List of included papers

The PhD thesis is based on the following three papers:

**Paper I:**
S Vuholm, MN Teisen, NG Buch, KD Stark, J Jakobsen, C Mølgaard, L Lauritzen, and CT Damsgaard.

*Is high oily fish intake achievable and how does it affect nutrient status in 8-9-year-old children?: the FiSK Junior trial.*

**Paper II:**
S Vuholm, JM Rantanen, MN Teisen, KD Stark, C Mølgaard, JH Christensen, L Lauritzen, and CT Damsgaard.

*Effects of oily fish intake on cardiometabolic markers in healthy 8- to 9-y-old children: the FiSK Junior randomized trial.*

**Paper III:**
S Vuholm, MN Teisen, C Mølgaard, L Lauritzen*, and CT Damsgaard*.

*Sleep and physical activity in healthy 8-9-year-old children are affected by oily fish consumption in the FiSK Junior randomized trial.*
The Journal of Nutrition, Feb 2020 (under review)

*These authors have contributed equally.
Other scientific contributions

Co-authorships on publications related to FiSK Junior


MN Teisen, J Niclasen, S Vuholm, J Lundbye-Jensen, KD Stark, CT Damsgaard, SS Geertsen, and L Lauritzen. Exploring correlations between neuropsychological measures and domain-specific consistency in associations with n-3 LCPUFA status in 8-9 year-old boys and girls. PLoS ONE, 2019; 14(5);e0216696. DOI: 10.1371/journal.pone.0216696.

S Vuholm and CT Damsgaard. Can citizen science give us new knowledge about Danes’ fish intake? [Can citizen science give us new knowledge about Danes’ fish intake?] Diætisten, 2019;157;7-11. (popular science paper in Danish).


MN Teisen*, S Vuholm*, J Niclasen, JM Rantanen, JH Christensen, CT Damsgaard, and L Lauritzen*. Effects of oily fish consumption on acute and long-term stress in healthy 8-9-year-old children: The FiSK Junior randomized trial. (draft, only reviewed by authors marked with *).

Co-authorships on publications related to other trials


**International conferences**

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<th>Date and location</th>
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<td>13th Congress by the International Society for the Study of Fatty Acids and Lipids (ISSFAL)</td>
<td>Oily fish intake improves the plasma lipid profile in healthy 8-9 year old children: The FiSK Junior randomized controlled trial (poster)</td>
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<tr>
<td>2019, 7-9 March Valencia, Spain</td>
<td>6th International Conference on Nutrition &amp; Growth</td>
<td>Oily fish intake affects cardiometabolic health in healthy 8-9-year-old children - The FiSK Junior trial (oral)</td>
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<td>2020, 26-28 March London, UK</td>
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Summary

**Background:** The fish intake is low in most Western countries, especially among children, and thus few meet the dietary guidelines for fish intake. Fish is the main dietary source of n-3 long-chain polyunsaturated fatty acids (LCPUFA) and vitamin D, but may substitute meat which is a better iron source. Many children have an insufficient vitamin D status, especially during winter, but also iron deficiency is prevalent among European children, why there is a need for studies that evaluate how an increased fish intake affects children’s nutrient status. Intake of n-3 LCPUFA is recognized for having beneficial effects on several cardiometabolic risk markers mainly blood pressure and triacylglycerol. However, few trials have investigated if similar effects can be achieved by a fish intake in line with the dietary guidelines, and studies are lacking among children. Moreover, novel but very limited evidence has indicated that n-3 LCPUFA might affect sleep and physical activity but findings are inconclusive and there is a need for studies which apply objective assessment methods.

**Objectives:** The overall objective of this PhD thesis was to investigate whether a high intake of oily fish affected health among healthy children, evaluated as the effects on nutrient status, cardiometabolic markers, sleep and physical activity. The secondary aim was to investigate if any of these effects were sex-specific.

**Design and methods:** All papers included in this PhD thesis are based on data from the FiSK Junior, which was a randomized controlled trial where 199 healthy Danish 8-9-year-old children received either oily fish or poultry (control) to be eaten 5 times/week (~300 g/week) for 12±2 weeks. At baseline and endpoint examinations, we measured blood pressure, heart rate, and heart rate variability via 3-h continuous electrocardiograms and collected fasting blood samples which were analyzed for erythrocyte EPA (20:5n-3) + DHA (22:6n-3), serum 25-hydroxyvitamin D (25(OH)D), blood hemoglobin, plasma ferritin, serum triacylglycerol, LDL and HDL cholesterol and plasma glucose and insulin. Prior to the examinations, 4-day dietary intake was recorded and the children wore accelerometers on their waist for 7 consecutive days while their parents registered sleep times. Sleep and physical activity measures were extracted from the actograms. The co-primary outcomes of FiSK Junior were defined as diastolic blood pressure and serum triacylglycerol.

