty acids can vary significantly depending on their various chemical forms, e.g., natural triglycerides, re-
esterified triglycerides or ethyl esters. Not only the type of chemical bonds but also the concomitant intake of food affect the uptake of LC n-3 PUFA. For example calcium ions can reduce the availability of LC n-3 PUFA by forming a complex with free fatty acids. Microencapsulation, as used in the present study, can possibly affect bioavailability by interacting with fat dispersion in the stomach due to the mechanical influence of stomach peristalsis. Pharmaceutical preparations and dietary supplements often use coatings that are resistant to gastric acid intended to reduce the gastrointestinal side-effects, such as reflux or heartburn, which occur in some people. Wallace et al. compared bioavailability of LC n-3 PUFA between microencapsulated fish oil-enriched foods to fish oil gelatin capsules with the same amount of LC n-3 PUFA. Their findings indicate that foods enriched with microencapsulated fish oil have the same bioavailability as LC n-3 PUFA fish oil capsules. This is in good agreement with our findings where LC n-3 PUFA from foods enriched with cod liver oil and LC n-3 PUFA from microencapsulated cod liver oil powder had comparable bioavailability. When looked at the absolute values in Table 3, the LC n-3 PUFA powder group seem to improve even more than the enriched foods group. However, none of these differences where close to statistical significance (results not shown).

Risk reduction and omega 3 index

The omega-3 index has been defined as the amount of EPA and DHA in red blood cell membranes (expressed as a percent of total fatty acids). It is a good measure of long-term incorporation of fatty acids in tissue and has also been used to estimate heart disease risk. Several studies have shown that higher omega-3 index is associated with a reduction of heart disease. Harris et al. estimated from literature a reasonable target for omega-3 index which
were associated with the lowest risk for CHD mortality. In their findings they proposed a cardio-
protective omega-3 index of 8%; an omega-3 index below 4% was associated with the greatest
risk for CHD death.\textsuperscript{18,21}

In our study we measured fatty acids in whole blood which represents a combination of two
different pools of LC n-3 PUFA, i.e., fatty acids in plasma and in red blood cell.\textsuperscript{24} It has been
shown that the EPA + DHA content of erythrocyte membrane is highly correlated with other
plasma-based measures of EPA + DHA content.\textsuperscript{18,21} With a simple equation as outlined by Harris
et al. it was possible to convert our results to get estimates of omega-3 index.\textsuperscript{18} In our study, the
increase in omega-3 index was, as expected, significantly higher in the intervention groups
(around 1.6\%) compared to the placebo group; however, the omega-3 index did not reach the
target concentration of 8\% after the 4 week intervention. A reason probably therefore is the short
study period of four weeks. However, a 1.6\% increase is still considerable. According to a study
which compared 768 patients with acute coronary syndrome to 768 matched healthy controls, an
increase in the omega-3 Index by 1\% was associated with 23\% lower odds for case status.\textsuperscript{25}

\textbf{Drop-out and adherence}

In this study, drop-out rate was 22.2\%. In group 2, which received the newly developed LC n-3
PUFA powder, drop-out was not higher than in the other two groups indicating that the
participants tolerated the powder. Drop-out was mostly related to lack of time or lack of interest.
Adherence to the study protocol seemed to be excellent. According to the questionnaires more
than 97\% of the meals were eaten during the intervention. Additionally, the changes in LC n-3
PUFA in blood were in good accordance with the different LC n-3 PUFA consumption in all of the three groups.

**Strengths and Limitations**

It is a strength of the present study that it was both doubly blinded and randomized and that there was a control group that did not receive LC n-3 PUFA during the intervention period. It is however a limitation that the study period was rather short. Nevertheless, the study detected still changes in LC n-3 PUFA in accordance with dietary composition of the three study groups.

**Conclusion**

The present study investigated the bioavailability of LC n-3 PUFA in microencapsulated cod liver oil powder and in ready-to-eat meals enriched with liquid cod liver oil. After 4 weeks of regular consumption, the amount of LC n-3 PUFA increased significantly in both intervention groups but not in the control group. Bioavailability of LC n-3 PUFA in encapsulated powder is very similar to bioavailability of LC n-3 PUFA in ready-to-eat meals enriched with liquid cod liver oil.

**Acknowledgments**

This project was funded by Nordic Innovation no 11057: Enriched Convenience Seafood Products. The test meals were provided by Grimur Kokkur ehf, Vestmanneyjar, Iceland; the microencapsulated powder was provided by BioActive Foods AS, Trondheim, Norway. None of these three entities participated in data collection, data interpretation or publication.
Conflict of interests

The authors declare no conflict of interests.
References


23 Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis* 2007; 193: 1-10.


<table>
<thead>
<tr>
<th></th>
<th>LC n-3 PUFA powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>630</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>10</td>
</tr>
<tr>
<td>Carbohydrates %</td>
<td>34</td>
</tr>
<tr>
<td>Sugars %</td>
<td>15</td>
</tr>
<tr>
<td>Ash %</td>
<td>2.5</td>
</tr>
<tr>
<td>Moisture %</td>
<td>2.5</td>
</tr>
<tr>
<td>Fat %</td>
<td>51</td>
</tr>
<tr>
<td>FA profile*</td>
<td></td>
</tr>
<tr>
<td>SFA g</td>
<td>15.4</td>
</tr>
<tr>
<td>MUFA g</td>
<td>22.9</td>
</tr>
<tr>
<td>Omega-9 g</td>
<td>18.3</td>
</tr>
<tr>
<td>PUFA g</td>
<td>12.1</td>
</tr>
<tr>
<td>Omega-6 g</td>
<td>1.7</td>
</tr>
<tr>
<td>Omega-3 g</td>
<td>9.4</td>
</tr>
<tr>
<td>EPA g</td>
<td>5.3</td>
</tr>
<tr>
<td>DHA g</td>
<td>2.4</td>
</tr>
<tr>
<td>Vitamin D µg</td>
<td>40.7</td>
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</tbody>
</table>

*Fatty acids profile is identical to liquid cod liver oil blend used in the fortified meals.
<table>
<thead>
<tr>
<th></th>
<th>Enriched meal group (n = 38)</th>
<th>LC n-3 powder group (n = 30)</th>
<th>Control group (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender female/male</td>
<td>17/10</td>
<td>16/9</td>
<td>19/6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 ± 6</td>
<td>56 ± 6</td>
<td>55 ± 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.3 ± 16.3</td>
<td>84.8 ± 16.3</td>
<td>78.6 ± 21.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5 ± 5.2</td>
<td>29.6 ± 6.0</td>
<td>27.0 ± 5.8</td>
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<tr>
<td>Waist circumf. (cm)</td>
<td>98.1 ± 14.1</td>
<td>103.0 ± 16.3</td>
<td>94.5 ± 18.2</td>
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<tr>
<td>Body fat (%)</td>
<td>33.7 ± 8.3</td>
<td>34.3 ± 8.6</td>
<td>33.0 ± 7.3</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>6.0 ± 1.7</td>
<td>5.9 ± 1.5</td>
<td>5.7 ± 0.9</td>
</tr>
</tbody>
</table>

Data are shown as means ± SD
Table 3 Whole blood fatty acids measurements from fingertip test (completers only).

<table>
<thead>
<tr>
<th></th>
<th>Enriched meal group (n = 27)</th>
<th>LC n-3 powder group (n = 25)</th>
<th>control group (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPA (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t0</td>
<td>1.15 ± 1.1</td>
<td>0.83 ± 0.3</td>
<td>1.06 ± 0.4</td>
</tr>
<tr>
<td>t1</td>
<td>1.91 ± 0.7*</td>
<td>2.02 ± 0.7*</td>
<td>1.13 ± 0.4</td>
</tr>
<tr>
<td><strong>DHA (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t0</td>
<td>3.04 ± 1.0</td>
<td>3.12 ± 0.7</td>
<td>3.40 ± 0.8</td>
</tr>
<tr>
<td>t1</td>
<td>3.62 ± 0.8*</td>
<td>3.69 ± 0.7*</td>
<td>3.42 ± 0.6</td>
</tr>
<tr>
<td><strong>n-6/n-3 ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t0</td>
<td>8.52 ± 3.8</td>
<td>10.25 ± 3.9</td>
<td>7.64 ± 2.6</td>
</tr>
<tr>
<td>t1</td>
<td>4.23 ± 1.6*</td>
<td>4.77 ± 3.1*</td>
<td>7.58 ± 2.3</td>
</tr>
<tr>
<td><strong>Omega-3 index (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t0</td>
<td>5.66 ± 1.7</td>
<td>5.51 ± 1.0</td>
<td>5.99 ± 1.2</td>
</tr>
<tr>
<td>t1</td>
<td>7.08 ± 1.5*</td>
<td>7.38 ± 1.5*</td>
<td>6.03 ± 1.0</td>
</tr>
</tbody>
</table>