**Results:** The fish group had a median (25th-75th percentile) fish intake of 375 (325-426) g/week which increased the children’s EPA+DHA and 25(OH)D status and improved their lipid profile by a dose-dependent lowering in serum triacylglycerol and increase in HDL cholesterol. The iron status was slightly but not markedly reduced compared to the poultry group while no effects were found on blood pressure, heart rate variability and glucose homeostasis. Furthermore, oily fish consumption appeared to improve sleep compared to poultry reflected by shorter latency time to sleep onset and less weekly variability in sleep duration. Also, physical activity was modified in the fish group as sedentary spare time was dose-dependently increased at the expense of light physical activity, while moderate-to-vigorous physical activity...
was increased during school hours. Several of the effects of oily fish intake indicated sex-specificity, as mainly the boys had a lowering in serum triacylglycerol and tended to have greater effects on physical activity compared to girls. On the other hand, the girls tended to reduce heart rate in response to oily fish intake and the sleep improvements appeared more pronounced in the girls than in the boys.

**Conclusion:** The evidence enclosed in this PhD thesis supports that a high oily fish intake among children contributes to beneficial health effects. An increased intake of oily fish can be nutritionally justified among healthy children since n-3 LCPUFA and vitamin D status was increased while not markedly compromising iron status. The improvements in children’s lipid profile support that classical n-3 LCPUFA effects can be achieved by consumption of fish *per se* and even in healthy children. These changes potentially contribute to substantial health improvements at a population level. Additionally, a high oily fish intake possibly improves children’s sleep and modifies their physical activity pattern. However, these findings should mainly be considered as hypothesis-generating and need to be substantiated by more human randomized controlled trials. Likewise, the indicated sex-specific effects of oily fish should be investigated further, optimally in studies that are sufficiently powered for sex-stratified analyses. Nevertheless, the presented evidence altogether supports the dietary guidelines for fish intake and highlights the relevance for initiatives that aim for increasing the fish intake even among healthy children and with a priority for oily fish types which are rich in n-3 LCPUFA and vitamin D.
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Sammendrag (Danish summary)

Baggrund: Indtaget af fisk er lavt i de fleste vestlige lande, især blandt børn, og kun få opfylder kostrådende for fiskeindtag. Fisk er den primære kilde til n-3 langkædet flerumættede fedtsyrer (LCPUFA) samt D-vitamin, men substituerer muligvis kød som er en bedre jernkilde. Mange børn har D-vitamin insufficiens, især om vinteren, men også jernmangel er udbredt blandt europæiske børn, hvorfor der er behov for klarhed over hvordan et øget fiskeindtag påvirker børns næringsstatus. Indtag af n-3 LCPUFA anses for at have gavnlige effekter på adskillige kardiometabolske risikomarkører, hovedsageligt blodtryk og triacylglycerol. Dog har få studier undersøgt, om lignende effekter kan opnås ved et fiskeindtag i overensstemmelse med kostrådene, og sådanne studier mangler helt blandt børn. Desuden har ny, men meget begrænset evidens indikeret, at n-3 LCPUFA muligvis påvirker søvn og fysisk aktivitet, men resultaterne er inkonsistente, og der er behov for studier som anvender objektive målemetoder.

Formål: Det overordnede formål med denne ph.d.-afhandling var at undersøge, om et højt indtag af fed fisk påvirker sundheden blandt raske børn, vurderet ud fra effekterne på næringsstatus, kardiometaboliske markører, søvn og fysisk aktivitet. Det sekundære formål var at undersøge, om nogle af disse effekter var kønspezifiske.


Resultater: Fiskegruppen havde et mediant (25.-75. percentil) fiskeindtag på 375 (325-426) g/uge, hvilket øgede børnenes EPA+DHA og 25(OH)D status samt forbedrede deres lipidprofil ved en dosisafhængig senkning af serum triacylglycerol og stigning i HDL-kolesterol. Deres jernstatus var let, men ikke markant reduceret sammenlignet med fjerkrægruppen, mens der ingen effekter var på blodtryk, hjerterytmevariabilitet og glukosehomeostase. Endvidere så det ud til, at indtaget af fed fisk i forhold til fjerkræ forbedrede børnenes søvn udtrykt som kortere latenstid til søvnindtræden og mindre ugentlig variation i søvnvarighed. Ligeledes havde fiskegruppen en ændring i fysisk aktivitet, idet stillesiddende fritid blev dosisafhængigt
forøget på bekostning af let fysisk aktivitet, mens moderat til høj fysisk aktivitet blev forøget i skoletiden. Flere af effekterne fra fed fisk indikerede kønspecificitet, idet hovedsageligt drengene havde en sænkning af serum triacylglycerol og samtidig havde en tendens til større effekter på fysisk aktivitet sammenlignet med pigerne. Derimod havde pigerne en tendens til sænkning i hjerterytmen efter indtag af fed fisk, og også søvneffekterne var mere udtalt hos pigerne end hos drengene.