Data are shown as means ± SD

* Significant difference between baseline and endpoint

$t_0$ = baseline
$t_1$ = endpoint
5 FUTURE PERSPECTIVES

This thesis discussed the bioavailability of LC n-3 PUFA from two different sources. First as fortified in ready-to-eat meals and second in powder form dissolved in water or other liquid. The results indicate that there is no difference in bioavailability between these two forms of ingestions meaning that consumption of n-3 FA in both powder form and as fortified meals are equally useful ways to increase n-3 FA \textit{in vivo}. Further research is needed to confirm the effects that LC n-3 PUFA have on the human body whereas there have been studies that show positive, negative and no effects at all from ingestion of the fatty acids.

A great deal of information that was collected in the study has not yet been analysed and could be researched further. Blood samples from vein were taken in the study that have not been analysed. If further analysis would be done it could be possible to look at potential changes in total cholesterol in serum, serum triglycerides and insulin. Some studies indicate that LC n-3 PUFA can have positive effect on blood pressure but the evidence is inconsistent, therefore it could be interesting to analyse this important outcome.\textsuperscript{56,57}

The general questionnaire provided information which could be interesting to dig deeper and for instance to see if smoking has any effect on the bioavailability of PUFA. Pawlosky et al. found in their study that smoking increased the n-3 bioavailability from plasma, accelerated the fractional synthetic rates, and heightened the percentage formation of some LC n-3 PUFA in men and women.\textsuperscript{58} These conclusions are in contrast to findings from cell culture but could be counteracted due to losses that occur through peroxidation. Further analyses of information could give us conclusions from this study whether the LC n-3 PUFA status raises more, less or equal, smokers compared to non-smokers.

LC n-3 PUFA are mostly found in fat fish, but due to various reasons not everybody has the opportunity to consume two portions of fat fish per week as recommended. Therefore it is necessary to have other options available to consume these important fatty acids. In this research we have shown two possible alternative ways, i.e., ingestion of microencapsulated LC n-3 PUFA powder and consumption of fortified meals.

Microencapsulated LC n-3 PUFA provide great opportunities in the field of product development. Whereas the powder is taste- and odour less it gives the opportunity to be added in many types of foods without having an effect on the original flavour.
6 REFERENCES


20 Styrkársdóttir A. Skóli og heilsa í 100 ár. [cited 07.05.2014]; Available from http://www.landlaeknir.is/um-embaettid/frettir/frett/item17093/Skoli-og-lydheilsa-


43 Green TJ, Liu Y, Dadgar S, Li W, Böhni R, Kitts DD. Wheat rolls fortified with microencapsulated L-5-methyltetrahydrofolinic acid or equimolar folic acid increase blood


7 APPENDICES

Appendix 7.1: Permission from the National Bioethics Committee
Appendix 7.2: Declaration from the Data Protection Authority
Appendix 7.3: Advertisement
Appendix 7.4: Introduction letter for participants
Appendix 7.5: Information letter for participants
Appendix 7.6: Informed written consent
Appendix 7.7: General questionnaire
Efni: Varðar: 13-039-S1 Lífaðgengi omega-3 fitusýra í matvæli og duftformi.


Fjallað var um svarbréf þitt og önnur innsend gögn á fundi Visindasíðanefndar 12.03.2013.

Rannsóknaráætlunin er endanlega samþykkt af Visindasíðanefnd.

Visindasíðanefnd bendir rannsakendum vinsamlegast á að birta VSN tilvisunarnúmer rannsóknarinnar þar sem vitnað er í leyfi nefindarinnar í birtum greinum um rannsóknina.

Jafnframt fer Visindasíðanefnd fram á að fá send afrit af, eða tilvisun í, birtar greinar um rannsóknina. Rannsakendur eru minntr á að tilkynna rannsóknarlok til nefindarinnar.

Áréttað er að allar fyrirhugaðar breytingar á þegar samþykktari rannsóknaráætlun þurfa að koma inn til nefindarinnar til umfjöllunar.

Jafnframt ber ábyrgðarmanni að látu stofnunir, sem veitt hafa leyfi vegna framkvæmdar rannsóknarinnar eða öflunar gagna vita af fyrirhugdum breytingum.

Með kveðju,

f.h. Visindasíðanefndar,

Gisli Ragnarsson, varaformaður
Harpa Hrund Hinriksdóttir
Píngaseli 8
109 Reykjavík

Persónuvernd
Rauðbrúarstræti 10 105 Reykjavík
sni: 510 9000 betennai: 510 9006
neðfang: postur@personuvernd.is
veffang: personuvernd.is

Reykjavík 27. mars 2013
Tilvísun: S6241/2013/ HGK/–

Hér með staðfestist að Persónuvernd hefur móttekið tilkynningu í yðar nafni um vinnslu persónuupplýsinga. Tilkynningin er nr. S6241/2013 og fylgir afrit hennar hjálagt.

Vakin er athygli á því að tilkynningin hefur verið birt á heimasíðu stofnunarinnar. Tekið skal fram að með móttöku og birtingu tilkynninga hefur engin afstaða verið tekin af hálfu Persónuverndar til efnis þeirra.

Vörðungarfyllst,

[Untegning]

Hjál.: - Tilkynning nr. S6241/2013 um vinnslu persónuupplýsinga.

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Þátttakendur óskast, vilt þú vera með?

Tilgangur verkefnisins „Lífaðgengi omega-3 fitusýra viðbættum í matvæli og í duftformi“ er að kanna hversu vel líkaminn nýtir omega-3 fitusýrur sem koma sem viðbót úr tilbúnunum réttum og bera þær saman við nýtingu omega-3 fitusýra sem eru í duftformi. Við erum að leita að fólki til að taka þátt í rannsókninni.

Þátttakendur:
- 50 ára eða eldri
- Borða fisk og fiskmáltíðir
- Þjáust ekki af alvarlegum eða langvarandi meltingarfærasjúkdóum

Það sem þú þarft að gera:
- Borða tilbúunar máltíðir sem þú færð FRÍTT 6 sinnum í viku í 4 vikur
- Drekka uppleyst omega-3 duft/lyfleysu 6 sinnum í viku í 4 vikur
- Mæta tvisvar í blóðsýnatöku og líkamsmælingar
- Svara almennum spurningalista um mataræði og heilsufarsþætti
- Svara spurningalista um máltíðirnar

Þeir sem ljúka við rannsóknina eiga möguleika á að vinna eitt af þremur 15.000 kr gjafabréfum sem dregið verður um.

Rannsóknarstofa í næringarfræði við Landspítala, Matvæla- og næringarfræðideild Háskóla Íslands og Matís óska eftir þátttakendum í rannsókn sem hlotið hefur samþykki Vísindasiðanefndar. Ábyrgðarmaður rannsóknarinnar er Alfons Ramel fræðimaður í næringarfræði (864-8330).

Áhugasamir sem uppfylla ofangreind skilyrði eru beðnir um að hafa samband við Hörpu Hrund Hinriksdóttur, meistaranema í næringarfræði í síma 694-2752 eða með því að senda tölvupóst á hhh15@hi.is
Kynningarbréf fyrir þátttakendur í rannsókninni

„Lífaðgengi omega-3 fitusýra viðbættum í matvæli og í duftformi“

Kæri viðtakandi,

Fyrirhugað er að hefja ofangreinda rannsókn á vegum Rannsóknarstofu í næringarfræði og Matís sem er styrkt af Visindasjóð Landspítala og NiCe. Markmið rannsóknarinnar er að kanna lífaðgengi omega-3 fitusýra hjá einstaklingum sem hafa annarsvegar neytt tilbúinna réttta með viðbættu omega-3 og hins vegar fengið omega-3 í duftformi sem blandað er í vatn. Þessi rannsókn er hluti af námsverkefni Hörpu Hrundar Hinriksdóttur sem er í meistaranámi í næringarfræði og Valgerður Lilju Jónsdóttur sem er í meistaranámi í matvælafræði við Matvæla- og næringarfræðideild Háskóla Íslands.