# 1 Introduction

Most Western countries have food-based dietary guidelines that state an intake of at least two weekly servings of fish and with half of the intake comprising oily fish types \(^{(1)}\). However, in most populations the fish intake is below the recommendation, especially among children \(^{(2–5)}\). Fish is the main dietary source of n-3 long-chain polyunsaturated fatty acids (LCPUFA), which are recognized for several health benefits \(^{(6,7)}\). Additionally, fish is rich in other relevant nutrients such as vitamin D which many children are insufficient of, especially during winter \(^{(8)}\). However, an increased consumption of fish may replace other animal protein sources such as meat, which is an important dietary source of iron. The European Food Safety Authority (EFSA) advocates that among infants and young children there should be particular attention on their intake and status of the n-3 LCPUFA docosahexanoic acid (C22:6n-3, DHA) as well as vitamin D and iron \(^{(9)}\). Thus, there is a need for studies that investigate how increases in fish intake would affect children’s diet and nutrient status.

A high intake of fish has been associated with lower cardiovascular disease (CVD) risk \(^{(10–12)}\), and several intervention trials among adults confirm that supplementation with fish oil lowers traditional CVD risk markers such as blood pressure and triacylglycerol (TAG) \(^{(13–16)}\). This evidence forms the primary basis for the dietary guidelines for fish intake \(^{(17)}\). However, most intervention trials have been conducted with fish oils rather than fish \emph{per se}, despite the fact that dietary guidelines are formulated for fish. Furthermore, the n-3 LCPUFA doses used are often higher than the expected dose from fish if consumed in amounts that correspond to the guidelines (about 1 g/d). To support the dietary guidelines, there is a need for intervention studies conducted with fish, and such trials are especially lacking among children. Children are generally at low risk for CVD and the immediate clinical impact of small improvements in cardiometabolic markers after fish intake might be limited in healthy children. However, such improvements could be important for health if sustained over time and could have a high impact at population level if the distributions of the markers are shifted in a favorable direction in the population.

Besides the classic effects of n-3 LCPUFA, studies have indicated that n-3 LCPUFA might also affect sleep and physical activity (PA) \(^{(18,19)}\), however the evidence is still very limited and inconclusive. Both sleep duration and PA intensity have declined through the last decades in most Western child populations \(^{(20,21)}\), which might have negative consequences for mental and cardiometabolic health \(^{(22–24)}\). Thus it is relevant to identify interventions that beneficially contribute to these lifestyle patterns, as it might be a strategy to indirectly improve children’s health. Animal models support plausible mechanisms by which n-3 LCPUFA improve sleep and lower hyperactivity \(^{(25–27)}\) and additionally indicate a linkage between vitamin D and sleep regulation \(^{(28)}\). Thus, an increase in fish intake could potentially affect sleep and PA patterns, but human trials are needed to substantiate this hypothetical relationship.
To evaluate the real-life health effects of regular fish consumption and strengthen the knowledge specifically within children, we conducted a randomized controlled trial (RCT) to investigate the health effects of a high oily fish intake in healthy 8-9-year-old children. Based on data from this oily fish intervention and in continuation of previous n-3 LCPUFA research, this PhD thesis aims to address the health effects of fish consumption in a public health perspective.

**Research questions and hypotheses**

With focus on young healthy children, the overall objective of this PhD thesis was to investigate how a high intake of oily fish affected the overall health profile, evaluated as nutrient status, cardiometabolic markers, sleep and PA.

The specific research questions and hypotheses were:

1. **Does oily fish intake affect diet quality and nutrient status in healthy children?** *(Paper I)*
   - Hypothesis: Intake of oily fish will dose-dependently increase n-3 LCPUFA and vitamin D status, but will slightly compromise iron status.

2. **Does oily fish intake affect markers of cardiometabolic health in healthy children?** *(Paper II)*
   - Hypothesis: Intake of oily fish will decrease blood pressure and TAG and the effects will show dose-dependency with n-3 LCPUFA status.

3. **Does oily fish intake affect sleep quality and PA patterns in healthy children?** *(Paper III)*
   - Hypothesis: Intake of oily fish will improve sleep quality and lower PA intensity towards more sedentary time.

4. **Are any of the health effects of oily fish intake different in boys and girls?** *(Paper II + III)*
   - Hypothesis: Some of the effects of oily fish intake might be sex-specific. I have no specific hypothesis of whether it will be in favor of boys or girls.