| Ábyrgðarmaður rannsóknarinnar: | Alfons Ramel fræðimaður  
| | Rannsóknarstofa í næringarfræði  
| | Landspítali Háskólasjúkrahúss  
| | Eiríkgötu 29, 1. hæð  
| | 101 Reykjavík  
| | Sími: 864-8330/ 543-8410  
| | Tölvupóstur: alfonsra@landspitali.is |

Aðrir rannsakendur

Alfons Ramel fræðimaður  
Rannsóknarstofa í næringarfræði  
Sími: 864-8330  
Tölvupóstur: alfonsra@landspitali.is

Harpa Hrund Hinriksdóttir MS nemi  
Rannsóknarstofa í næringarfræði  
Sími: 694-2752  
Tölvupóstur: hhh15@hi.is

Valgerður Lilja Jónsdóttir MS nemi  
Matís  
Sími:691-9878  
Tölvupóstur: valgerdur@matis.is

Öflun þátttakenda og þátttökkuskilyrði

Þátttakendur eru einstaklingar 50 ára og eldri sem eiga ekki við alvarlega eða langvarandi melingarsjúkdóma að striða. Auglýst verður eftir þátttakendum. Þátttakendum er óheimilt að neyta ákveðinna matvæla á meðan rannsókn stendur. Sjá fylgiskjal.
**Hvað felst í þátttöku?**

Þátttakendur fá kynningu á verkefninu við upphaf rannsóknar þar sem þeir eru beðnir um að gefa skriflegt samþykki, lagður er fyrir almennur spurningalisti sem tekur u.þ.b. 10 mínútur að svara, útskyrt verður nákvæmlega hverning rannsókn fræ fram og lista um matvæli sem ber að forðast úthlutað. Annar spurningalisti er afhentur um gæðjun á fiskréttingum sem er svarað á meðan rannsókn stendur og tekur það u.þ.b. 5 mínútur að svara hverjum lista. Einum spurningalisti er svarað fyrir hvern rétt, alls 6 spurningalistar. Tvað blöðsýnatökur á umsöðum tíma, önnur í upphafi rannsóknar og hin í lok rannsóknar. Mælingar verða gerðar á þloðhag, þloðglúkósa, insúlíni og þloðfitum. Þátttaka felur einnig í sér að neyta tilbúinna retta og drekka duftlausn út í vatn 6 sinnum í viku í 4 vikur. Einstaklingar sem taka þátt í rannsókninni þurfa að forðast matvæli sem innihalda omega-3 fitusýrur í 3 vikur áður en þilutun hefst.

**Kostnáður/greiðslur**

Þátttaka mun hvorki fela í sér kostnáð né er greitt fyrir þátttökuna. Rannsakendur munu afla þátttakendum tilbúnum réttum til neyslu 6 daga vikunnar sem samvarar einnig heitri máltil þann daginn auk duftlausnar. Þeir sem ljúka við þátttöku eiga möguleika á að vinna eitt af þremur 15.000 króna gjafabréfum sem dregið verður um að rannsókn lokinni.

**Áhætta og ávinningur**


**Trúnaður við þátttakendur**


**Hætt við þátttöku**

Það er þitt val hvort þú tekur þátt í rannsókninni eða ekki. Þér er frjálst að hafna þátttöku eða hættu í rannsókninni á hvaða stigi sem er, án útskyringa.

Kær kveðja,
með von um góðar undirtektir,

Alfons Ramel, Harpa Hrund Hinriksdóttir, Valgerður Lilja Jónsdóttir

Rannsóknin er unnin með samþykki Vísindasafnsins og tilkynning hefur verið send til Persónuverndar. Ef þú hefur spurningar um rétt þinn sem þátttakandi í vísinarrannsókn eða vill hættu þátttöku í rannsókninni getur þú snuð þér til Vísindasafnsins, Hafnarhúsinu við Tryggvägötu 17, 150 Reykjavík. Sími: 551-7100,
7.5 Information letter for participants

Komutímar fyrir omega-3 rannsókn

Nafn þátttakanda:

_______ 1. Heimsókn – MÆTA FASTANDI
Rannsókn byrjar
- Svara almennum spurningalista
- Blóðprufa
- Blóðsyskur mældur
- Blóðbrýstingur mældur
- Likamsmælingar
  o BIA
  o Ummáls mælingar
  o Þyngd
  o Hæð
- Réttir og duft fyrir fyrstu viku afhent
- Spurningalisti 1 afhentur

_______ 2. Heimsókn
- Réttir og duft fyrir viku 2 afhent
- Spurningalisti 1 skilað

_______ 3. Heimsókn
- Réttir og duft fyrir viku 3 afhent

_______ 4. Heimsókn
- Réttir og duft fyrir viku 4 afhent
- Spurningalisti 2 afhentur

_______ 5. Heimsókn – MÆTA FASTANDI
Rannsókn lýkur síðasta koma MUNA að skila spurningalistum
- Blóðprufa
- Blóðsyskur mældur
- Blóðbrýstingur mældur
- Likamsmælingar
Matvæli sem bera að forðast á meðan þátttaka er í rannsókn
„Lífaðgengi omega-3 fitusýra viðbættum í matvæli og í duftformi“

Vinsamlegast neytið ekki eftirfarandi matvæla í 3 vikur yfir þátttöku og á meðan þátttöku stendur. Ástæðan fyrir því er að ákveðin matvæli geta skekkt niðurstöður rannsóknarinnar.

Ekki má borða:

**Fiskur, allur feitur fiskur eða sem inniheldur hátt hlutfall omega-3**

- Lax
- Makrill
- Túnfiskur
- Bleikja
- Lúða
- Makrill
- Loðna
- Rauðmagi
- Silungur
- Kaviar
- Hrogn

**Fæðubótaefni**

- Lýsi
- Lýsisperlur
- Omega-3
- Heilsutvenna
- Sportþrenna
- Æskubrunnur
- Öll önnur fæðubótaefni sem innihalda omega-3, EPA eða DHA í innihaldslýsingu, t.d.
  - Udos blend
  - Hörfræolía

**Annað**

- Öll matvæli með viðbættu omega-3, EPA eða DHA í innihaldslýsingu
- Mikið magn af hnetum
- Mikið magn jurtaolía
Allar heimsóknir fara fram á sama stað.

Kópavogsbraut 5-7
7.6 Informed written consent

UPPLÝST SAMÞYKKI FYRIR ÞÁTTTÖKU
Í VÍSINDARANNSÓKNI “Lífaðgengi omega-3 fitusýra viðbættum í matvæli og í duftformi”

Ég undirrituð/aður samþykki að taka þátt í visindarannsókninni “Lífaðgengi omega-3 fitusýra viðbættum í matvæli og í duftformi”.

Þátttaka í rannsókninni felst í því að mæta tvisvar á umsömdum tíma í blóðsýnatöku, neyta tilbúinna réttu og duftlausnar.

Ég samþykki einnig að svara almennum spurningalista og spurningalista um geðjun fiskréttu.

Ég samþykki að ég er búinn að fá góða kynningu á verkefninu og skil til hvers er ætlast af mér í rannsókninni.

Ég samþykki að öll dulkóðuð gögn og þar með talin blóðsýni, sem safnað er saman í rannsókninni verði ekki eytt eftir að rannsókn líkur og megi nýta í visindalegum tilgangi í framtíðinni s.s. í samanburðarrannsóknnum, gegn leyfi visindasiðanefndar.

Niðurstöður rannsóknarinnar munu verða sendar til birtingar í virtum erlendum visindatímaritum og verða kynntar fagfólk.

Þátttakandi hefur lesið kynningarbréf fyrir “Lífaðgengi omega-3 fitusýra viðbættum í matvæli og í duftformi”.
Undirskrift þátttakanda:

Ég ________________________________________________________________

 lísi því hér með yfir að ég gef samþykki mitt af fúsum og frjálsum vilja fyrir því að taka þátt sem sjálfbóðaliði í þessari hlutandi rannsókn.
Ég hef fengið nauðsynlegar upplýsingar og lesið þær yfir.

Mér hefur verið kynnt eðli og umfang þessarar visindarannsóknar og ég er samþykk(ur) þátttöku og skrifa því undir þessi tvö eintök:

Dagsetning og staður:

Undirskrift þátttakanda

Undirritun þess sem aflar samþýkks


UPPLÝST SAMÞYKKI ÞETTA ER Í TVÍRITI, ÞÁTTTAKANDI HELDUR EFTIR EINU EINTAKI, SÁ SEM AFLAR SAMÞÝKKIS HELDUR EFTIR ÖDRU EINTAKI
7.7 General questionnaire

Spurningalisti fyrir þátttakendur

Nafn: ___________________________  Aldur: ____________

Þátttakendanúmer: _______  Dagssetning: _______

A) Likamsmál

Hæð (m): __________  BMI (kg/m²): __________

Þyngd (kg): __________

Mittismál (cm): __________

Mjaðmamál (cm): __________

B) Bioimpedans

Fituhlutfall (%): __________

Fita (kg): __________

Fitufrír massi (kg): __________

Grunnefnaskipti (kcal/d): __________

Total body water (TBW) (l): __________

C) Aðrar mælingar

Blóðþrýstingur: __________

Blóðóskursgildi: __________
Almennar spurningar

1) Hver er hjúskaparstaða þín?

Ég...

☐ er gift/-ur
☐ er í sambúð
☐ er fráskilin/-n
☐ er einhleyp/-ur
☐ bý hjá foreldrum

2) Hversu mórg börn átt þú? ___

3) Hversu margir búa á heimilinu? ___

4) Hver er menntun þín?

☐ Grunnskólamenntun
☐ Framhaldsskólamenntun
☐ Háskólamenntun

☐ B.Sc/B.A/B.Ed
☐ M.Sc/M.A/M.Ed/Cand. psyc

☐ Verknám
☐ Annað nám: ____________________________

5) Hvert er póstnúmerið í hverfinu þínu? ______
Spurningar um reykingar

6) Hefur þú einkvern tíma reykt?
   □ já
   □ nei (ef svarað nei þann farðu beint að spurningu 8)

7) Reykir þú núna?
   □ já (ef svarað já þann farðu beint að spurningu 7)
   □ nei

8) Ef þú ert hætt/hættur að reykja, hvað ertu búin/nn að vera hætt/hættur lengi?
   □ < 1 ár
   □ 1-5 ár
   □ 6-10 ár
   □ >10 ár

9) Ef þú reykir eða hefur reykt, hve margar sigarett/vindla reykir þú/reyktir þú að meðaltali?
   □ “Social smoker” – minna en 10 stk að meðaltali á viku
   □ <10 stk á dag
   □ 11-15 stk á dag
   □ 16-20 stk á dag
   □ > 20 stk á dag
Spurningar um áfengisneylsu

10) Drekkur þú áfengi?

☐ já
☐ nei (ef svarað nei tandi farðu beint að spurningu 10)

11) Ef þú drekkur áfengi hve marga drykki af áfengi drekkur þú að meðaltali á viku? (einn drykkur er 1 lítil bjór, 1 léttvínsglas, 1 einfaldur sterkur drykkur)

☐ 1 drykk eða minna á viku
☐ 2-5 drykki á viku
☐ 6-10 drykki á viku
☐ >10 drykki á viku
Spurningar um heilsu

12) Hefur þú fæðuofnæmi og/eða fæðuóþol fyrir einhverju svo þú vitir?
   ∎ já (fæðuóþol _____ fæðuofnæmi ___)
   ∎ nei (ef svarað neitandi farðu beint að spurningu 12)

13) Fyrir hverju hefur þú ofnæmi/óþol (merktu við allt sem á við)?
   ∎ Fiskofnæmi
   ∎ Skelfiskofnæmi
   ∎ Eggiaofnæmi
   ∎ Mjólkurofnæmi
   ∎ Mjólkursykursóþol (laktósaóþol)
   ∎ Hnetur og/eða möndlur
   ∎ Jarðhnetur og/eða ertur
   ∎ Glútenóþol
   ∎ Sojaofnæmi
   ∎ Annað ofnæmi – hvaða: __________________________
Spurningar um lyf

14) Tekur þú núna einhver lyf, þ.m.t. náttúrulyf?
   - ja
   - nei (ef svarað neitandi sleppa spurningu 13)

15) Hvaða lyf tekur þú að staðaldri (merkið við fleiri en eitt svar ef við á)?
   - Hormónalyf (nafn lyfs: ____________________________)
   - Bólögueyðandi lyf (nafn lyfs: ____________________________)
   - Blóðþrýstingslækkandi lyf (nafn lyfs: ____________________________)
   - Blóðvíritutækkandi lyf (nafn lyfs: ____________________________)
   - Blóðþynningarlyf (nafn lyfs: ____________________________)
   - Ofnæmislyf (nafn lyfs: ____________________________)
   - Lyf við sykursýki (nafn lyfs: ____________________________)
   - Sýklalyf (nafn lyfs: ____________________________)
   - Verkjalalyf (nafn lyfs: ____________________________)
   - Steralyf (nafn lyfs: ____________________________)
   - Ónnur lyf – (hvaða: ____________________________)
   - Vitamín – (hvaða: ____________________________)
   - Náttúrulyf – (hvaða: ____________________________)
**Spurning um hreyfingu**

16) Hreyfir þú þig reglulega (vinsamlegast lestu yfir listann fyrir neðan áður en þú svarar) ?

- Ja
- Nei

17) Hve miklum tíma eyðir þú í eftirfarandi athafnir (miðað við síðasta ár) ?

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<td>Hjólað á kyrfstæðu hjöld (t.d í likamsreaktarstöð)</td>
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<td>Knattspyrma</td>
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<tr>
<td>Annað (vinsamlegast takð fram hvað)</td>
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</tbody>
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54
## Spurningar um innkaup og neyslu

### 1. Ég kaupi fisk eða annað sjávarfang

<table>
<thead>
<tr>
<th>Aldrei</th>
<th>1-4 sinnum á ári</th>
<th>5-8 sinnum á ári</th>
<th>1 sinni í mánuði</th>
<th>2-3 sinnum í mánuði</th>
<th>1 sinni í viku</th>
<th>2 sinnum í viku</th>
<th>3-4 sinnum í viku</th>
<th>Daglega/ nær daglega</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

### 2. Ég kaupi tilbúna fiskrétti (eins og t.d. fiskrétti í fiskiborði eða frosna fiskbollur til upphitunar)

<table>
<thead>
<tr>
<th>Aldrei</th>
<th>1-4 sinnum á ári</th>
<th>5-8 sinnum á ári</th>
<th>1 sinni í mánuði</th>
<th>2-3 sinnum í mánuði</th>
<th>1 sinni í viku</th>
<th>2 sinnum í viku</th>
<th>3-4 sinnum í viku</th>
<th>Daglega/ nær daglega</th>
</tr>
</thead>
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</tbody>
</table>

### 3. Hversu oft borðar þú fisk sem aðalrétt?

<table>
<thead>
<tr>
<th>Aldrei</th>
<th>1-4 sinnum á ári</th>
<th>5-8 sinnum á ári</th>
<th>1 sinni í mánuði</th>
<th>2-3 sinnum í mánuði</th>
<th>1 sinni í viku</th>
<th>2 sinnum í viku</th>
<th>3-4 sinnum í viku</th>
<th>Daglega/ nær daglega</th>
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</thead>
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</table>

### 4. Hversu oft borðar þú tilbúna fiskrétti sem aðalrétt?

<table>
<thead>
<tr>
<th>Aldrei</th>
<th>1-4 sinnum á ári</th>
<th>5-8 sinnum á ári</th>
<th>1 sinni í mánuði</th>
<th>2-3 sinnum í mánuði</th>
<th>1 sinni í viku</th>
<th>2 sinnum í viku</th>
<th>3-4 sinnum í viku</th>
<th>Daglega/ nær daglega</th>
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</tbody>
</table>

### 5. Hversu oft tekur þú inn vítamin, lýsi eða steinefni?

<table>
<thead>
<tr>
<th>Aldrei</th>
<th>Sjaldnar en 1 sinni í mánuði</th>
<th>1 sinni í mánuði</th>
<th>2-3 sinnum í mánuði</th>
<th>1 sinni í viku</th>
<th>2 sinnum í viku</th>
<th>3-4 sinnum í viku</th>
<th>Daglega/ nær daglega</th>
<th>Oftar en 1 sinni á dag</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

### 6. Skoðar þú innihaldsupplysingar á umbúðum matvæla?

<table>
<thead>
<tr>
<th>Aldrei</th>
<th>Sjaldan</th>
<th>Stundum</th>
<th>Oft</th>
<th>Alltaf</th>
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</thead>
<tbody>
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